THE UNIVERSITY OF HULL

Characterising the drivers of tropical freshwater fish dynamics and abundance in the Mekong river, under environmental change

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by

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Abstract

The Mekong river's monsoon driven annual flood pulse creates a range of diverse habitats, with high levels of connectivity and primary productivity that support and trigger fish migratory dynamics and abundance. This abundance is reflected in the fact that the Mekong is the World's most productive inland fisheries, supporting between 2.8-3.2 million tonnes of catch annually, underpinning the food security of over 70 million people.

This thesis explores the impacts of climate change and anthropogenic activity on Mekong hydrology and system function, and the impacts these have on fish and fisheries. It combines an interdisciplinary approach utilising earth observation, historical fisheries data and hydrological records, alongside the application of hydrological modelling tools and the testing of new environmental DNA metabarcoding analyses, in order to explore how changes in Mekong hydrology will affect fish populations into the future.

The results highlight a range of projected negative impacts of hydropower development, irrigation expansion and climate change on the *dai* fishery resources, and in turn a range of significant impacts on regional fish protein availability, which primarily result from the blockage of key migratory routes caused by dam construction as well as changes in the timing of the flood pulse in key areas in the basin such as Tonle Sap Great Lake. The findings in this study urge a need to prioritise environmental conservation action centred on a need to maintain the historical flood pulse hydrologic regime of the Mekong river. Such a pulse is shown to be important in sustaining floodplain flood dynamics and habitat connectivity that maintains the critical *dai* fishery, enables migratory fish dynamics, and is thus key to overall regional food security.

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Content

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Equations

- **Chapter 4**: “Applying Earth Observation to assess historical variations in the flood pulse of the Tonle Sap Great Lake system: importance to the *dai* fishery and species composition”:

- **Chapter 5:** “Quantifying the impacts of hydropower, irrigation and climate change scenarios on the dynamics and productivity of the Tonle Sap Great Lake system”:

Nomenclature

Nomenclature

$\sum$= Sum
A= Area (km^2)
CPUE= Catch Per Unit Effort (kg *dai*$^{-1}$ day^{-1})
D= days
Fa= Flood area (km^2)
FI= Flood Index (km^2 days)
F_{in}= flow in (m^3 s^{-1})
F_{out}= flow out (m^3 s^{-1})
La= Lake area (km^2)
Q= Discharge/Flow/run-off (m^3 s^{-1})
ONI= Oceanic Niño Index
WL= Water level (meters)
Y= Year

Acronyms

3S	Se Kon, Se Prok, Se San
ADCP	Acoustic Doppler Current Profiler
AMCS	Asian Monsoon Climate System
amsl	above mean sea level
ANOSIM	Analysis of Similarities
ANOVA	Analysis of Variance
APHRODITE	Asian Precipitation – Highly-Resolved Observational Data Integration Towards Evaluation
ATTZ	Aquatic Terrestrial Transition Zone
BBC	British Broadcasting Corporation
BLAST	Basic Local Alignment Search Tool
bp	base pair
CC	Climate Change
CIA	Cumulative Impact Assessment
CMIP5	Coupled Model Intercomparison Project 5
COI	Cytochrome oxidase subunit 1
CSI	Connectivity Status Index
Cyt-b	Cytochrome-b
DBM	Digital Bathymetric Model
df	Degrees of freedom
DNA	Deoxyribonucleic acid
eDNA	environmental DNA
ENSO	El Niño Southern Oscillation

FAO	Food and Agriculture Organisation
FPC	Flood Pulse Concept
GCMs	Global Circulation Models
GIS	Geographical Information Systems
GPS	Global Positioning System
GSO	General Statistics Office
HSY	Hoo Som Yai
INCA Model	Integrated Catchment Model
IPCC	International Panel on Climate Change
ITCZ	Inter-Tropical Convergence Zone
IUCN	International Union for Conservation of Nature
KL	Kampong Luong
Lao PDR	Lao People Democratic Republic
LMB	Lower Mekong Basin
MARD	Ministry of Agriculture and Rural Development
MFD	Mekong Fish Database
MIRCA	Monthly Irrigated and Rainfed Crop Areas
MRC	Mekong River Commission
NCBI	National Center for Biotechnology Information
NGS	Next Generation Sequencing
NMDS	Non-metric Multidimensional Scaling
NOAA	National Oceanic and Atmospheric Administration
OAA	Other Aquatic Animals
PCR	Polymerase Chain Reaction
PK	Prek Kdam
PP Port	Phnom Penh Port
qPCR	quantitative Polymerase Chain Reaction
RCPs	Representative Concentration Pathways
rRNA	ribosomal ribonucleic acid
RNA	Ribonucleic acid
SE	Southeast
SRES	Special Report on Emission Scenarios
SRTM	Shuttle Radar Topography Mission
TM	Thematic Mapper
TSGL	Tonle Sap Great Lake
UK	United Kingdom
USA	United States of America
UV-radiation	Ultraviolet radiation
WATCH	Water and Global Change
WWF	World Wildlife Fund

Declaration of authorship

The candidate confirms that the work submitted is her own and acknowledge the collected data sources and where data has been shared in academic collaboration.

- The hydrologic, water quality and fisheries data presented in Chapter 3 and 4 was gathered from the Mekong River Commission Data Portal (www.portal.mrcmekong.org/index), MRC Official Fisheries Logbook (Halls *et al.,* 2013a;b) and acknowledged published studies (e.g. DHI);
- The Landsat-TM images and ENSO data presented in Chapter 4 was gathered from www.earthexplorer.usgs.gov and www.ncdc.noaa.gov respectively;
- The data for the extended flow and DBM Model analyses presented in Chapter 4 were shared in academic collaboration from Kummu *et al.,* (2014).
- The VMod modelling flow data for the individual and combined hydropower, irrigation and climate change scenarios were gathered in shared academic collaboration from Hoang *et al.,* (2016;2019) study and reported in Chapter 5;
- The water samples collected in Cambodia and Lao PDR for the eDNA analyses (Chapter 6) were collected by Rita Santos.

The data obtained and collected during this thesis was processed and interpreted by Rita Santos.

CHAPTER 1

Introduction

Freshwater environments represent nearly 0.02% of the available volume of aquatic habitat and cover approximately 0.8% of the Earth's surface (Dawson, 2012). Despite such a small area, freshwater environments sustain a disproportionally high level of biodiversity, hosting nearly 126,000 species, which represent almost 10% of all reported species for all environments across the Earth (Tedesco *et al.,* 2017). Furthermore, from the 126,000 species, 15,750 freshwater fish species have been described, representing 48% of the global described fish diversity (i.e. 48% for freshwater and 52% for marine environments) and approximately 25% of all reported vertebrates (Eschmeyer and Fong, 2013). Freshwater fish play a crucial part in the function of global ecosystems (Lévêque and Mounolou, 2004; Hoffman *et al.,* 2010; Lynch *et al.,* 2016). They are of considerable interest for the scientific community, with every year a significant proportion of published studies focusing on their taxonomic diversity, adaptive strategies and competition, life histories, genetic evolution, among others (Lévêque and Mounolou, 2004; Liermann *et al.,* 2012; Closs *et al.,* 2016; Lynch *et al.,* 2016). Additionally, freshwater fish contribute to over 40% of the World's inland catch, underpinning food security at the global scale (UNEP, 2010; Cooke *et al.,* 2016; Lynch *et al.,* 2016). Significantly, developing countries rely heavily on freshwater fish for high proportions of calorific intake and protein provision (FAO, 2018). Nearly 90% of the tropical inland fisheries (cumulative percentage for capture and aquaculture) is used for human consumption. For example, up to 80% of the animal protein consumed in the Mekong basin comes directly from fish (Hortle, 2009; Baran, 2010; Ziv *et al.,* 2012; DHI, 2015).

Yet, despite their notorious importance, until now there has been no globally comprehensive assessment of freshwater fishes' conservation status, and it is likely that many species are disappearing without record (IUCN, 2013; Closs *et al.,* 2016). An overall freshwater biodiversity decay of 52% was recorded from 1970 to 2010, being significantly higher than the 30% observed for the marine environment during the same period (Millennium Ecosystem Assessment, 2005;

WWF, 2014). This issue raises more awareness due to the rate at which rivers, lakes and wetlands are disappearing or becoming imperilled (Liermann *et al.,* 2012). According to Vörösmarty *et al.,* (2010), 65% of inland habitats worldwide are already classified as moderately to highly threatened by anthropogenic stressors. Thieme *et al.,* (2011) summarizes the major threats to freshwater ecosystems affecting biodiversity as i) the position of freshwater in the landscape favours the deposition of pollutants, heavy sediment loads and other run-offs from the terrestrial environment; ii) competition for freshwater resources with growing human population (hydropower, irrigation for agriculture, etc.); iii) harvest pressures, especially fisheries; iv) high level of connectivity making it accessible to the widespread of invasive species and v) climate change, with increase seasonal periods of flooding and droughts, and changes in water quality (Arthington *et al.,* 2010; Dugan *et al.,* 2010; Corlett, 2014; Turak *et al.,* 2017).

Many regions are poorly assessed for their overall freshwater fish diversity. This is particularly true in the sub-tropical and tropical regions (roughly delimited by the Tropic of Cancer ($23^{0}27'$N) and the Tropic of Capricorn ($23^{0}27'$S), where the highest known freshwater species number, richness and endemism (ecological concept to define species that are unique to a defined location) prevail (Lucas and Baras, 2001; Sodhi *et al.,* 2010; Oberdorff *et al.,* 2011; Corlett, 2014). Abell *et al.,* (2008) provide a global comprehensive assessment of freshwater fish diversity according to delimited ecoregions (Figure 1.1). The study is still considered as one of the most reliable global estimates of the geographical distribution of freshwater fish diversity (Pimm *et al.,* 2014; Closs *et al.,* 2016), showing unequivocally that over 50% of the known freshwater fish species diversity and endemism is located within the tropics (Sodhi *et al.,* 2010).

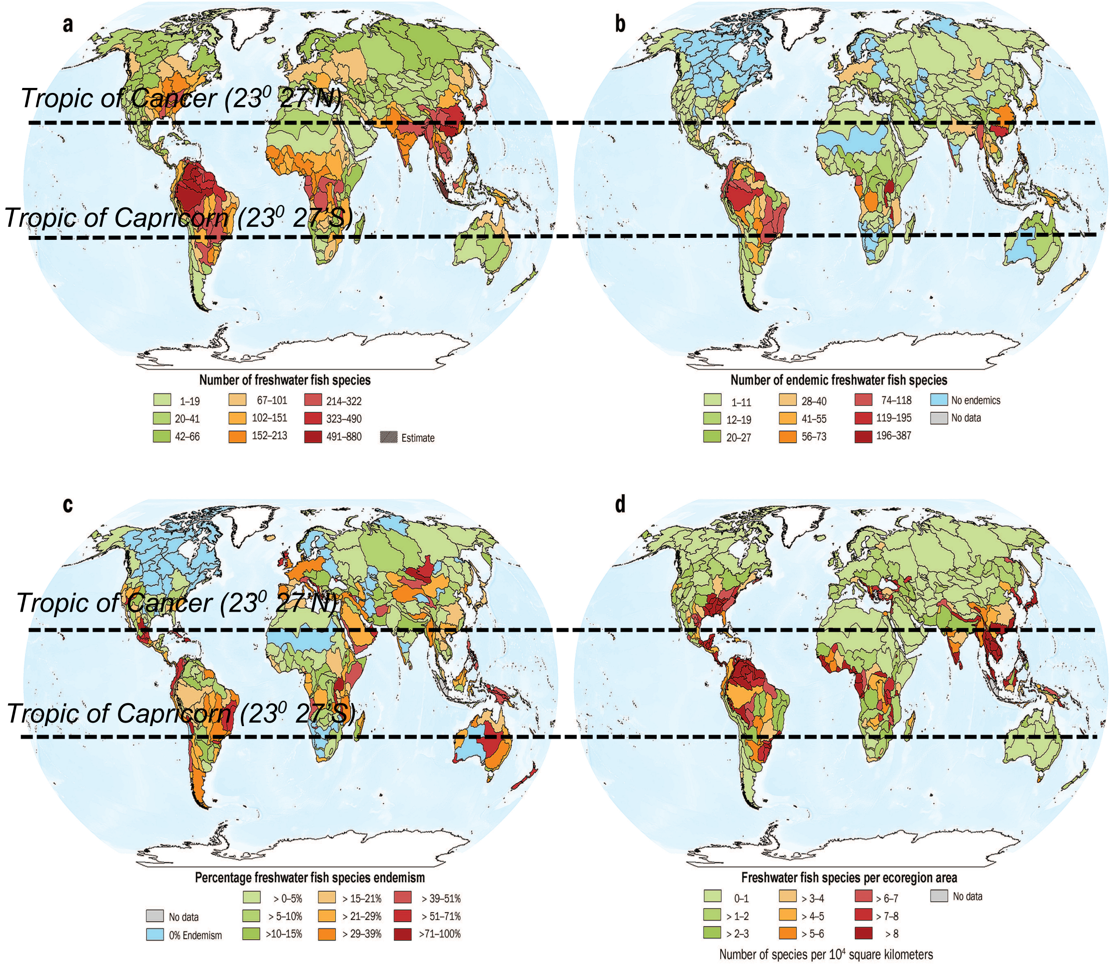


FIGURE 1.1 - WORLDWIDE FRESHWATER FISH SPECIES NUMBER (A), ENDEMIC NUMBER (B), PERCENTAGE OF ENDEMISM (C), AND NUMBER OF SPECIES PER 10^4 KM^2 OF THE DIFFERENT ECOREGIONS (D). THE DARK DASHLINE REPRESENTS THE LONGITUDINAL BOUNDARIES OF THE TROPICS OF CANCER AND CAPRICORN FOR THE DELIMITATION OF THE TROPICAL AND SUB-TROPICAL REGIONS, RESPECTIVELY (SOURCE: ABELL *ET AL.*, 2008).

Although temperate freshwater regions have a long history of being human-altered, mostly due to water diversion, clearance of habitats and water pollution (Closs *et al.,* 2016), the tropical and sub-tropical freshwater regions have been experiencing unprecedented levels of threats compared to temperate regions. This is associated with: 1) the location, mainly flowing through developing countries, with the populations living under high poverty and lacking on developed institutional services (e.g. sanitation, water treatments, among others), releasing waste waters directly to the river; 2) Southern Asia hosts some of the most heavily populated areas in the World, while sustaining the largest tropical river basins and deltas, with over 4 billion people mainly concentrated in the riparian areas and directly depending on water-pressure activities (e.g. fisheries and agriculture) for their livelihoods and food security; 3) the political pursuit for economic development, in light of growing human populations, results in unsustainable continuous exploration of the freshwater environment, neglecting the needs to preserve it for the ecosystem services provided.

The increasing demand for new energy sources and investments, and the fact that the tropics have seen reduced hydropower development, compared to temperate regions, make this region a new area to explore for hydropower (Thieme *et al.,* 2011; Winemiller *et al.,* 2016). Consequently, the threats to the tropical freshwater environment are mainly characterised by hydropower, water abstraction, water pollution, landscape diversion, clearance of habitats and climate change, ultimately affecting the aquatic biota (Dudgeon *et al.,* 2006; Lamoureux *et al.,* 2006; Ficke *et al.,* 2007; Döll *et al.,* 2010; Dugan *et al.,* 2010; Liermann *et al.,* 2012; Lynch *et al.,* 2016). Thus, it not surprising that the highest percentage of imperilled freshwater fish is also concentrated in this region (Sodhi *et al.*, 2010; Figure 1.2).

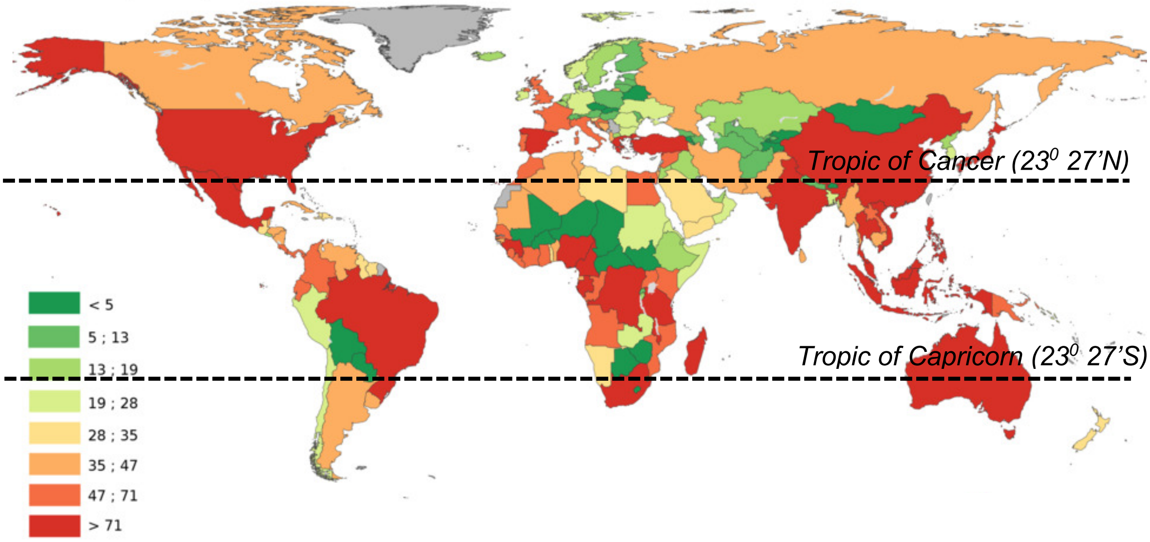


FIGURE 1.2 - PERCENTAGE OF THREATENED FISH SPECIES. THE DARK DASHLINE REPRESENTS THE LONGITUDINAL BOUNDARIES OF THE TROPICS OF CANCER AND CAPRICORN FOR THE DELIMITATION OF THE TROPICAL AND SUB-TROPICAL REGIONS, RESPECTIVELY (SOURCE: THE WORLD BANK, 2015).

1.1 THE TROPICAL AND SUB-TROPICAL ENVIRONMENT: INFLUENCE ON THE DRIVERS OF FISH DYNAMICS, ABUNDANCE AND FISHERIES

The tropical and sub-tropical climate zone occupies nearly 40% of the Earth’s surface (Val *et al.,* 2006) and is roughly delimitated by Latrubesse *et al.,* (2005) as bounded by the Tropic of Cancer ($23^{0}27'$N) and the Tropic of Capricorn ($23^{0}27'$S). This zone supports some of the largest rivers in the World, with a total combined drainage area of nearly 29 $\times 10^{6}$ km^{2}, compared with the nearly 23 x 10^{6} km^{2} from other regions (e.g. temperate). Moreover, it possesses a diversity of seasonally connected and inundated habitats (e.g. floodplains, swamps, mainstream river, estuary, rice fields, lakes, caves, among others) and supplies the highest sediment fluxes and run-off downstream to their deltas, contributing to their sustainability and profitable economic activities that develop (Abell *et al.,* 2008; Darby *et al.,* 2016; Winemiller *et al.,* 2016; Kondolf *et al.,* 2018). The mean annual run-off and mean annual sediment flux in the tropics are estimated at approximately 13,000 km^{3} and 3,500 Mt year^{-1} respectively, compared with the approximately 4,000 km^{3} and 450 Mt year^{-1} from other non-tropical regions of the World (Table 1.1; Latrubesse *et al.,* 2005; Milliman and Farnsworth, 2011; Sinha *et al.,* 2012; Syvitski *et al.,* 2014; Darby *et al*., 2016; Lynch *et al.,* 2016).

According to Latrubesse *et al.,* (2005) and Syvitski *et al.,* (2014), tropical river systems can be broadly classified as 1) lying within a tropical geographic region; 2) showing little intra-annual variability on the experienced warm temperature, and 3) the location of floodplains or deltas within the tropical climate belt. On a global scale, the tropical and sub-tropical region experience relatively stable average annual temperatures yet display large variations in the annual rainfall patterns. This is mainly associated with the Inter-Tropical Convergence Zone (ITCZ), which connected with the Hadley Cell Trade Winds, makes this atmospheric upwelling zone combine with the equatorial moisture and expand to the troposphere, creating an epicentre of convective storms (Latrubesse *et al.,* 2005; Peel *et al.,* 2007; Syvitski *et al.,* 2014). Consequently, seasonal and regional variability in tropical precipitation results in the delimitation of distinct predictable climate systems (e.g. Asian Monsoon Climate System - AMCS) that affects large transboundary river basins like the Mekong, Ganges-Brahmaputra, Yangtze and Irrawaddy, regarding the flow dynamics and sediment flux (Peel *et al.,* 2007).

TABLE 1.1 - THE 30 LARGEST RIVERS IN THE WORLD, DEMARKING IN RED THE ONES BELONGING TO THE TROPICAL AND SUB-TROPICAL REGIONS (ADAPTED FROM: DARBY *ET AL.*, 2016).

BASIN NAME	DRAINAGE AREA (10^6 KM^2)	MEAN ANNUAL RUN-OFF (KM^3 YR^{-1})	MEAN ANNUAL SEDIMENT FLUX (MT YR^{-1})
AMAZON	6.3	6300	1200
CONGO	3.8	1300	43
MISSISSIPI	3.3	490	120
OB	3.0	390	16
NILE	2.9	30	0.2
YENISEI	2.6	620	4.1
PARANA	2.6	530	90
LENA	2.5	520	20
NIGER	2.2	160	40
AMUR	1.9	350	52
YANGTZE	1.8	900	470
MACKENZIE	1.8	310	100
ZAMBEZI	1.3	100	9
ST. LAWRENCE	1.2	340	4.6
NELSON	1.1	89	-----
MURRAY - DARLING	1.1	7.9	1
ORINOCO	1.1	1100	210
ORANGE	1.0	4.5	17
GANGES	0.98	490	520
INDUS	0.98	5	10
RIO GRANDE	0.87	0.7	0.7
YUKON	0.85	210	54
DANUBE	0.82	210	42
SHEBELLE	0.81	19	-----
MEKONG	0.80	450	110
TOCATINS	0.76	370	75
YELLOW	0.75	15	150
BRAHAMAPUTRA	0.67	630	540
COLUMBIA	0.67	240	9.7
KOLYMA	0.60	120	10

The tropical climate, by the predictable delimitation of wet and dry seasons, acts, therefore, as the main driving force on the spatial and temporal variations in water level, flow and sediment flux, with these inter-connected variables influencing the seasonal connectivity and inundation pattern of the diverse and extensive habitats found, and resulting in some of the highest productivity levels in the World. Additionally, these variables affect water quality, notably dissolved oxygen, pH and nutrient recycling (Dudgeon, 2000; Latrubesse *et al.,* 2005; Ducharne, 2008). Thus, the combination of seasonal habitats' availability, connectivity and the food sources established by the recycling of nutrients, trigger different fish migratory behaviour and adaptive strategies, ultimately influencing the fish abundance found (Dudgeon, 2000; Kolding and van Zwieten, 2012) and the productive inland fisheries that develop (Baran and Myschowoda, 2009; Dugan *et al*., 2010; Cooke *et al.,* 2016; Lynch *et al.,* 2016). For this reason, these variables are known as the drivers of tropical freshwater fish dynamics and abundance, developing a cascade of events, with changes in one or all of the drivers directly threatening the resilience of the system, posing challenges on fish diversity, composition, abundance and inland fisheries (Figure 1.3).

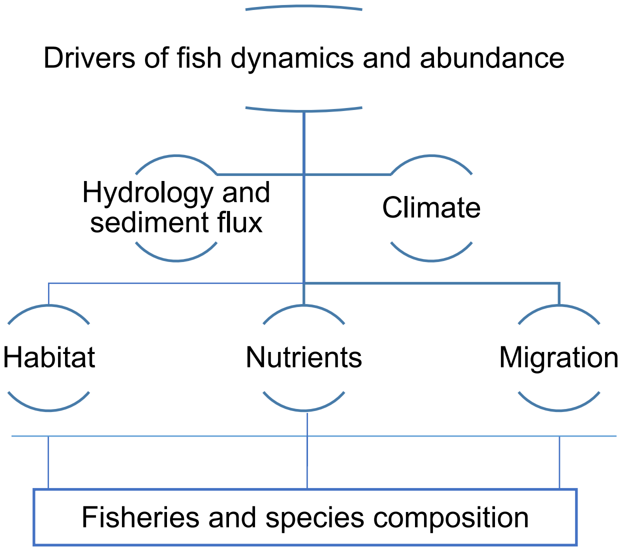


FIGURE 1.3 - THE DRIVERS OF FISH DYNAMICS AND ABUNDANCE IN TROPICAL FRESHWATER ENVIRONMENTS.

Acreman *et al.,* (2014) define the term "environmental flows" as describing the quantity, quality and timing of flows, strictly dependent on the seasonal predictable climatic influence, which are required to sustain freshwater and estuarine ecosystems, and human livelihoods. The term centres on the benefits to the society provided by the eco-hydrologic interactions established in freshwater systems, which result in productive agriculture and fisheries activities. The inland fisheries of the tropics are a result of the exceptional fish production linked with the seasonal flow pattern that drives the species richness found in the unique diversity of habitats. Furthermore, tropical inland fisheries are regarded as the most important in the World for securing fish protein to approximately 60% of the human population who live in the tropics, particularly in developing countries, to whom fish represent a key source for protein intake (Hortle, 2009; Darby *et al.,* 2013; Kondolf *et al.,* 2018). The tropical inland fisheries of Asia and Africa are estimated to provide nearly 80% of the World’s total inland catch (with

Asia as a whole region, accounting for two-thirds of the global percentage; FAO, 2018), which increased exponentially from about 5 million tonnes in 1980 to about 11.63 million tonnes in 2016 (Bartley *et al.,* 2015; FAO, 2018).

However, high data constraints, and inaccuracies surround the reported statistics on the global tropical inland fisheries. Welcomme *et al.,* (2010) reviewed the reasons behind this paucity of data assessment and the identified weakness in the available statistics for the tropics, compared with the relatively well-known inland production in temperate regions, stating five main reasons: 1) inadequate data collection systems; 2) selective data collection; 3) double counting of landings; 4) confusion with aquaculture production, and 5) political pressure. Consequently, the preservation of tropical inland fisheries and efforts to effectively describe its trends, should be pursued for food security issues, mostly in developing countries, where the majority of populations live under high poverty and rely on fish as key source for protein intake (estimated as 40 kg person^{-1} year^{-1}; FAO, 2018).

1.2 THE ANTHROPOGENIC IMPACTS ON THE DRIVERS OF TROPICAL FRESHWATER FISH DYNAMICS AND ABUNDANCE

Historically, the most industrialised areas of the World are all concentrated near lowland water catchments and large river basins, with influences of marine and/or freshwater environments (e.g. the deltas; Kundzewicz *et al.,* 2009; Centre for Research on the Epidemiology of Disasters, 2011; Latrubesse and Parks, 2017). Moreover, an estimated 4 billion of the World's population (nearly 60%; Darby *et al.,* 2013) is concentrated in the tropical and sub-tropical regions, particularly near freshwater and across delta plain lowland areas, and thus being highly dependent on the freshwater resources for poverty alleviation and food security (Kondolf *et al.,* 2018). This dependency is intrinsically connected with the ecosystem services provided. These are related to the easy access to drinking water (Millenium Ecosystem Assessment, 2005; Corlett, 2014), productive fields for agriculture (Orr *et al.,* 2012; Kondolf *et al.,* 2018), food security by fishing (Dudgeon *et al.,* 2006; Baran and Mischowoda, 2009; Dugan *et al.,* 2010; Cooke *et al.,* 2016), navigation and transport. However, above all, the fact that freshwater ecosystems are confined spaces in the landscape means they are intrinsically affected by changes made to it (Vörösmarty *et al.,* 2010; Lehner *et* *al.,* 2011; Closs *et al.,* 2016; Latrubesse and Parks, 2017). Furthermore, as the worldwide human population rises towards a projected peak of 9.7 billion people by 2050 (UN, 2017), comparing to the estimated 7 billion people in 2012, the forecasted water demand is expected to rise to 55% between 2000 and 2050. Moreover, an 80% increase in energy is also required by 2050 along with a 60% rise in food demand expected by 2050 (OECD, 2012). Such projections are alarming for the sustainability of freshwater resources and to human populations, who rely on it for poverty alleviation and food security, with the issue being particularly dramatic in tropical developing countries (World Water Resources, 2015; UN WCR, 2016). It is, therefore, necessary to identify the direct and indirect human impacts on freshwater systems, to then predict future modifications in a system that not only is expected to support high population density, but it also hosts high fish diversity, directly depending on the quantity and quality of the freshwater resources to survive (Abell *et al.,* 2008).

As mentioned previously the freshwater environment is confined by the landscape where it passes, the coastal area where it finishes and influenced by the region's climate and anthropogenic actions (Nijssen *et al.,* 2001; Kundzewicz *et al.,* 2009). Direct and indirect anthropogenic actions in the freshwater watercourses can be summarised under five broad interrelated categories: 1) ecosystem destruction; 2) habitat alteration and degradation; 3) water pollution and altered water chemistry; 4) species additions and removals and 5) climate change (Dudgeon *et al.,* 2006; Closs *et al.,* 2016; Gordon *et al.,* 2018). Thus, water abstraction for irrigation purpose and the modification of the landscape for agriculture, have contributed to the clearance of habitats (e.g. mangrove forests in the Pantanal and floodplain riparian areas in the Lower Mekong Basin; Lamberts, 2008; Closs *et al.,* 2016). An intensification in bankside erosion connected with losses in vegetation, result in water pollution due to wash loads of sediments from the terrestrial environment, particularly from agriculture, often spreading pesticides and other toxins, which deteriorate the water quality for fishes. Moreover, changes in water quality often result in the reduction of dissolved oxygen levels, increase in turbidity, changes in pH, and increased levels of nitrates and phosphates, ultimately affecting species' physiology, behaviour, reproduction and growth, linked to species' tolerance range to modifications in water quality parameters. Importantly, dams and climate change have dramatically modified the flow dynamics, habitat connectivity and water quality, being regarded as major anthropogenic factors impacting upon the fish species diversity, abundance and composition, indirectly facilitating the spread of invasive species and affecting fisheries yields. All of these direct and indirect human interventions in the freshwater environment have profoundly shaped riverine systems, in which freshwater fishes are one of the most affected groups (Figure 1.4; Dudgeon, 2000; Kummu *et al.,* 2010; Vörösmarty *et al.,* 2010; Closs *et al.,* 2016; Winemiller *et al.,* 2016; Gordon *et al.,* 2018).

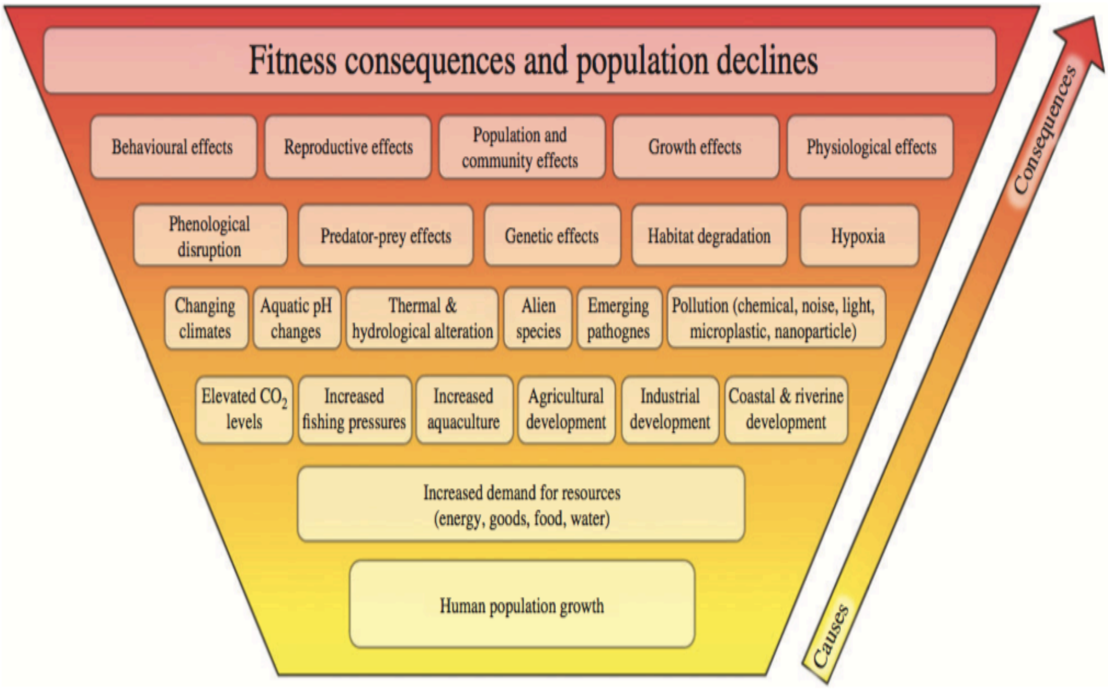


FIGURE 1.4 - HIERARCHICAL STRUCTURE OF THE CAUSES AND CONSEQUENCES OF A WIDE RANGE OF INTER-LINKED THREATS THAT FISHES FACE GLOBALLY, DRIVEN BY ANTHROPOGENIC ACTIONS, WHICH ULTIMATELY AFFECTS THEIR FITNESS, RESULTING IN POPULATION DECLINES (SOURCE: GORDON *ET AL.,* 2018).

1.2.1 HYDROPOWER DEVELOPMENT

Hydropower development has been intensely pursued for energy generation since the Industrial Revolution, when the World’s first hydroelectric power scheme was developed in England in 1878, followed by a boom in the United States of America and Canada, where by 1889 there were 200 small and large schemes already in place (WDC, 2000). In 2012 hydropower generated approximately 17% of the World’s total electricity and 70% of all renewable electricity, with the trend expected to increase each year by 3.1% for the next 25 years (Chao *et al.,* 2008; REN21, 2014). China is the largest energy producer, with an estimated 896.3 TWh in 2013, representing 16.9 % of the total domestic electricity use (Hu, 2014; IPCC, 2011; REN21, 2014). Yet, the high hydropower development in temperate regions contrasts with tropical and sub-tropical regions, where large river basins had remained relatively pristine (i.e. free-flowing waters) until nearly 40 years ago, when the largest South American Itaipu dam opened in 1984, producing 14,000 MW. The record was then surpassed in 2008 by the Three Gorges dam in China, with a 22,500 MW power generation (WDC, 2000; Hu, 2014). Of note, the recent assessment by Grill *et al.,* (2019; Figure 1.5) shows that 63% of the World's rivers have lost connectivity due to dams, with only 37% remaining free-flowing for their entire length, mainly in the tropics in the Amazon and Congo basins. The difficulty in accessing free-flowing inland waters, together with differences in their geomorphology, and the fact that most large river basins are transboundary and affected by each countries' economic power and development policies, have all contributed to these relative pristine conditions (Lehner *et al.,* 2011; Winemiller *et al.,* 2016; Grill *et al.,* 2019).

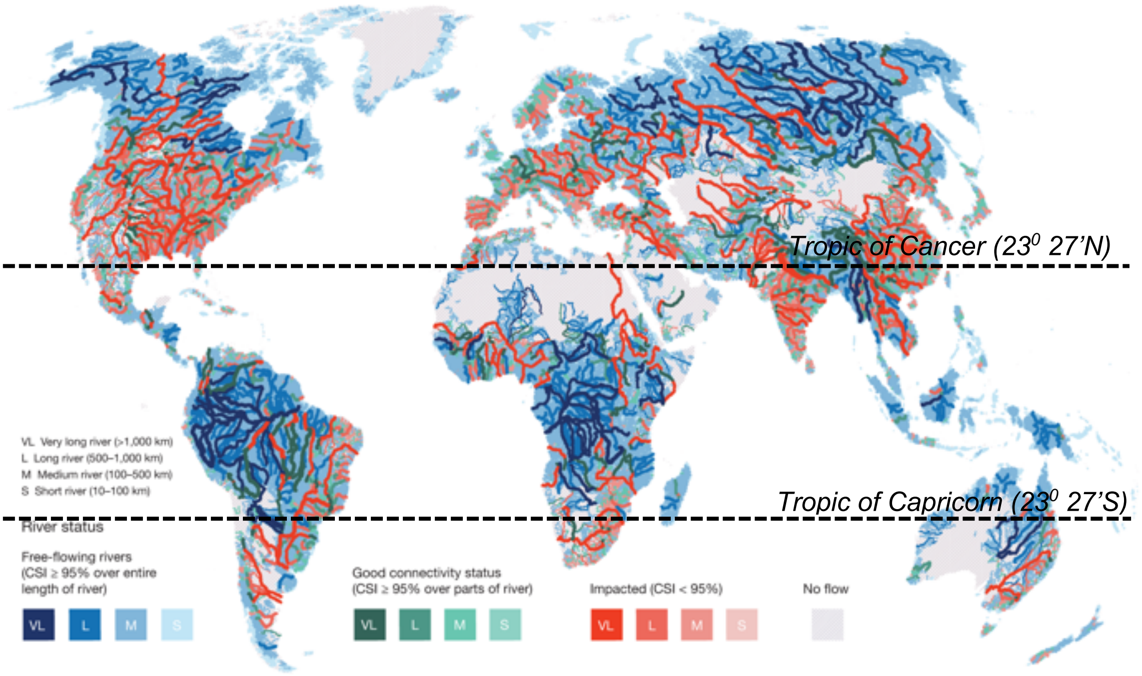


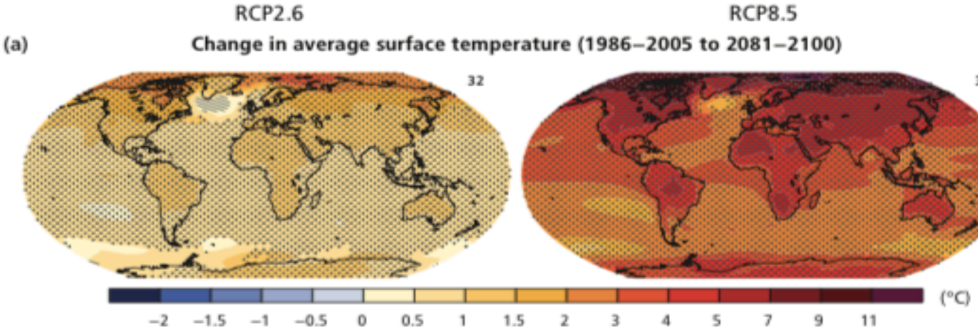
FIGURE 1.5 – THE MAP OF THE WORLD'S FREE-FLOWING RIVERS, SHOWING THE LOSS IN CONNECTIVITY FOR 63% RIVERS OF VARYING LENGTH DUE TO DAM IMPOUNDMENT. ONLY 37% RIVERS LONGER THAN 1,000 KM REMAIN FREE-FLOWING FOR THEIR ENTIRE LENGTH, MAINLY IN THE AMAZON AND CONGO BASINS, DELIMITED BY THE DARK DASH LINE FOR THE TROPICAL AND SUBTROPICAL REGION. THE CONNECTIVITY STATUS INDEX (CSI) SHOWS THE RELATIVE PRISTINE FLOW CONDITIONS IN THE TROPICAL ENVIRONMENT (SOURCE: GRILL *ET AL.,* 2019).

Nowadays, however, hydropower dams are a reality in the tropics, with a set of negative impacts on previously unregulated large transboundary river basins (Keskinen *et al.,* 2010; Kondolf *et al.,* 2014;2018; Winemiller *et al.,* 2016). Examples of impacts include the decline of total catch and fish abundance change in tropical inland fisheries (e.g. the Lake Victoria fishery; Sharpe *et al.,* 2012), human populations needing to be re-allocated (e.g. Amazon basin; Latrubesse *et al.,* 2017), modifications in lateral habitats' seasonal hydrologic connectivity, inundation and productivity, by modifications in the flood pulse dynamics (Lamberts, 2006;2008; Castello and Macedo, 2016), and sediment starvation (Kummu *et al.,* 2010; Kondolf *et al.,* 2014;2018), are some of the negative impacts observed in large tropical transboundary river basins like the Mekong, Amazon, Congo and Nile (Dudgeon *et al.,* 2006; MRC, 2009a; Baran, 2010; DHI, 2015; Winemiller *et al.,* 2016; Latrubesse *et al.,* 2017). Consequently, one can infer the importance of freshwater resources to policy makers of developing countries, where the needs to meet energy demand, and promote economic development have resulted in a continuous increase in hydropower schemes (Hirsch, 2010). Furthermore, this has raised questions on how well-informed the public and investors are to the reasons behind those projects and the short to long-term consequences to the river hydro-ecological dynamics, the fisheries supported and protein provision (Baran, 2010; Keskinen *et al.,* 2010).

1.2.2 CLIMATE CHANGE

The Industrial Revolution marked a dramatic transformation in the history of the World, with fast rates of population growth (Ficke *et al.,* 2007). This was possible by, among other factors, the rise in use of fossil fuels, which have caused profound improvements in people's quality of life (ACIA, 2004). Yet, fossil fuels are responsible for some of the most harmful impacts on the global climate, by burning and realising toxic gases to the atmosphere (i.e. dioxide of carbon, methane, etc.), which have resulted in modifications in global temperature and rainfall, directly affecting freshwater resources and fishes (Bolin *et al.,* 1986; ACIA, 2004; Ficke *et al.,* 2007; IPCC, 2014).

Climate change projections all come with a level of uncertainty, from the inherent assumptions and variables used in the numerical models applied to the Special Report on Emission Scenarios (SRES) and the Representative Concentration Pathways (RCPs) used in the projection studies (Hoanh *et al.,* 2010; Keskinen *et al.,* 2010; Kingston *et al.,* 2011; Beilfuss and Triet, 2014; IPCC, 2014; Thompson *et al.,* 2014). Nevertheless, every effort to increase the knowledge on climate change impacts on natural environments, the species and ultimately the people, should be encouraged. The IPCC (2014), projects that the increase of global mean surface temperature by the end of the 21st century (2081-2100) relative to 1986-2005 is likely to be 0.3-1.7 degrees Celsius under RCP2.6[1]; 1.1-2.6 degrees Celsius under RCP4.5; 1.4-3.1 degrees Celsius under RCP6.0, and 2.6-4.8 degrees Celsius under RCP8.5 (Figure 1.6).



(b) Change in average precipitation (1986–2005 to 2081–2100)

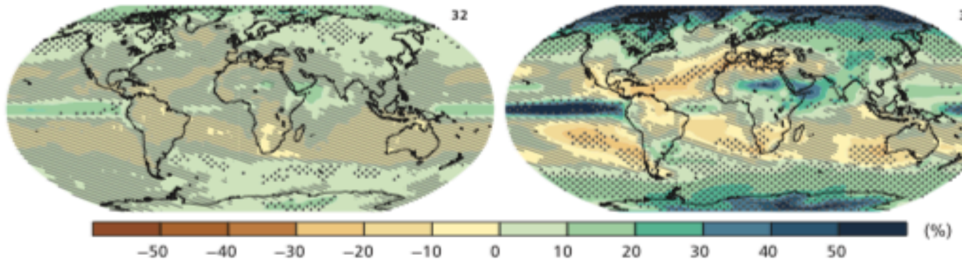


FIGURE 1.6 - A) GLOBAL CHANGE IN AVERAGE SURFACE TEMPERATURE AND B) GLOBAL CHANGE IN AVERAGE PRECIPITATION, BASED ON MULTI-MODEL MEAN PROJECTIONS FOR 2081-2100 RELATIVE TO 1986-2005 UNDER RCP2.6 (LEFT) AND RCP8.5 (RIGHT) SCENARIOS. THE RIGHT TOP CORNER OF BOTH PICTURES STATES THE NUMBER OF MODELS USED TO CALCULATE THE MULTI-MODEL MEAN VARIABILITY AND WHERE AT LEAST 90% OF THE MODELS AGREED ON THE SIGN OF CHANGE (SOURCE: IPCC, 2014).

These projections come with significant regional differences, with the rises in temperature predicting to mostly impact high latitudes (van Vliet *et al.,* 2013). This will manifest itself in an increase in freshwater temperature, threatening the temperature-tolerance range for most fish species (Sharma *et al.,* 2007; Heino *et al.,* 2009). In the tropics, which are under seasonal predictable monsoon climates and weather events like the El Niño Southern Oscillation (ENSO; Räsänen and Kummu, 2013), temperature increase is not projected to significantly impact the freshwater fish abundance, which show a higher thermal tolerance than their temperate counterparts (Lucas and Baras, 2001). On the other hand, climate

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[1] The IPCC (2014) report on global climate change projections is based on modelling scenarios using four Representative Concentration Pathways (RCP's), each contemplating different greenhouse concentration gas. The RCP2.6; RCP4.5; RCP6.0 and RCP8.5 are named after a possible range of radiation predictions for the year 2100 relative to pre-industrial levels, being this 2.6; 4.5; 6.0 and 8.5 W m^{-2}, respectively.

change projections in the tropics are related to the increased variability in the rainfall frequency and intensity (Figure 1.6; Nijssen *et al.,* 2001; Syvitski *et al.,* 2014), which will likely modify the wet/dry season pattern, by alterations in the timing and duration of water availability and quantity, threatening habitat connectivity and inundation pattern, disrupting fish migratory dynamics, affecting spawning behaviour and fish abundance. These projections will also likely amplify the variability and intensity of extreme flooding and droughts events, affecting flood regimes, the stability and sustainability of freshwater fish resources and ultimately the fisheries (Keskinen *et al.,* 2010; Frappart *et al.,* 2018). Consequently, for large tropical freshwater river basins, this magnifying pressure on watercourses can severely disrupt the food security and livelihoods of the people who rely on fish resources as their source of poverty alleviation and protein intake (Dinar *et al.,* 2015; World Water Assessment Programme, 2009).

1.3 THE IMPORTANCE OF APPLYING MOLECULAR-BASED APPROACHES FOR TROPICAL FRESHWATER FISH MONITORING AND CONSERVATION

Knowledge concerning fish species' distribution, abundance and composition are still a significant gap in ecology and conservation biology fields, especially in the tropics where the majority of the freshwater fish diversity is found and approximately 70% of the known fish species perform migrations to complete their life cycle (Abell *et al.,* 2008; Dugan *et al.,* 2010; Tedesco *et al.,* 2017). It is commonly acknowledged that without a proper assessment of the freshwater fish ecology, any attempts to preserve species while restoring riverine systems, will result in failed plans (Vörösmarty *et al.,* 2010; Turak *et al.,* 2017). Notably, the fact that the tropical freshwater environment is under severe threat due to environmental change projections (e.g. hydropower, irrigation schemes and climate change), magnifies the uncertainty regarding the future of tropical fishes (Ziv *et al.,* 2012; Irvine *et al.,* 2016; Winemiller *et al*., 2016). One can expect a decline in the fish species composition, abundance, and losses in the genetic diversity, due to the blockage of migratory pathways, disruption of life cycles, and consequent population declines (Myers *et al.,* 2000; Poff *et al.,* 2007; Winemiller *et al.,* 2016). On the other hand, the lack of full understanding on most tropical fish species ecology and synergistic importance of the drivers affecting tropical fish dynamics and abundance, make it challenging to infer the impacts of environmental modifications in the system to the fish communities and fisheries supported, and pursue conservation plans (Arthington *et al.,* 2010; Fischer, 2013; Corlett, 2014; Closs *et al.,* 2016). Accordingly, one of the utmost challenges in tropical fish conservation ecology is to improve the knowledge on tropical fish species composition, diversity, abundance and migratory behaviour, by developing and applying effective methodologies (Myers *et al.,* 2000; Markovic *et al.,* 2012; Fischer, 2013; Closs *et al.,* 2016).

Traditional methods to survey tropical freshwater fish species rely on visual census, tagging, hydroacoustics and electrofishing (Fischer, 2013). However, the lack of taxonomic expertise has resulted in the misidentification of species that are morphologically similar. Moreover, worldwide, the taxonomy field is increasingly diminishing, posing challenges to the correct assemblage and identification of species for conservation purpose (Lévêque and Mounolou, 2004; Closs *et al.,* 2016). Additionally, most traditional methods are constrained by the visual presence/absence of fish, the time and place where the survey is taken, the number of times performed, the individuals' knowledge, among other variables; all bias species surveillance (Fischer, 2013). These techniques are also time-consuming and ultimately uncover the real estimation of species. Detection of rare or cryptic species is another difficulty in monitoring for conservation purposes, leading to false assumptions of species extinction or under threat (Pauls *et al.,* 2014; Thomsen and Willerslev, 2015; Deiner *et al.,* 2016; Taberlet *et al.,* 2018). Furthermore, the approach taken often focus only in one or few species (e.g. *flagship* species[2]) instead of a community level approach, as a result of the large catchment area of tropical freshwater systems and reduction in the capacity level (e.g. number of people employed for surveillance, time and monetary constrains, and lack of effective tools to monitor a system, or to focus in more than one species; Fischer, 2013; Pauls *et al.,* 2014; Thomsen

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[2] *Flagship* species is a common denomination given to certain iconic species inhabiting the freshwater system of a single or transboundary region, being one of the principal attractions (i.e. for fisheries and tourism) of the country or countries where these species are found. Example of a *flagship* species is the Mekong giant catfish (*Pangasianodon gigas*), one of the largest freshwater fish in the World (nearly 3 meters length; Bellemain *et al.,* 2016), only found in the Mekong basin. Usually these species possess particular characteristics (e.g. body length, shape and colour) that make them valuable for the region. Moreover, these species are part of the cultural history and myths of the region. Therefore, it is not surprising that these species are often classified as being threatened, usually resulting from modifications in the habitats and continuous and unsustainable effort in catch (both for economic income and fishery's trophy; Mattson *et al.,* 2002).

and Willerslev, 2015; Deiner *et al*., 2016).

The demand for novel approaches capable of documenting and improving conservation actions of freshwater fish species have been urged over the past decades by the scientific community. Advances in molecular tools to sequence and provide genetic referenced databases of identified species offer such an advance (e.g. GenBank). The focus also changed from a species-specific approach to a community level approach, and over larger geographic areas (Thomsen and Willerslev, 2015; Deinar *et al.,* 2016; Taberlet *et al.,* 2018).

The use of molecular techniques to measure genetic variation and species evolutionary history across different environments, have revolutionised most fields of science (Pauls *et al.,* 2014; Taberlet *et al.,* 2018). Thus, freshwater biologists have implemented molecular methods for over 40 years to answer questions regarding population structure and evolutionary history, for taxonomic and systematic studies, and more recently for biomonitoring. By applying new molecular-based approaches (e.g. Environmental DNA - eDNA), to monitor freshwater fishes, it is possible to significantly decrease the misleading errors in species' identification, while improving the knowledge on the distribution dynamics and fish community composition and abundance (Taberlet *et al.,* 2018). Also, eDNA, by detecting the species inhabiting a certain catchment, help to understand the levels of adaptation to different habitats and, therefore, predict the consequences of environmental change. Subsequently, this results in enhancing the taxonomic knowledge on freshwater biodiversity, while integrating this information to improve management and monitoring plans for conservation purpose. Moreover, the species identification from DNA sequences are often recognised to be cost-effective and able to detect a variety of aquatic species from a single water sample (Thomsen and Willerslev, 2015; Deinar *et al.,* 2016; Taberlet *et al.,* 2018). Yet, the implementation of state-of-the-art molecular survey tools in the tropics is still in its infancy, compared to temperate regions. This is a priority area that urges to be tackled, for the high fish abundance and migratory behaviour found in a significant percentage of the known tropical fish species, while addressing the environmental threats to tropical freshwater systems.

1.4 RATIONALE

The increasing threats to the tropical freshwater environment, together with its recognised importance for fish abundance, fisheries, and people who rely on fish for food security and economic income (particularly in developing countries), make river's flows, habitat dynamics and fish preservation and conservation, a vital and challenging area of study (Myers *et al.,* 2000; Pimm *et al.,* 2014). However, this can only be achieved by increasing the knowledge on tropical freshwater fish diversity, abundance and distribution dynamics. Traditional surveys have proven their limitations, urging new molecular-based approaches to be applied. Such approaches can be a challenge in large transboundary river basins, where the economic and political interests of each country often overcome the recognition of environmental priority areas to be implemented in development policies. Also, the lack of understanding on the interconnection of climate, environmental flows, habitats, migratory behaviour and nutrient flux as drivers of fish dynamics and abundance, ultimately influencing the fisheries, will magnify the threats by the multiple drivers of environmental change (i.e. hydropower, irrigation and climate change).

This thesis recognises the role of environmental flows and habitat dynamics in the fisheries and fish abundance of a large tropical transboundary river system, the Mekong. The Mekong sits at the apex of environmental change; changes that are expected to threaten the seasonal migratory dynamics and adaptive strategies of the fish fauna, impacting upon the lives of over 70 million people who rely on freshwater fisheries for food security. Environmental DNA is also applied to increase the knowledge on fish diversity, abundance and seasonal migrations. The characterisation of the vital role of the environmental flows and habitat dynamics as main drivers of the fisheries yields, species abundance and migratory behaviour, will then be used to understand how the individual and combined actions of the multiple drivers of environmental change on the system, will impact upon the fisheries, fish abundance and dynamics of the system, particularly in the Tonle Sap Great Lake system (TSGL; Cambodia), a hotspot of fish diversity and fisheries, where complex floodplain eco-hydrologic interactions happen driven by the Mekong river's flow regime.

1.5 RESEARCH QUESTIONS AND OBJECTIVES

The overarching aim of this research is to quantify the individual and combined impacts of the multiple drivers of environmental change on fish dynamics and abundance of the Mekong river, and estimate the consequences for the *dai* fishery (bag-net fishery) and species compositions across the TSGL system. This overall aim will be addressed through the establishment of four interconnected research questions, each with a set of specific objectives:

1) *How important is the Mekong river's hydrologic regime and sediment flux to the habitats, fish diversity and ecosystem services supported?*

O1: Characterise the historical importance of the Mekong river for its climate, hydrologic regime and sediment flux, influencing the water quality, habitat flood dynamics, fish abundance and migratory behaviour, which affect the ecosystem services supported, key to people for economic income and food security;

O2: Assess the long-term (1960-2017) mean annual variations in the basin's water level and discharge, regarding the pre and post-dam period (i.e. after the Manwan dam 1993 operation start);

O3: Evaluate the historical status of the Mekong river fishery resources and the relation with the drivers of fish dynamics and abundance;

O4: Describe the threats by the multiple drivers of environmental change on the historical hydrologic regime, sediment flux, habitat connectivity and availability, for the gaps in knowledge on the impacts upon fish abundance, migratory behaviour and the fisheries supported, affecting people for food security.

2) *Can remote sensing tools detect historical variations in the flood pulse dynamics of the Tonle Sap Great Lake (TSGL) system, and how it influenced the dai fishery and species composition of the catch?*

O4: Develop a flood inundation model of the TSGL system, by applying Landsat-TM images with historical water level measurements from a gauging station inside the lake derived from **O2**;

O5: Assess the relationship between variations in the flood pulse variables (water amplitude, duration, timing, continuity and smoothness) to the *dai* fishery yields and species composition of the catch, and infer the importance of floodplain habitats' predictable seasonal inundation to different species' guild abundance;

O6: Estimate how historical El Niño Southern Oscillation events can influence the flood level and fishery yields of the lake.

3) *How will the flood pulse and floodplain flood dynamics of the TSGL system be impacted by future individual and combined action of the multiple drivers of environmental change scenarios of the Mekong basin? What are the consequences to the dai fishery, species composition and food security?*

O7: Quantify future (2036-2064) modifications in the lake's flood pulse and floodplain flood dynamics, by applying individual and combined modelling scenarios of the basin's hydropower development, irrigation and climate change projections, using the Landsat flood model (**O4**) and VMod hydrological model;

O8: Estimate future modifications in the *dai* fishery resources and food security, due to the predicted modifications in the flood pulse and flooded habitats **(O7)**.

4) *Can eDNA metabarcoding analyses detect the Mekong river's fish communities and identify patterns of seasonal dispersion, demonstrating the needs to improve the biomonitoring and environmental conservation actions in the basin, in light of the projected environmental threats?*

O9: Detect the Mekong river's fish communities, by applying eDNA metabarcoding analyses on water samples collected along the basin's sites;

O11: Assess seasonal differences in species composition, relative abundance and distribution, for the habitats explored, driven by the seasonal flood regime;

O12: Predict future consequences, for the identified species, on the migratory behaviour and contribution to the *dai* fishery and food security, linked to the key findings from the previous research questions, for the needs to improve biomonitoring and environmental conservation actions in the Mekong basin.

1.6 THESIS SUMMARY

This interdisciplinary work joins together fields of tropical freshwater ecology, fish biology, conservation, tropical inland fisheries, molecular ecology, numerical modelling, fluvial dynamics, climate and anthropogenic impacts. All of these will be covered in a set of seven chapters in which this thesis is organised (Figure 1.7) addressing each of the research questions and objectives.

In **Chapter 2** a critical review is covered to identify the present gaps in knowledge, which will be attempted to fill by this work. First, the ecology of tropical freshwater fishes and the migratory behaviour are discussed for large tropical rivers. Next, the flood pulse concept is covered for the eco-hydrodynamics supported that justifies the productivity associated with floodplain habitats, which fishes rely on (e.g. TSGL system, Amazon River and Lake Victoria). The impacts of hydropower, irrigation and climate change in the flood regime, habitat eco-hydrologic interactions and sediment flux of large tropical rivers (Amazon, Mekong, Congo and Nile) are characterised for the consequences on the ecosystem services provided, and the gap of studies focusing on the future impacts on fisheries and fish abundance. The last section of this chapter will review the importance of Environmental DNA as a new molecular-based approach to detect freshwater fish species, improve the knowledge on the migratory behaviour, the necessity of further developments and the paucity of eDNA studies in the tropics.

Chapter 3 addresses the historical importance of the Mekong river as supporting the most productive inland fisheries of the World, and the strict relation with the river's flood regime and habitat dynamics. The chapter covers the status of the profitable fisheries for the food security of the estimated over 70 million people inhabiting the basin. Also, the threats to the system's sustainability are characterised, regarding the multiple drivers of environmental change scenarios. The significance of the historical flood regime of the Mekong river will further be assessed in **Chapter 4** for the unique bi-directional flow reversals observed in the

TSGL system. This chapter will demonstrate the applicability of remote sensing tools to predict a flood inundation model of the lake and assess its historical importance to the profitable *dai* fishery and species composition. How ENSO climate events influence the flood level and annual catch, will also be explored.

The Landsat flood model, the historical fishery assessment and species composition of the catch, will then be used in **Chapter 5**, to quantify future variations in the lake's flood pulse and floodplain flood dynamics, driven by individual and combined scenarios of hydropower, irrigation schemes and climate change of the Mekong river. The quantified alterations will be used to estimate future impacts upon the *dai* fishery, species composition and food security.

Chapter 6 will improve the knowledge on the Mekong river's fish communities and seasonal distribution along the basin, by applying eDNA metabarcoding analyses on collected water samples from the two fieldworks performed. The threats to the identified fishes and role on the *dai* fishery, linked to the projections on environmental change, will also be discussed. This will demonstrate the needs to establish biomonitoring and environmental conservation actions in the Mekong.

Chapter 7 will present a synthesis of the key findings of this thesis and the answers to the research questions, while discussing the limitations found. This will result in an outlook for further work and a discussion of the wider implications of the research, before stating the take-home messages.

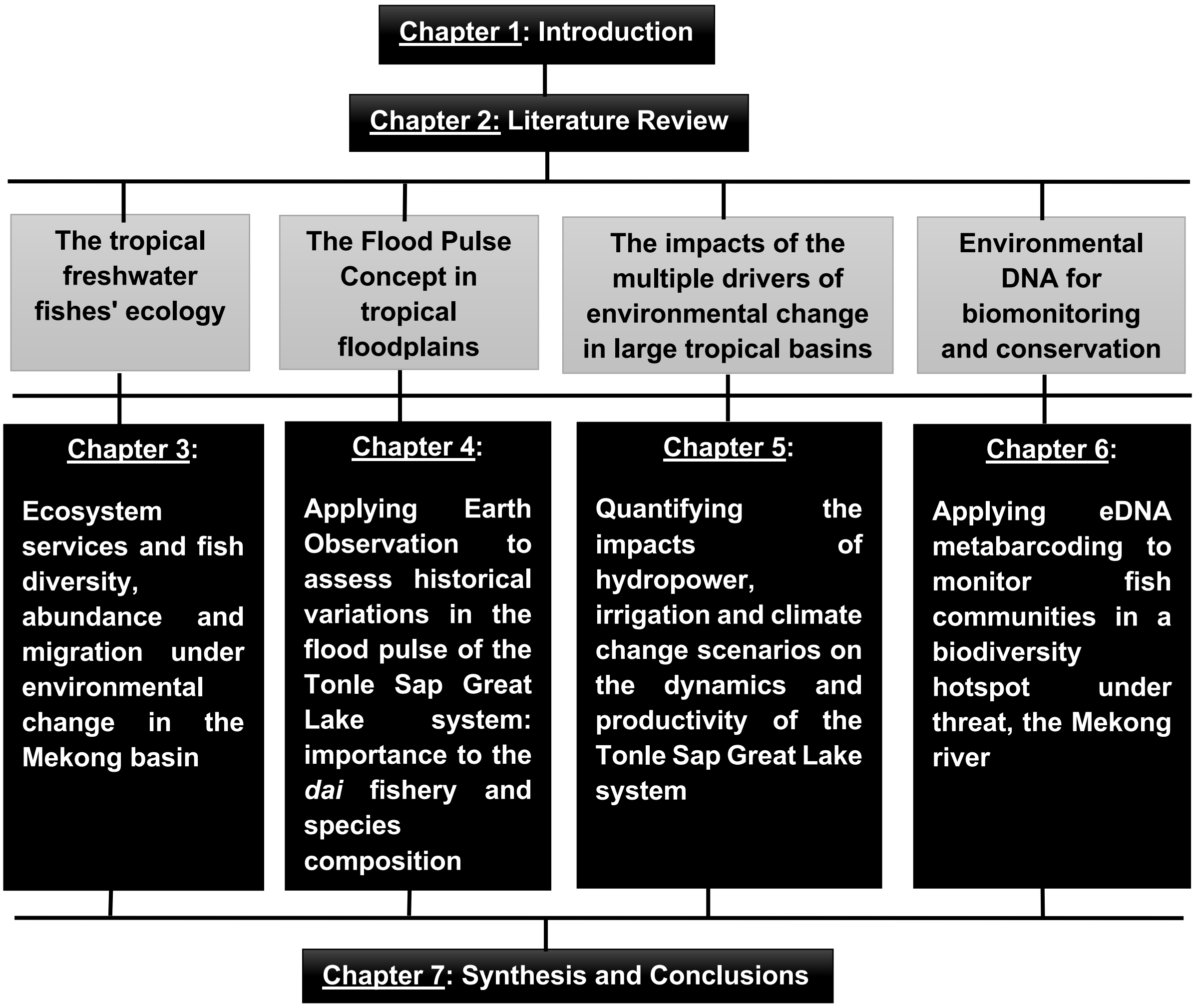
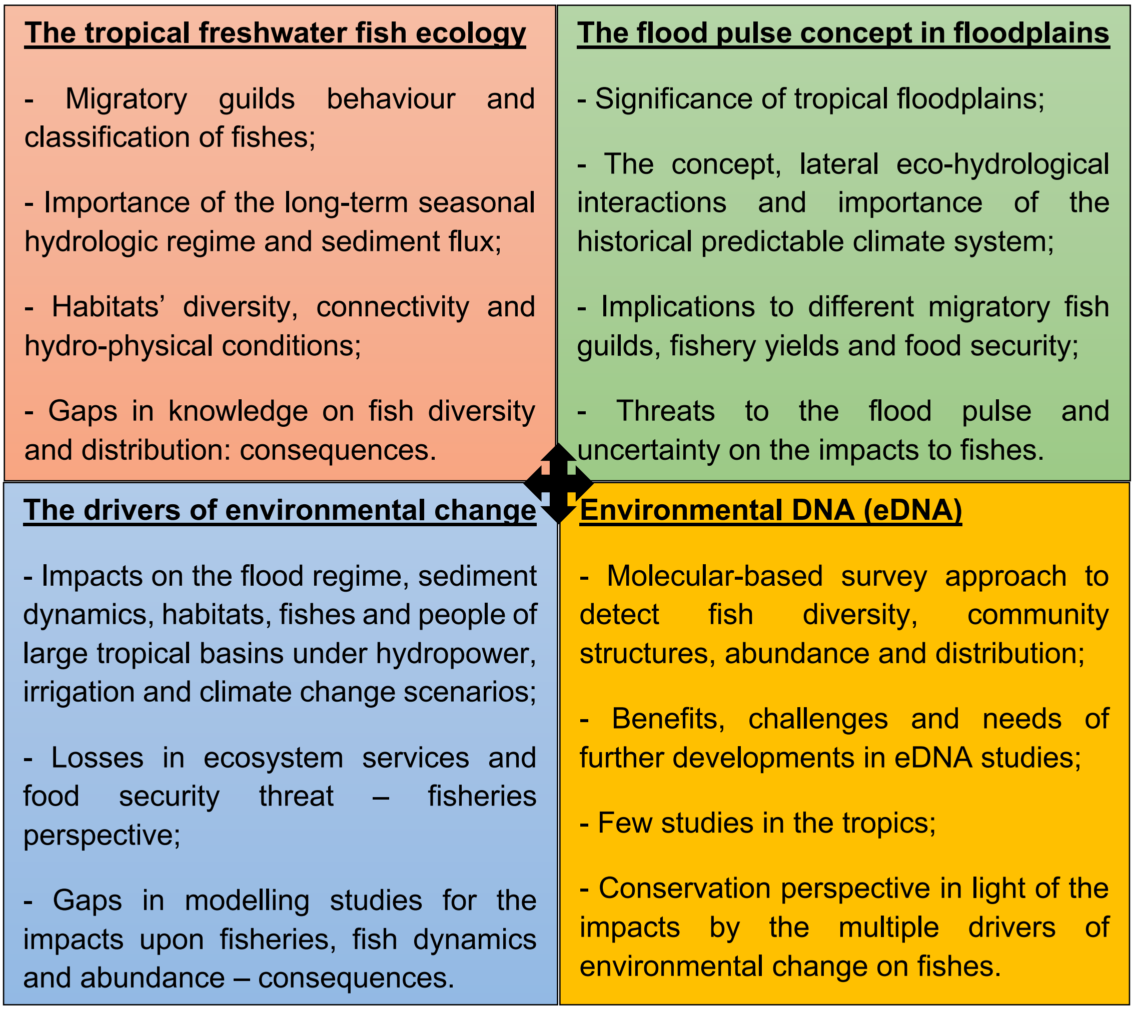


FIGURE 1.7 – LAYOUT OF THE THESIS' STRUCTURE.

CHAPTER 2

Literature Review

Overview of the chapter: Figure 2.1 provides an overview of the inter-linked issues reviewed in this chapter, to establish the gaps in knowledge that will be addressed throughout this thesis.



The tropical freshwater fish ecology

- Migratory guilds behaviour and classification of fishes;

- Importance of the long-term seasonal hydrologic regime and sediment flux;

- Habitats' diversity, connectivity and hydro-physical conditions;

- Gaps in knowledge on fish diversity and distribution: consequences.

The flood pulse concept in floodplains

- Significance of tropical floodplains;

- The concept, lateral eco-hydrological interactions and importance of the historical predictable climate system;

- Implications to different migratory fish guilds, fishery yields and food security;

- Threats to the flood pulse and uncertainty on the impacts to fishes.

The drivers of environmental change

- Impacts on the flood regime, sediment dynamics, habitats, fishes and people of large tropical basins under hydropower, irrigation and climate change scenarios;

- Losses in ecosystem services and food security threat – fisheries perspective;

- Gaps in modelling studies for the impacts upon fisheries, fish dynamics and abundance – consequences.

Environmental DNA (eDNA)

- Molecular-based survey approach to detect fish diversity, community structures, abundance and distribution;

- Benefits, challenges and needs of further developments in eDNA studies;

- Few studies in the tropics;

- Conservation perspective in light of the impacts by the multiple drivers of environmental change on fishes.

FIGURE 2.1 - SCHEMATIC VIEW OF THE KEY INTERCONNECTED TOPICS REVIEWED HEREIN.

2.1 THE TROPICAL FRESHWATER FISH ECOLOGY

Tropical fishes are vital components of the tropical freshwater environments. Their life cycles are dependent and triggered by the predictable variations in the hydrologic regime and physical conditions (i.e. modifications in water level, flow, sediment flux, turbidity, dissolved oxygen, habitats availability and connectivity) found in the unique diversity of habitats (for examples see Chapter 1; Lucas and

Baras, 2001; Val *et al.,* 2006). These predictable variations in the tropical catchment conditions are driven by monsoonal climate and other weather events, delimiting periods of intense rainfall (with increases in water level, run-off, sediment flux, dissolved oxygen and habitat connectivity), and periods of scarce rainfall (with decreases in water level, run-off, sediment flux, dissolved oxygen, increases in turbidity and habitat fragmentation; for further information see sub-section 2.2.1; Lamberts, 2006;2008; Val *et al.*, 2006; Kolding and van Zwieten, 2012). Additionally, the relative low level of long-term variations in the seasonal monsoonal climate influence (i.e. for the temperature and precipitation) on the flood regime and sediment flux (see sub-section 2.3.2; Syvitski *et al.,* 2014), linked to the diversity of lateral and longitudinal habitats, have promoted high levels of dispersion, speciation and different adaptative strategies of fishes. Consequently, studies argue that the predictability and seasonality of the climate-driven hydrologic regime and physical conditions found in the diversity of habitats, affect different species' swimming capability, morphology, reproduction, feeding behaviour and other physiologic responses (Lucas and Baras, 2001; Baran, 2006; Val *et al.,* 2006; Winemiller *et al.,* 2008; Halls, 2010).

These factors result in species performing periodical migrations to different sections of the catchment, and others, lacking on migratory capabilities, either performing sporadic local movements within the same section or developing physiologic adaptive strategies to cope with the aquatic conditions (Lucas and Baras, 2001; Farrell, 2011; Closs *et al.,* 2016). Hence, it is not surprising that the ecology of tropical fishes is described by the different levels of movement that all fishes perform, either to avoid predators, complete life cycles, find shelter, feed and/or as a response (clue) to the periodical variations in the hydrologic and physical habitat conditions (Lucas and Baras, 2001; Campbell *et al.,* 2006; Lamberts, 2006;2008; Val *et al.,* 2006; Winemiller *et al.,* 2008).

2.1.1 THE MIGRATORY BEHAVIOUR AND CLASSIFICATION OF TROPICAL FISHES: ROLE OF THE HYDROLOGIC REGIME, SEDIMENT FLUX AND HABITAT DYNAMICS

Northcote (1984) defines fish migrations as "movements that result in an alternation between two or more separate habitats, which occur within a regular periodicity, and involves a large proportion of the population". Rainboth (1996) Poulsen *et al.,* (2002;2004), Baran (2006) and Halls (2010) corroborate this definition for some of the known Mekong fish species (see Chapter 3 for further information), recognising that the spawning, feeding and sheltering migratory behaviours occur in response to periodical variations in the environmental factors (i.e. modifications in water level, discharge, turbidity, lunar phase and insects' appearance). However, Lucas and Baras (2001), supported by Baran (2006) and Val *et al.,* (2006) argue that little is known on the long-term dynamics of tropical flood regimes and the complex interactions established on the diversity of habitats, to effectively understand the role on tropical fishes´ ecology and adaptative strategies. Therefore, it is vital to promote studies that help to mitigate these gaps in knowledge, notably concerning the impacts that alterations in the environmental factors might pose to the availability, connectivity and productivity of habitats, and how it will reflect on different species abundance, dispersion dynamics and system's resilience.

Many of these alterations have been linked to the impact of anthropogenic activities, such as hydropower development, irrigation schemes and climate change scenarios (see section 2.3; Dudgeon, 2000; Pringle *et al.,* 2000; Winemiller *et al.,* 2016; Hoang *et al.,* 2019), with consequences to the ecosystem services provided, with onward impacts to people who rely on the periodical migration of fishes for the inland fisheries (e.g. subsistence fisheries of the Mekong, Amazon and Congo basins; Baran and Myschowoda, 2009; Ziv *et al.,* 2012; Castello *et al.,* 2015; Winemiller *et al.,* 2016; Gordon *et al.,* 2018).

The tropics host the highest known freshwater fish diversity, endemism and abundance found in the World (see Chapter 1; Dudgeon, 2000; Pringle *et al.,* 2000; Abell *et al.,* 2008). Therefore, it is a challenging task to assess the ecological aspects of individual tropical fishes, for the diversity of life-forms and physiologic responses found in different basins, and high uncertainty on the estimates of species' numbers (see sub-section 2.1.2; Dudgeon, 2000; Pringle *et al.,* 2000; Winemiller *et al.,* 2008). Moreover, the regional differences in the climatic influence (e.g. periodicity of the monsoon climate) among tropical basins (see sub-section 2.3.2), resulting seasonal flood regimes, sediment flux and diversity of connected habitats found (i.e. from freshwater to brackish and marine habitats), which promote longitudinal and lateral high dispersion among fishes, make it unreliable to individually assess the ecology of tropical fish species (Lucas and Baras, 2001; Winemiller *et al.,* 2008). For this reason, freshwater fish (including fish inhabiting temperate and tropical environments) are primarily grouped based on their physiologic evolutionary history as “primary” freshwater fishes (fishes that only live in the freshwater environment), “secondary” (fishes that exhibit some salt tolerance) and “peripheral” (species from marine families that live in the freshwater environment for part or all of their lives, usually in estuaries; Berra, 2001; Val *et al.,* 2006). Additionally, this general classification is linked to the migratory behaviour for spawning purpose, where fishes are classified as (Lucas and Baras, 2001; Poulsen *et al.,* 2002;2004; Val *et al.,* 2006):

- **Anadromous:** fishes that live most of their lives in the sea and enter the freshwater realm to spawn;
- **Catadromous:** fishes that breed in the sea, but enter the freshwater realm, where they stay until being ready to spawn again;
- **Potamodromous:** fishes that live their entire lives in the freshwater realm but perform seasonal medium to long distance migrations in the mainstream river (longitudinal pattern) and connected lateral tributaries (lateral pattern) or short migrations within tributaries and other lateral habitats from the mainstream river (lateral pattern) to spawn.

The three groups (i.e. primary, secondary and peripheral) are found in tropical freshwater environments, providing vital biological indicators of the past evolutionary history of tropical freshwater catchment conditions (see sub-section 2.1.2) and the eco-physiologic adaptations of fishes (e.g. barbels present in carps to detect food in turbid waters; Lucas and Baras, 2001; Val *et al.,* 2006).

A number of studies refer that the potamodromous group exhibits the highest known fish diversity found in the Mekong, Amazon and Congo basins (Winemiller *et al.,* 2008; Poulsen *et al.,* 2002;2004; Castello and Macedo, 2016). In these tropical lowland areas, fishes belonging to this group are usually described under three general migratory guilds[3] (blackfish - no migratory behaviour, whitefish – high migratory behaviour and greyfishes – medium migratory behaviour; see Chapter 3 for more information; Baran, 2010), connected to the ability to explore the same habitats for spawning, feeding and shelter purpose (see Chapter 3 for examples of species and habitats for the Mekong river). Also, these fish guilds

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[3] The guild concept classifies different fish species according to the ability to behave and exploit different resources (e.g. habitat, spawning behaviour, food preferences, among other characteristics) in the same way (see Chapter 4 for the 11 guilds detailed to the Mekong river; Simberloff and Dayan, 1991; Welcomme *et al.,* 2006; Halls, 2010).

are described to perform longitudinal upstream/downstream (white and greyfishes) migrations and local movements (blackfishes), driven by periodical variations in the flood regime, physical habitat conditions and food provision in the recent inundated habitats (i.e. in the flood season; see section 2.2; Poulsen *et al.,* 2002;2004). The spawning usually occurs when waters start to rise in the mainstream river, floodplain and other habitats (Winemiller *et al.,* 2008; Valbo-Jorgensen *et al.,* 2009), with larvae being drifted to lateral habitats with high nutrient content, from the loads of sediments carried in the flow and lateral eco-hydrologic interactions established in the aquatic and riparian habitats (e.g. in floodplains; see section 2.2; Junk *et al.,* 1989; Lamberts, 2008). When the flood level starts to recede (onset of the dry season) and the flood area starts to shrink, fishes that require higher flood levels and exhibit low tolerance to an increase in turbidity and decrease in dissolved oxygen, migrate to mainstream habitats (white and greyfishes) or remain in the floodplain (some greyfish and blackfishes; Lucas and Baras, 2001; Baran, 2006; Val *et al.,* 2006; Winemiller *et al.,* 2008).

The fishes' ecology, habitats explored and role of the flood regime and sediment flux in driving the migratory behaviour, are usually well understood in temperate regions, compared to the tropics (Lucas and Baras, 2001; Val *et al.,* 2006; Closs *et al.,* 2016). This is because temperate freshwater rivers support a proportional lower number of species concentrated in few habitats, less habitat diversity and catchment area, compared to tropical freshwater systems (Lucas and Baras, 2001). Moreover, the flood regime and sediment loads fluctuate less than in the tropics, where the majority of large river basins are under different monsoonal systems and the seasonal rainfall plays a major role in driving the water availability, quantity and sediment flux (see sub-section 2.3.2; Holmes *et al.,* 2009; Darby *et al.,* 2013; Syvitski *et al.,* 2014).

Therefore, the diversity of habitats and the longitudinal and lateral hydrologic connectivity, promoting high levels of dispersion, combined to the seasonal interactions established between the aquatic and terrestrial riparian areas (see section 2.2 for floodplain habitats), contribute to the high uncertainty surrounding the effective estimations on fish diversity in tropical catchments and their ecology (Dudgeon, 2000; Pringle *et al.,* 2000). This is also linked to significant gaps in the literature, regarding the establishment of long-term monitoring datasets to identify and map the distribution of different fish species across tropical basins, a decrease in taxonomic skills, with consequent misidentification of species, paucity of long-term hydrologic time-series to assess how historical temporal and spatial modifications in the hydrologic regime influence habitat's availability and connectivity, and consequent scarcity of full understanding on fishes' ecology and genetic diversity (Dudgeon, 2000; Val *et al.,* 2006; Winemiller *et al.,* 2008).

The fact that studies that have tried to fill these gaps, refer the limitations (for the fish numbers reported, available fish databases, sections of the basin surveyed, periodicity, among other issues; Dudgeon, 2000; Abell *et al.,* 2008; Winemiller *et al.,* 2008; Halls, 2010; Kano *et al.,* 2016) on the overall projections and the necessity to develop further studies, reflect the few efforts made to effectively enhance the knowledge on tropical freshwater fishes' ecology. Additionally, if compared to efforts made in temperate regions (i.e. studies on the migratory pattern of salmon species in the USA and Canada; Lucas and Baras, 2001; Ferguson *et al.,* 2011), where the long-term surveillance has provided important insights on the population dynamics, genetics and role of the hydrology in triggering the spawning migratory behaviour; in the tropics the efforts made to identify fish species, monitor the seasonal distribution, habitats explored and adaptive strategies, have mainly focused on a few species (e.g. nearly 50 fish species in the Mekong river out of an estimated over 700 species; see Chapter 3; Poulsen *et al.,* 2002;2004; Baran, 2006; Valbo-Jorgensen *et al.,* 2009), mostly with economical value (e.g. fisheries; Dudgeon, 2000; Lucas and Baras, 2001; Poulsen *et al.,* 2002;2004; Val *et al.,* 2006; Winemiller *et al.,* 2008).

Consequently, it is certainly true that tropical freshwater catchments sustain high diversity of freshwater fish (based on basin-wide and few global estimations; e.g. Poulsen *et al.,* 2002;2004; Abell *et al.,* 2008; Kano *et al.,* 2016). However, the scarcity of scientific knowledge assessing the role of the flood dynamics and habitats to fish ecology, is a significant issue that needs to be addressed (Campbell *et al.,* 2009; Kolding and van Zwienten, 2012; Castello and Macedo, 2016). This is related to the role that fishes play to the fisheries and people inhabiting the tropics, and the projected environmental change impacts in all tropical basins. These threatens the ecology and adaptive strategies of tropical fishes, through modifications in the migratory behaviour, associated with disruptions in the flood regime and sediment flux clues and shifts in habitat availability and connectivity (see section 2.3; Dudgeon, 2000; Thieme *et al.,* 2011; Winemiller *et al.,* 2008;2016; Hecht *et al.,* 2019).

2.1.2 THE UNCERTAINTY ASSOCIATED WITH TROPICAL FRESHWATER FISH DIVERSITY AND DISTRIBUTION DYNAMICS

Winemiller *et al.,* (2008) state that South and Central American rivers (particularly the Amazon and Orinoco rivers) contain the highest number of freshwater fish species on Earth, with the estimates ranging as high as 8000 species, being nearly 25% of the global fish species richness (compiling marine, brackish and freshwater species). Yet, in the Amazon basin, only nearly 2000 species have been effectively reported in studies trying to assess the adaptative strategies to the seasonality of the flood regime, migratory behaviour and role on the productive fisheries (Castello *et al.,* 2015;2017; Castello and Macedo, 2016; Pinaya *et al.,* 2016). The Mekong river in Southeast Asia is argued to possess the second highest fish richness, with the estimates ranging between 750-1200 species (see Chapter 3; Valbo-Jorgensen *et al.,* 2009; Baran, 2006;2010). In Africa, the highest fish diversity is found in the Congo basin (690 species), followed by the Niger (211 species), Volta (137 species) and Nile basins (127 species; Winemiller *et al.,* 2008). Despite the high number of fish species supported by these basins, these are representative of a small number of orders. Thus, Siluriformes (catfishes), Perciformes (represented mostly by the Cichlidae family), Cypriniformes (barbs and loaches) and the Cyprinodontiformes (killifishes), are the main fish orders found in those basins (Dudgeon, 2000; Pringle *et al.,* 2000; Winemiller *et al.,* 2008). Pringle *et al.,* (2000) and Winemiller *et al.,* (2008) argued that the past evolutionary history of the Earth's landscape (for the convergence of tectonic plates, mountainous' elevations and plain areas) and climatic modifications (for temperature and precipitation), have promoted the levels of dispersion and speciation of different freshwater fishes to previous connected large tropical basins. Yet, and due to the evolution of the landscape morphology (e.g. separation of the American continent from the mainland) and hydrologic fragmentation among basins, intra-specific differences are seen regarding the species found from each order, which reflects the diversity of adaptative strategies (Pringle *et al.,* 2000; Lucas and Baras, 2001; Poulsen *et al.,* 2002;2004; Baran, 2006; Winemiller *et al.,* 2008).

The evolutionary history of tropical freshwater fishes reflects, the past and present modifications in tropical catchments, with the majority of fish species exhibiting higher tolerance to the seasonal changes in water temperature, compared to their temperate counterparts (Lucas and Baras, 2001; Winemiller *et al.,* 2008). This is mainly because in the tropics the annual water temperature does not fluctuate greatly (Lucas and Baras, 2001; Val *et al.,* 2006). Yet, variations in dissolved oxygen and turbidity, reflect the seasonal interactions of the fluvial dynamics and sediments flux with the riparian areas, thus influencing differently fish species with higher/lower tolerance to, for example, low dissolved oxygen and high turbidity as found during the dry season in the Mekong and Amazon basins and Lake Victoria (Lucas and Baras, 2001; Val *et al.,* 2006; Winemiller *et al.,* 2008; Campbell *et al.,* 2009; Kolding and van Zwieten, 2012).

To sum up, it has been argued that the high fish diversity and migratory behaviour found in large tropical freshwater basins, result from high levels of speciation, driven by a combined diversity of habitats, seasonal hydrologic regime, physical conditions and climatic influence. However, this recognition only covers a fraction of the overall estimated fish diversity. Additionally, the scarcity of studies leaves a series of open questions on how modifications in the seasonal hydrologic regime and sediment flux impact the eco-hydrological interactions in habitats? What are the consequences to fish dynamics and abundance? What guilds are likely to be more affected by modifications in the hydrologic regime, habitats flood dynamics and connectivity? What are the consequences to the system's sustainability and ecosystem services provided? Hence, it is vital to develop effective studies, aiming to answer these questions, in light of the high pace in which tropical environments are becoming imperilled by anthropogenic-driven disturbances (see section 2.3; Abell *et al.,* 2008; Winemiller *et al.,* 2016; Thieme *et al.,* 2011; Grill *et al.,* 2019).

2.2 THE FLOOD PULSE CONCEPT IN TROPICAL FLOODPLAIN HABITATS AND ROLE ON FISH DYNAMICS, ABUNDANCE AND FISHERIES

Tropical freshwater systems (e.g. rivers and lakes) support a diversity of lowland lateral riparian areas (e.g. floodplains, swamps, marsh, forests, among others; Junk *et al.,* 1989;2010; Kolding van Zwieten, 2012; Castello *et al.,* 2015;2017; Isaac *et al.,* 2016), commonly known as wetlands and perceived as some of the most productive habitats on Earth, covering between 2.0-12.8 million km^2 globally, which corresponds to nearly 3-9% of the land surface (Junk *et al.,* 1989;2010;2013; Kvist and Nebel, 2001; Millennium Ecosystem Assessment, 2005; Wantzen *et al.,* 2008; Arias *et al.,* 2012;2013;2014). Floodplains are amongst the most biologically productive and ecologically diverse regions in the World (Davies *et al.,* 2008; Wohl, 2014; Dang *et al.,* 2018) and cover an extensive riparian area. In the Amazon basin for example, floodplains and other wetlands account for approximately 1,000,000 km^2 of the entire basin (Junk *et al.,* 2010; Castello *et al.,* 2015;2017; WWF, 2016), supporting important subsistence fisheries, the first global fish richness (estimated as 2000 species; Castello *et al.,* 2012;2015;2017; Castello and Macedo, 2016; Isaac *et al.,* 2016; WWF, 2016; Lima *et al.,* 2017), terrestrial and aquatic invertebrates and other vertebrates (e.g. birds and mammals; Junk *et al.,* 2010; WWF, 2016). Additionally, floodplains support a diversity of vegetation (e.g. macrophytes) with different tolerance to the flood/drought annual cycle (see sub-section 2.2.1), providing vital sources of food and habitats to terrestrial and aquatic biota (Davies *et al.,* 2008; Pacini and Harper, 2008; Winemiller *et al.,* 2008; Castello *et al.,* 2017). In the Mekong basin, floodplains compose nearly 72% of the entire Vietnamese area, mainly in the delta, where high agriculture activities rely on the seasonally inundated areas, the nutrients' exchange and sediment flux for the productivity of soils to rice yield (see Chapter 3 for more information on rice farming in Vietnam; Baran *et al.,* 2007; Chapman and Darby, 2018; Dang *et al.,* 2018; Kondolf *et al.,* 2018). The Tonle Sap Great Lake (TSGL) system in Cambodia (total catchment area of 85,790 km^2, representing 11% of the entire Mekong basin; Kummu *et al.,* 2014) is surrounded by an extensive area of predictable seasonally inundated floodplains, lasting over 5 months, supporting the 4th global lake fish richness (nearly 200 species) and providing vital ecosystem services (e.g. fisheries) to over 1.2 million people (see Chapter 4; Lamberts, 2006;2008; Campbell *et al.,* 2006;2009; Halls *et al.,* 2013a).

2.2.1 THE FLOOD PULSE CONCEPT (FPC) AND KEY ECO-HYDROLOGIC DYNAMICS

Junk *et al.,* (1989;2010;2013) and Junk and Wantzen (2004) argue that tropical floodplains are dynamic components of the freshwater environment, being periodically coupled (flood stage) and decoupled (dry/terrestrial stage) from the aquatic parental body (e.g. river and/or lake). Furthermore, in large mostly unregulated tropical freshwater systems, the long-term annual predictability and relative unchangeable conditions of the monsoonal climate and other weather events (i.e. for the timing, duration and magnitude of the rainfall; see sub-section

2.3.2), have been argued to drive a period of high flood level in the aquatic parental body, with resulting lateral floodplain connectivity and inundation (coupled phase; wet season), and a period of low flood level and habitat fragmentation (decoupled phase; dry season), with the annual hydrograph presenting a single flood peak seen in the Amazon, Mekong and Nile basins (Amarasekera *et al*., 2007; Adamson *et al.,* 2009; Delgado *et al.,* 2012).

Junk *et al.,* (1989) named the climate-driven lateral hydrologic interactions established between the aquatic environment and the riparian floodplains the aquatic-terrestrial-transition-zone (ATTZ). Notably, the interactions on the ATTZ drive the anatomical, physiological, phenological and/or ethological adaptations of biota, producing characteristic community structures (Junk *et al.,* 1989;2010;2013; Junk and Wantzen, 2004; Lamberts, 2006;2008; Winemiller *et al.,* 2008; Kolding and van Zwieten, 2012). Therefore, in tropical freshwater catchments, the role of the predictable climate-driven flood dynamics in driving floodplain seasonal lateral inundation and connectivity have been argued to play a major role on the adaptive strategies of different biota, particularly migratory fish guild species (see sub-section 2.2.2; Junk *et al.,* 1989;2010; Lucas and Baras, 2001; Lamberts, 2006;2008; Val *et al.,* 2006; Welcomme *et al.,* 2006).

The Flood Pulse Concept (FPC) is an eco-hydrologic concept proposed by Junk *et al.,* (1989) and acknowledged by Castello and Macedo (2016) for the Amazon basin, Lamberts (2006;2008) and Arias *et al.,* (2012;2013;2014) for the TSGL system and Lake Victoria (Kolding and van Zwieten, 2012), to explain the seasonal flood interactions established in the ATTZ. These interactions result in high productivity rates (i.e. primary and secondary production by the recycling of nutrients; Davies *et al.,* 2008) and variations in the chemical and physical water conditions (e.g. dissolved oxygen, turbidity, pH and temperature), driving the adaptative strategies of terrestrial and aquatic biota (e.g. vegetation, fishes, invertebrates, etc; Pacini and Harper, 2008; Wantzen *et al.*, 2008; Winemiller *et al.,* 2008). The concept implies that the timing, duration, amplitude, continuity, rapidity of change and smoothness of the flood (also described as flood regime; Figure 2.2; Kummu and Sarkkula, 2008; Kummu *et al.,* 2014) are key variables to understand the coupled and decoupled phases in floodplains, which different tropical migratory fish guilds have adapted their life cycles (Lucas and Baras, 2001; Lamberts, 2006;2008; Val *et al.,* 2006; Welcomme *et al.,* 2006).

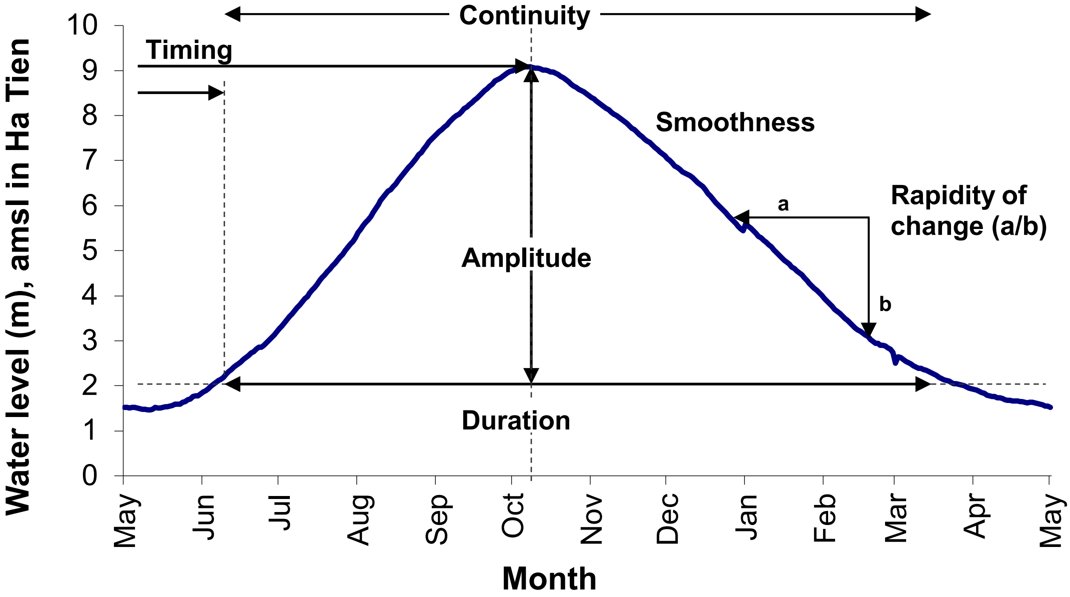

FIGURE 2.2 – THE FLOOD PULSE CONCEPT PROPOSED BY JUNK *ET AL.,* (1989) AND ACKNOWLEDGED IN THE TSGL SYSTEM, WITH THE KEY VARIABLES RESPONSIBLE FOR THE ECO-HYDROLOGIC INTERACTIONS ESTABLISHED IN THE FLOODPLAIN HABITATS. THE HYDROLOGIC YEAR (MAY-APRIL) IS HERE PRESENTED WITH CORRECTED WATER LEVEL RECORDS FROM THE KAMPONG LUONG GAUGING STATION (SEE CHAPTER 4; REDRAW AFTER KUMMU *ET AL.,* 2014).

The onset of the monsoonal rains (wet season) sets the timing of the rise in the flood level until it reaches the peak flood, where a decrease in flood amplitude sets the timing of the dry season, driven by a reduction in the monsoonal rains (Figure 2.2; Junk *et al.,* 1989; Adamson *et al.,* 2009). Importantly, during the wet season, the continuous and smoothness increase in the flood amplitude in the aquatic parental body, although multiple short flood peaks are observed linked to the variability in the daily water level, results in lateral floodplain inundation, whose variability in the duration and extent drive key eco-hydrologic processes affecting terrestrial and aquatic biota (Junk *et al.,* 1989;2010; Kolding and van Zwieten, 2012). Thus, in the TSGL system, the diversity of vegetation species exhibits different tolerances to the variability in the inundation period. Additionally, terrestrial biota able to move to dryland areas, are more capable of surviving the periods of flood than species lacking on movements. For this reason, the variability in the flood duration and rapidity of change in the inundation dynamics, bring important and complex ecological processes, with the death of plants and other biota, being decomposed by bacteria and serving as food sources to migratory fishes that seek these newly inundated habitats for feeding, sheltering and spawning purpose (Campbell *et al.,* 2006; Lamberts, 2006;2008; Arias *et al.,* 2012;2013;2014; Kolding and van Zwieten, 2012). Moreover, the local detritus and sediments (ecologically termed as autochthonous material; Junk *et al.,* 1989;2010; Davies *et al.,* 2008; Wantzen *et al.,* 2008) containing organic and inorganic nutrients from the terrestrial environment are washed to the aquatic environment. Also, nutrient bounded-sediments are transported with the flow (ecologically known as allochthonous material; Junk *et al.,* 1989; Davies *et al.,* 2008; Wantzen *et al.,* 2008), with important recycling of nutrients occurring, boosting the productivity of floodplains and flow of energy and matter from the different trophic levels established (Figure 2.3; Junk *et al.,* 1989; Sarkkula *et al.,* 2003; Davies *et al.,* 2008; Wantzen *et al.,* 2008). Additionally, the annual sunlight intensity and the diversity of vegetation species, result in high photosynthetic activity, with consequent increase in primary production and higher trophic interactions (Davies *et al.,* 2008).

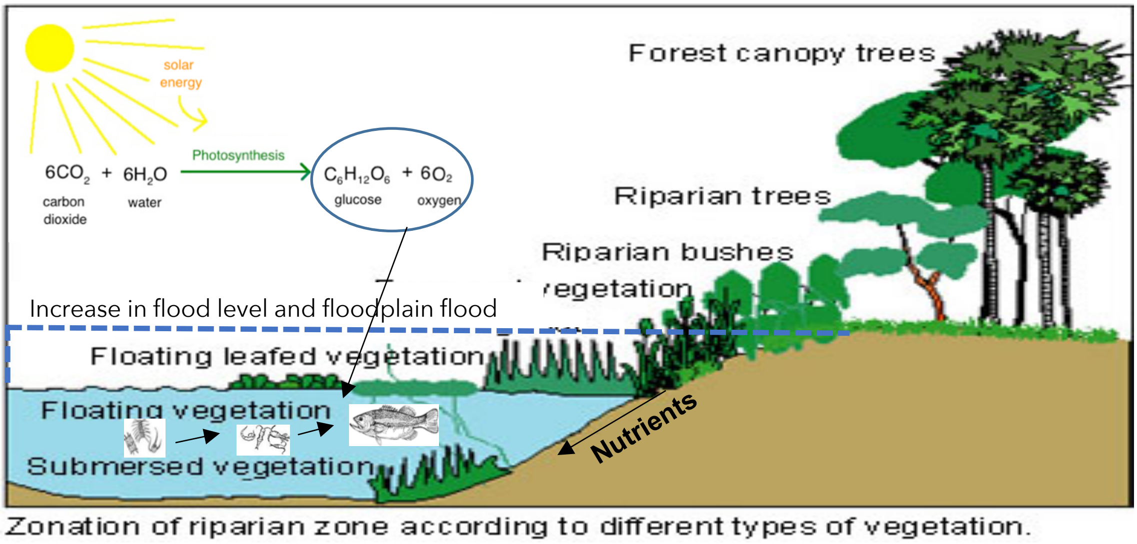

FIGURE 2.3 – DIAGRAM OF THE PHOTOSYNTHETIC ACTIVITY OF DIFFERENT VEGETATION SPECIES FOUND IN TROPICAL ENVIRONMENTS AND CONSEQUENT RELEASE OF OXYGEN AND GLUCOSE. THE SEASONAL INUNDATION PATTERN IN FLOODPLAINS CREATES NUTRIENT RECYCLING AND WASHLOAD OF DEAD PLANTS, ANIMALS AND DETRITUS TO THE AQUATIC ENVIRONMENT, BOOSTING THE HIGH PRODUCTIVITY OF TROPICAL FLOODPLAINS (FROM THE LEFT TO THE RIGHT: PHYTOPLANKTON, ZOOPLANKTON AND FISH; ADAPTED FROM: HTTP://MEKONG.RIVERAWARENESSKIT.ORG/HTML/1.7.3C_PLANTS.HTML).

2.2.2 THE LONG-TERM IMPORTANCE OF ASSESSING THE FPC IN FLOODPLAINS TO THE FISH DYNAMICS, ABUNDANCE AND FISHERIES SUPPORTED

The flood pulse dynamics established in floodplains have been considered to play a vital role on different migratory fish guilds dynamics and abundance. In the TSGL system, Baran *et al.,* (2001) and Halls *et al.,* (2013a) assessed the importance of the annual variability in floodplain inundation timing, extension and duration to species biomass, and consequent effect on the observed annual *dai* fishery catch (see Chapter 4 for the definition). The authors argued that longer/reduced flood duration, amplitude and extension significantly influenced the biomass of species composing the catch (R^2>50% in both studies), with large body-size whitefish species requiring longer floodplain flood duration and extension to growth, linked to an increase in food sources. Moreover, small body-size species (e.g. *Henycorhynchus sp.*), residents and blackfish were triggered by the timing, smoothness and rapidity of change of the coupled/decoupled flooding dynamics to migrate in/out, being particularly driven by the decrease in flood level, at the onset of the dry season, to migrate out of floodplains (Baran *et al.,* 2001; Campbell *et al*., 2009; Baran, 2010; Halls *et al.,* 2013a).

The authors demonstrated the importance of assessing the variability in the flood pulse for the key influence on the species and fisheries supported, driven by the annual variations in the monsoonal rains (i.e. rainfall quantity). Of note, the variability in the flood pulse is related to the daily water level fluctuations, for the higher rainfall in a given day compared to the following, with multiple short periods of high/low flood peaks occurring within the rise and falling phases of the flood, and this intrinsic variability influencing differently the species biomass. Moreover, a high flood peak in a given year does not necessarily translate in the same flood magnitude in the following year, with this variability affecting the annual fish community composition and abundance. Consequently, it is likely that the contributing role of different species to the fisheries is influenced by the inter and intra-annual variability in the pulse, with the maturation and spawning of small and large body-size species being influenced by the flood phases. Large body-size species are likely to be highly influenced by the variability in the flood pulse, with the maturation and spawning being favoured by continued periods of higher and prolonged floods. However, small body-size species (i.e. quick life-spanners) are likely to take advantage of the multiple flood peaks of short duration observed in the flood pulse to growth and spawn more than once a year, with their higher population numbers being reflected in the annual percentage contribution to the total catch (Baran *et al*., 2001; Baran, 2010; Halls *et al.,* 2013a). Junk *et al.,* (2010;2013) and Castello and Macedo (2016) for the Amazon basin, have also discussed the importance of the flood pulse variability in floodplains to fish abundance, which are then reflected on the productive inland fisheries. The authors linked the long-term predictability of the monsoon system to the timing, duration and magnitude of the flood pulse influence on floodplains, resulting in high annual fish production, abundance and consequent high fishery yields (Castello *et al.,* 2015;2017; WWF, 2016).

The significance of the predictable and seasonal variable flood pulse to tropical floodplain flood dynamics, goes beyond the eco-hydrologic interactions established (i.e. for the physical and chemical processes occurring, influencing the trophic levels established and flow of energy and matter), with the synergistic effect of seasonal habitat connectivity, inundation and food sources playing a key role to fish production. This is then reflected on the fishery yields and fish protein to availability to people. Notably, studies argue that marked variations in the long-term predictability and seasonality of the flood pulse dynamics are likely to pose great threats to the adaptative strategies of different fish guilds, affecting the annual fishery yields and species composition of the catch (Junk *et al.,* 1989;2010;2013; Lamberts, 2006;2008; Winemiller *et al.,* 2008; Baran, 2010; Kolding and van Zwieten, 2012; DHI, 2015; Castello and Macedo, 2016).

Tropical floodplains have been disappearing and/or modified in all tropical systems, linked to direct and indirect anthropogenic disturbances on the system (e.g. agriculture development, irrigation schemes, flood defences, dam development and climate change; Lamberts, 2006;2008; Castello and Macedo, 2016; Arias *et al.,* 2012;2013;2014; Hoang *et al.,* 2016;2019). Hydropower dams, irrigation schemes and climate change are arguably the most high impact factors affecting the long-term variability of the flood pulse dynamics in floodplains (see section 2.3 for further information). Castello and Macedo (2016) reported the loss of nearly 30% of the entire floodplain areas in the Amazon basin during the last decade, linked to the clearance of riparian habitats for agriculture production, dam construction and irrigation schemes, with the percentage likely to reach 50% in the near future for the increase in hydropower development and irrigation expansion schemes under changing climate. Moreover, the authors warned the profound modifications likely to occur on the ecological wealth status and to migratory fish guilds supported, linked to significant changes in the seasonal flood pulse-driven floodplain inundation regime and connectivity, and consequent clearance of suitable nursery, feeding and sheltering areas, while expecting marked decrease in nutrient recycling, primary and secondary production. However, quantified scenarios on the consequences to different fish guilds and the ecosystem services provided (e.g. losses in annual fishery yields and species composition of the catch) are absent, bringing alarming uncertainty on the future sustainability of floodplains, fishes, fisheries and food security in the region.

Arias *et al.,* (2012;2013;2014), by applying remote sensing tools and modelling scenarios of the individual and cumulative impacts of hydropower and climate change on the historical flood pulse dynamics and extensive floodplain areas of the TSGL system, reported expected decreases in floodplain inundation area, flood level and duration in the wet season and increases in flood extent, duration and flood level in the dry season, with these projections profoundly shaping the vegetation pattern, land cover and net primary production (see Chapter 5 for detailed values). Additionally, the authors discussed the likely modifications in the timing of floodplain flooding dynamics and the expected disruptions on the vital fisheries, migratory fish guilds composition and production. Yet, the incorporation of a fish component on the modelling exercises is a significant gap, opening questions on the future of the fishery resources of the most productive lake in Southeast Asia (Lamberts, 2006;2008; Campbell *et al.,* 2009; Hortle, 2009).

Few studies have assessed the long-term influence of the flood pulse and floodplain inundation dynamics on fish production and fishery yields, by establishing predictive linear and non-linear models (Baran *et al.,* 2001; Halls *et al.,* 2013a; Castello *et al.,* 2015;2017; Isaac *et al.,* 2016). These models quantified the influence of variations in some of the flood pulse variables (e.g. flood timing, duration and extent (i.e. flood index); Baran *et al.,* 2001; Halls *et al.,* 2013a) on the migratory species composition of the catch and on the annual fishery yields (by using the total annual catch and/or catch effort; Halls *et al.,* 2013a; Castello *et al.,* 2015;2017; Isaac *et al.,* 2016). Moreover, the majority of studies focusing on the Amazon basin, argue the same year and yearly delays for the flood to affect the fishery catch and effort, and be significantly felt (Castello *et al.*, 2015;2017; Isaac *et al.,* 2016). Yet, for systems like the Mekong, where official fishery statistics, although with gaps, report long-term yearly stable catch yields (see Chapter 3; Hortle, 2007;2009; Baran, 2010; Halls *et al.,* 2013b), the few studies assessing the influence of the flood pulse dynamics on the reported fisheries and species composition of the catch have focused on applying a hydrologic year approach (Baran *et al.,* 2001; van Zalinge *et al.,* 2004; Halls *et al.,* 2013a), acknowledging the influence of the single peak flood pulse regime on the fish dynamics and fisheries of the system. Of note, the few attempts made on the Mekong lack a long-term assessment (i.e. the mentioned studies focused on

<10 years assessment). This gap needs to be tackled for the importance the flood pulse place to the Mekong floodplains, and the consequent importance to fish dynamics and abundance, reflected in the fisheries of the TSGL system (see Chapter 4; Lamberts, 2006;2008; Campbell *et al.,* 2009), vital to food security and livelihoods (Hortle, 2007;2009). Only then can future predictions on the impacts upon fishery resources from the multiple drivers of environmental change (i.e. hydropower, irrigation schemes and climate change) can be assessed.

2.3 HYDROPOWER, IRRIGATION AND CLIMATE CHANGE IMPACTS ON LARGE TROPICAL TRANSBOUNDARY RIVER BASINS: LOSSES IN ECOSYSTEM SERVICES TO PEOPLE

2.3.1 THE IMPACTS OF WATER INFRASTRUCTURE DEVELOPMENT

Rivers by the interplay action of winds, precipitation and channel morphology, result in dynamic flow regimes, offering a natural source of energy and water, which is transported, shared and modified along the course by the interactions with the rivers' components (i.e. lateral tributaries, vegetation, bedform morphology, landscape, among others; Wohl, 2014). For centuries, humans have recognised and utilised the energy and water from rivers for their livelihoods, by developing artisanal watermills and continuously enhancing the performance and technology of novel infrastructures (e.g. hydropower dams), to maximise and extract the energy produced by natural flows (Nilsson *et al.,* 2005; Wohl, 2014). Particularly in large tropical river systems, the seasonal distinct flow regimes, influenced by the climatic conditions in place (e.g. monsoons, ENSO; see later), offer a window of opportunity for hydropower development, which has been highly pursued for the needs to provide energy and water to the increasing populations (OECD, 2012; Varis *et al.,* 2012; Wohl, 2014; Hecht *et al.,* 2019). Nowadays, hydropower is a well-established technology for electricity production, generating an estimated 80% of the renewable energy globally (Zarfl *et al.,* 2015).

Zarfl *et al.,* (2015) state records of over 3700 hydropower dams (>1 MW generation power) globally that are either operating, under construction or planned. In the tropics, the number of hydropower schemes have remained relatively low compared to the north hemisphere (see Chapter 1). Yet, the past 40 years have seen an unprecedent escalating number of infrastructures at different stages of development, along with the continuous demand for more dams in the near future (Tollefson, 2011; Liermann *et al.,* 2012; Winemiller *et al.,* 2016; WWF, 2016; Latrubesse *et al.,* 2017; Kondolf *et al.,* 2018; Hechet *et al.,* 2019). Sáenz and Mulligan (2013) estimate that more than 18,000 dams (combined number for hydropower and other man-made structures) regulate approximately 32% of the drainage in the tropics, for the recognised potential on flow regulation and energy production. Moreover, according to Winemiller *et al.,* (2016), the tropical freshwater environment is projected to host the highest global number of dams in the next 10-20 years, confined to three large transboundary river systems (i.e. Amazon, Mekong and Congo basins; Figure 2.4).

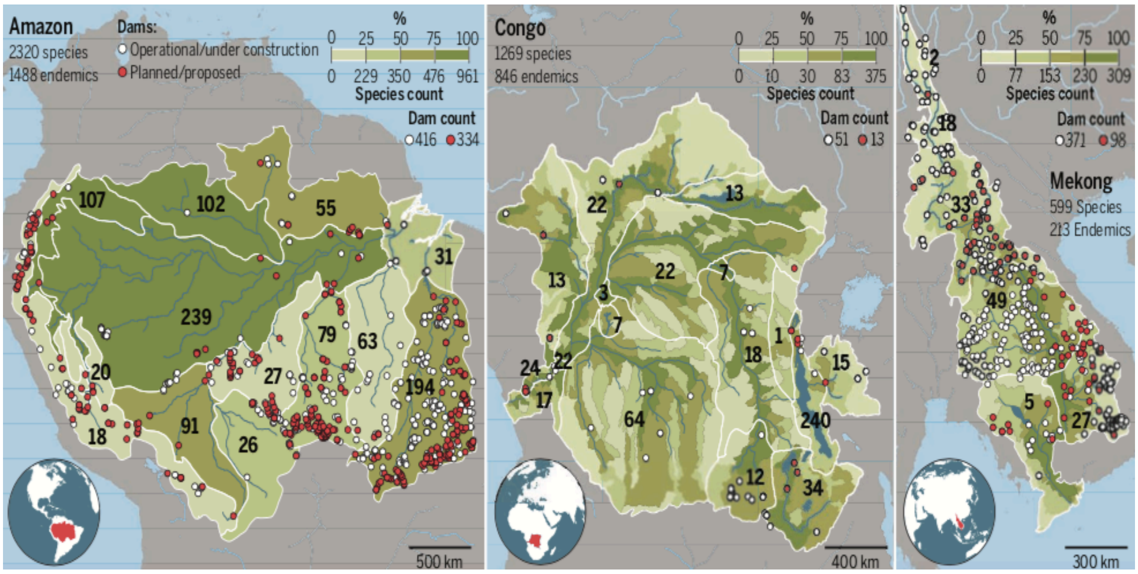

FIGURE 2.4 - FISH DIVERSITY AND DAM LOCATIONS (AT DIFFERENT STAGES OF DEVELOPMENT) IN THE AMAZON, CONGO AND MEKONG BASINS THAT TOGETHER SUSTAIN 75% OF THE KNOWN GLOBAL FRESHWATER FISH DIVERSITY. ESTIMATED TOTAL SPECIES AND ENDEMIC NUMBERS ARE PROVIDED IN THE UPPER LEFT SECTION (RIGHT SECTION FOR THE MEKONG BASIN), AND % SPECIES COUNTS ARE PROVIDED FOR EACH BASIN IN THE RIGHT SECTION. WHITE BOUNDARIES DELIMIT THE ECOREGIONS AND NUMBERS REPRESENT THE SPECIES THAT ARE ONLY FOUND IN A SINGLE ECOREGION. IN ADDITION, WITHIN A RIVER BASIN, THE SPECIES COUNT CAN DIFFER HIGHLY, ILLUSTRATED BY SHADES OF GREEN (SOURCE: WINEMILLER *ET AL.,* 2016). NOTEWORTHY THE OVER AND UNDER-ESTIMATION OF THE CONGO AND MEKONG FISH SPECIES NUMBERS, RESPECTIVELY, ACCORDING TO THE REPORTED NUMBERS IN SUB-SECTION 2.1.2.

The observed highest mean annual run-off (see Chapter 1), promoting energy production, and the needs to regulate the seasonal distinct flow regime, for annual water provision (i.e. for both wet and dry seasons), are the main reasons argued for the expected impoundment on those basins (Latrubesse *et al.,* 2005; Milliman and Farnsworth, 2011; Sinha *et al.,* 2012; Syvitski *et al.,* 2014; WWF, 2016). Additionally, economical boost (i.e. usually by foreign investment on hydropower development and consequent electricity-selling value) is a major appeal to the countries hosting hydropower schemes, for the needs to provide energy to their increasing populations (Hirsch, 2010; Latrubesse *et al.,* 2017). Consequently, the reasons advocating hydropower schemes often centre on the improvements made to the livelihoods of the populations and to the economies of the countries where dams are built (Hirsch, 2010; Varis *et al.,* 2012; Winemiller *et al.,* 2016; WWF, 2016; Latrubesse *et al.,* 2017; Olson and Morton, 2018). Hirsch (2010) reviewed the politics of hydropower in the Mekong basin, expressing the goals of the six riparian countries of the river in boosting their own economies, while providing electricity to the increasing populations. Yet, transboundary issues surrounding the Mekong are seen in the Amazon and Congo basins, where the riparian countries continuously develop water infrastructures, without implementing joint strategic development plans, seeking a compromise between sustainable exploration and preservation of the river's resources. These three transboundary rivers support nearly 75% of the global known freshwater fish diversity, with over 50% exhibiting migratory behaviour, while also sustaining high number of endemic species (Figure 2.4). Hence, how water infrastructures will impact the migratory behaviour, endemism, connectivity and eco-hydrologic interactions in habitats are essential questions to be tackled, for the importance fishes play to the ecosystem and major source of protein intake and livelihoods (i.e. inland fisheries) to the populations inhabiting those basins (Vörösmarty *et al.,* 2010; Ziv *et al.,* 2012; Kano *et al.,* 2016; Winemiller *et al.,* 2016; WWF, 2016; Latrubesse *et al.,* 2017; Hecht *et al.,* 2019).

The boom in hydropower dam development and other man-made structures in the tropics (e.g. reservoir dams, weirs and dykes), connected to the increase in irrigation schemes (mainly for agriculture), have shaped and altered landscape morphology, river courses and flood regimes, with profound consequences to habitats connectivity and inundation dynamics, nutrient recycling, sediment transport, freshwater biota, ecosystem services and to people (Xenopoulos *et al.,* 2005; Vörösmarty *et al.,* 2010; Liermann *et al.,* 2012; Varis *et al.,* 2012; Kano *et al.,* 2016; WWF, 2016; Hoang *et al.,* 2016;2019; Konfolf *et al.,* 2018). While it has been acknowledged that these water schemes promote flow regulation (i.e. for storage of water during the wet season and release in the dry season, decreasing overland flood events during the flood season) and increase water availability (e.g. irrigation schemes for agriculture in the dry season), which are seen as fundamental issues in tropical large rivers under monsoonal flood regimes, these often called "benefits" mask the medium to long-term negative effects, when assessing the multiple complex interactions established in tropical freshwater systems (Friend and Blake, 2009; Liermann *et al.,* 2012; Winemiller *et al.,* 2016; WWF, 2016; Latrubesse *et al.,* 2017; Olson and Morton, 2018; Hecht *et al.,* 2019). Dams are claimed as the single most impacting anthropogenic action to freshwater systems, their ecosystem services (e.g. fisheries) and natural biodiversity (Baran and Mychowoda, 2009; Vörösmarty *et al.,* 2010; Castello and Macedo, 2016; Chapman and Darby, 2018; Kondolf *et al.,* 2018). In fact, when assessing the complexity of interactions established in tropical freshwater systems (i.e. flood regime-sediment flux-habitats-nutrient recycling-fishes; see diagram in Chapter 1), the first impact by dams is on the long-term flood dynamics (i.e. for the run-off, water level and connectivity; Castello and Macedo, 2016; Latrubesse *et al.,* 2017). Large tropical river systems, previously characterised by continuous fast-flowing waters (lotic systems), have been heavily fragmented by dams to stagnant waters (lentic systems), in sections of the catchment associated to the formation of extensive reservoirs and irrigation schemes, where waters are stored and seasonally released (Nilsson *et al.,* 2005).

Grill *et al.,* (2019) recently provided the first worldwide assessment of free-flowing rivers, showing that 67% of the World's rivers have already lost connectivity due to dams, with only 37% remaining free-flowing for their entire length, mainly in the Amazon and Congo basins (see Figure 1.5 of Chapter 1). Fearnside (2014) reported the problems raised by the Madeira dams in the Madeira river (tributary of the Amazon), stating the loss in hydrological connectivity and fragmentation of longitudinal (mainstream river) and lateral habitats (e.g. floodplains). Additionally, change in the historical inundation timing, duration and extent in floodplain habitats, by early/delays in the flow pattern, decreases in flood level during the wet season and increases in flood level in the dry season, were projected to significantly impact the productivity of the system. Also, projected decreases in nutrient recycling were inferred by the expected losses in the vegetation pattern, since the flooded forest in some areas could not cope with the permanent inundation from the established reservoir, that according to modelling analysis would result in an increase of the permanent flooded area from 241 km^2 to 529 km^2 and variations in water quality for dissolved oxygen levels and turbidity. The authors' main argument was that these eco-hydrological changes would severely threat over 800 species, known to seasonally and permanently seek floodplain inundated habitats for spawning, feeding and sheltering purposes. Moreover, expected losses in the inland fisheries were argued, however no quantification was provided. Similarly, Castello and Macedo (2016) argued disruptions on the migratory pattern of more than 50% of the known fish fauna of the Amazon basin and fisheries loss, as a direct consequence of current and future scenarios of dam and irrigation effects in the seasonal predictable flood pulse regime, habitats inundation profile and migratory pathway blockage. Yet, the authors did not provide quantifications of the percentage loss in the fishery yields nor on species composition of the catch. Likewise, Baran *et al.,* (2007) brought into attention the failed plans from the Pak Mun dam in the Mun River (Mekong tributary), which resulted in 67-96% decrease in the annual catches in Thailand, linked to 80% decrease in the migratory behaviour of nearly 200 fish species, since this dam trapped the migratory pathway. Hence, the importance of maintaining hydrologic connectivity and preserving the historical flood pulse regime, for the eco-hydrologic interactions established in lateral habitats, have been extensively debated among studies for the fundamental role on driving fish migratory dynamics, production and abundance, which are then reflected on the inland fisheries, key for food security to people.

Abell *et al.,* (2008), Kano *et al.,* (2016) and Tedesco *et al.,* (2017) argue that over 70% of the known fishes of the tropics are triggered by rises in the flood level to perform spawning migrations. Thus, hydropower, by storing water in the wet season and releasing it in the dry season, dramatically disrupts the long-term timing of the migratory behaviour, with expected severe consequences to fish life cycles for early/delay spawning and mortality increase of larvae and eggs, for the modifications in food sources and nursering areas, with consequent decrease in fish abundance (Junk *et al.,* 1989; Winemiller *et al.,* 2008). Additionally, irrigation schemes, linked to dam development, have been related to disruptions on the flood dynamics and clearance of key riparian habitats in the Amazon and Mekong basins, with profound, yet largely unquantified, consequences to fish abundance, production and migratory dynamics (Castello and Macedo, 2016; Hoang *et al.,* 2019). Ultimately, alarming losses in the inland fisheries, composed by over 50% of migratory fishes, are expected from the resulting losses in fish abundance and migratory pathway blockage (Baran and Myschowoda, 2009; Ziv *et al.,* 2012).

The paucity of studies quantifying the future impacts of hydropower and irrigation schemes on tropical inland fisheries and fish production (i.e. for the species composition of the catch) is a major gap that needs to be addressed, for the vital role that inland fisheries place to human populations, to whom fish represent the major (and sometimes only) source of protein intake (Hortle, 2009; FAO, 2018). According to FAO (2018), the highest annual fish protein consumption (>40 kg year^{-1}) is in tropical under-developed countries. Remarkably, the diversity of modelling studies applied to predict different impact scenarios of hydropower (and irrigation schemes from few studies; e.g. Hoang *et al.,* 2019) on the flood regime, seasonal habitat inundation dynamics and sediment flux (see below), contrast with the lack of quantification on the resulting consequences to the inland fisheries, species abundance and migratory dynamics, with only statements on the expected disruptions on the migratory pattern and losses in the annual catches (Baran and Mychowoda, 2009; Ziv *et al.,* 2012; Piman *et al.,* 2013; Fernside, 2014; Winemiller *et al.,* 2016; WWF, 2016; Latrubesse *et al.,* 2017). This issue is highlighted for the Mekong basin in Chapter 3, but also observed in the Amazon, Congo, Nile and other tropical large river systems, with limitations in the long-term fisheries data (see Chapter 1), constraining future predictions based on modelling analysis (Welcomme *et al.,* 2010). Nevertheless, available tropical inland fishery data (although with long-term limitations), should be recognised for the key information provided to understand the historical fisheries annual catches, species composition and the influence of the system's historical flood regime, to then allow the quantification of future ecosystem services loss from environmental changes in the system (Ziv *et al.,* 2012; DHI, 2015).

Sediment starvation is another consequence of dams (Kummu *et al.,* 2010; Kondolf *et al.,* 2014;2018), raising particular alarming issues in tropical lowland deltas. This is linked to the projected sea level rise, under changing climate, resulting in sinking of terrestrial land, increase in bankside erosion, threats to flood control and disruption on the coastal interactions between the freshwater and marine waters (i.e. variations in salinity and pH of water, which influence fish species and fisheries; Milliman and Syvitski, 1992; Closs *et al.,* 2016). Not only freshwater fish will be affected by increases in salinity levels, but also the densely populated areas observed in lowland tropical deltas (e.g. Mekong, Amazon, Nile, Niger, Ganges, together accounting to nearly 60% of the World's population; Darby *et al*., 2013) will suffer from this sediment clearing and losses in bounded nutrients, particularly for agriculture disruption, as already seen in Vietnam (Balica *et al.,* 2014; Kondolf *et al.,* 2018). Intralawan *et al.,* (2018) settle the agriculture productivity in the Vietnamese delta at $220 million per year, highly dependent on the sediment-nutrient bounded transport and recycling from seasonally inundated riparian areas. Chapman and Darby (2018) claim that the reported value might be underestimated, however, and perhaps more importantly, the authors agree with the views from Intralawan *et al.,* (2018) for the risks by sediment starvation from upstream hydropower development, combined to climate change influence on sea level rise, on the system's natural resources.

Population displacement is another major social problem linked to hydropower development, where people are resettled from their lifelong villages, due to flood events of previous drylands (Latrubesse *et al.,* 2017; Olson and Morton, 2018).

In the Mekong river, plans to build Cambodia's largest hydropower scheme, Sambor (expected completion date: 2025-2027; 1,800 MW; Sithirith, 2016) in Kratie province, has been under severe controversy for the negative impacts expected to the historical flood regime, sediment flux, fisheries and people (WWF Mekong, 2017). The potential displacement of over 19,000 people, estimation on 30% loss in fishery yields and a dramatic impact on freshwater fish abundance, particularly to the already IUCN critically endangered status of iconic species like the Irrawaddy dolphins (*Orcaella brevirostris*) and Mekong giant catfish (*Pangasianodon gigas*), along with disruptions on the migratory pathway of more than 50% of the migratory fish species that leave the TSGL system on the onset of the dry season to find shelter in the upstream deep pools around Kratie, have been argued to profoundly threaten the ecological wealth status of the basin and food security of one of SE Asia's poorest country, which annual fish protein consumption is the highest (see Chapter 3; Hortle, 2007;2009; So, 2010; Sithirith, 2016; Brownell Jr., 2017). Additionally, claims of disruption on the unique bi-directional flow reversals on the TSGL system and sediment flux, highlights the threats to floodplain productivity and to the important *dai* fishery (see Chapter 4; Lamberts, 2008). Consequently, the location of the dam has been argued to be the worst possible place for such infrastructure, whose impacts are expected to extent further downstream to the Vietnamese Delta (Sithirith, 2016).

It is vital to increase the understanding on the multiple impacts of hydropower on the freshwater resources, to seek a balanced equilibrium, in which the anthropogenic pressures are made in a sustainable manner and for the preservation of the natural system and the biodiversity supported. Olson and Morton (2018) recently pointed out that Lao PDR plans in becoming the "battery of Southeast Asia" lack on multiple environmental impact assessment, for the location of the dams. The authors proposed specific sections of the mainstream river and tributaries to be impounded, without significantly impacting upon fish migratory dynamics and abundance, the productive fisheries, the historical flood regime, habitats and sediment flux. Thus, the authors reinforced Winemiller *et al.,* (2016) view that basin development plans should move towards an integration analyses of the medium to long-term impacts on the natural resources, the ecosystem services supported and the consequences to the populations they integrate, particularly in light of projected climate change scenarios.

2.3.2 THE IMPACTS OF CLIMATE CHANGE

The tropical climate of large transboundary river basins like the Mekong, Amazon, Congo, Nile and Ganges-Brahmaputra, is characterised by monsoon systems (Amarasekera *et al.,* 1997; Delgado *et al.,* 2012; Syvitski *et al.,* 2014). These monsoon systems possess spatio-temporal and regional disparities among basins, reflecting the interactions of the soil moisture, evapotranspiration and temperature of the land-ocean-atmosphere convective winds' pattern across tropical regions (Syvitski *et al.,* 2014). Moreover, the large nature of the drainage area, connected to the heterogeneity in the vegetation pattern, topography, geomorphology and biogeochemical processes in the landscape, means that the interactions of the land-ocean-atmosphere, result in the delimitation of distinctive monsoon components that ultimately affect sections of the basin differently, for the precipitation and temperature pattern (e.g. the Asian monsoon system, with the Indian and Western-North Pacific components, influence differently the Upper and Lower Mekong basin. Also, the African monsoon system, with the east and west components, mark variations in the precipitation timing and duration (i.e. delimitation of wet and dry seasons) in the Nile and Congo basins; Dhar and Nandargi, 2000; Delgado *et al.,* 2012; Aisch *et al.,* 2014).

Syvitski *et al.,* (2014) assessed 35 large tropical rivers for the influence of annual changes in climate-driven variables (i.e. temperature and precipitation), on the long-term (50 years) discharge and sediment transport pattern, by applying model simulations of sediment transport from historic discharge data and sediment flux. The study demonstrated long-term significance of the variations in the annual rainfall pattern across basins, as the main driver for the observed seasonality on periods of low and high peak discharge and consequent changes in sediment transport. Moreover, it revealed low level of change, during the 50 years period, on the timing and duration of periods of rainfall and dry weather and resulting timing and duration of rise and fall discharge pattern across basins, suggesting a long-term seasonality and predictability on the flood regime dynamics. Consequently, the long-term seasonality and predictability of the rainfall pattern, is argued to define a single annual wet and dry season (exception for the Congo basin that exhibits two rainy (dry) seasons: March-May (June-August) and September – November (December-February); Amarasekera *et al.,* 1997; Dyer *et al.,* 2017). Additionally, these large river systems experience periodic predictable climatic events (e.g. ENSO and tropical cyclones, with exception for the Amazon basin linked to the rainforest tropical climate; Syvitski *et al.,* 2014), which heighten periods of rainfall and droughts (Amarasekera *et al.,* 1997; Castello *et al.,* 2012; Darby *et al.,* 2013;2016). As so, the historical periodicity and the seasonality on the monsoonal rainfall pattern and other weather events influence the annual hydrograph (i.e. rise and fall), driving the variations in the flood regime of tropical catchments and affecting the eco-hydrologic interactions established on habitats, connectivity, productivity and aquatic biota supported, as previously mentioned and further developed in this section (Junk *et al.,* 1989;2010; Val *et al.,* 2006; Lamberts, 2006;2008; Kolding and van Zwieten, 2012; Castello and Macedo, 2016).

Studies have also focused on the role of long-term fluctuations of the rainfall pattern, to the eco-hydrologic interactions established between the freshwater catchment and the riparian landscape, the importance of the timing, duration and extent of the flood in connecting previously fragmented habitats (during the dry season), the flux of sediments and recycling of nutrients and the significance of these interactions to the fish diversity sustained (Junk *et al.,* 1989; Kundzewicz *et al.,* 2009; Milliman and Farnsworth, 2011; Arias *et al.,* 2012;2014). During the dry season months (with monthly variations among basins), characterised by significant decreases in the rainfall and consequent decrease in flood magnitude, some areas experience complete droughts and habitat fragmentation (e.g. in shallow lakes in Nile and Congo basins; Aisch *et al.,* 2014; Dyer *et al.,* 2017), challenging the survival of fishes. The wet season months, characterised by heavy rainfalls (with La Niña years and tropical cyclones exacerbating the amount of rainfall in the catchments), although exhibiting monthly variations among basins, can be broadly described as resulting in higher flood magnitudes and discharge, with consequent transport of heavy sediments loads from upstream-downstream unidirectional pattern (with exception to the unique bi-directional flow reversals in the TSGL system; see Chapter 4; Lamberts, 2008; Kummu *et al.,* 2014). This promotes habitat connectivity, inundation extent and productivity, with Lucas and Baras (2001), Dudgeon (2000), Castello and Macedo (2016) and Lima *et al.,* (2017) recognising the importance that the wet season places to fish migratory behaviour, production and to the productive fisheries that develop.

The attempts made by Pinaya *et al.,* (2016) to quantify the role of historical ENSO events on the migratory pattern and catch effort in the Lower Amazon fisheries, only described that higher floods resulted in higher fish production, while lower floods resulted in decrease in fish production, with both effects being highlighted in next year's fishery yields. No quantification was made linked to ENSO data constrains. For the Mekong river the gaps in knowledge are emphasised, not only by the paucity of studies focusing on the fish dynamics, fisheries and habitats explored, but also by the lack of studies describing the role of ENSO events on fish production and how historical variations in the seasonal monsoonal climate, influence the fisheries and fish production of the system. These open questions need to be answered in light of the already experienced modifications in the Asian monsoonal climate, as described by Delgado *et al.,* (2012), and the projected climate change scenarios (Kundzewicz *et al.,* 2009; Delgado *et al.,* 2012; IPCC, 2014). Therefore, the reviews made by Kundzewicz *et al.,* (2009) and Syvitski *et al.,* (2014), highlight the fundamental role of the long-term and low level of variations in the timing and duration of the rainfall pattern, in driving the seasonal flood regime in large tropical river basins. Importantly, the eco-hydrological interactions established, the distribution behaviour and adaptive strategies of fishes to these flood alterations, are argued to be severely impacted by modifications in the monsoonal climate-driven flood regime. Yet, the scarcity of studies quantifying those impacts to fish dynamics is a major gap that needs to be filled (Lamberts, 2008; Castello *et al.,* 2012; Castelo and Macedo, 2016).

Recent scientific evidences suggests that major environmental changes are already occurring across the globe, associated to climate change (temperature and precipitation), through variations in floods, droughts, sea level rise and storm events (Falkenmark, 2007; Ludwig and Moench, 2009; Michel and Pandaya, 2009; Van Manh *et al.,* 2015). The IPCC (2014) report on climate change shows distinctive projections on the increase in temperature and precipitation until 2100, with the impacts expected to be felt differently among regions (see Chapter 1). For large tropical river basins, climate change projections are likely to significantly disrupt the predictable seasonality on wet and dry season's flood regime, as well as increase the variability and intensity of weather events like ENSO and tropical cyclones. These are argued to result in some of the highest and unpredictable challenges, regarding the resilience of the systems, the people and the directions to take on implementing adaptive measures for the increasing concern on future water stress and likely losses in ecosystem services (Boko *et al.,* 2007; Palmer *et al.,* 2008; Keskinen *et al.,* 2010; Delgado *et al.,* 2012; Aich *et al.,* 2014).

The severity of the impacts on tropical systems, compared to other regions, is linked to supporting the highest percentage of human population (60% of the World's population live in Asia; Darby *et al.,* 2013), living under severe poverty (UNEP, 2006) and depending on an estimated more than 40 kg of fish protein per year for protein intake (FAO, 2018). Also, the tropics sustain over 50% of large river basins (see Chapter 1), where the highest diversity of habitats and known fish diversity found, have been claimed to be driven by the long-term predictable monsoonal climate and low variation in the periodicity of climatic events (Abell *et al.,* 2008; Kundzewicz *et al.,* 2009; Syvitski *et al.,* 2014). However, the scarcity of studies assessing the future consequences of climate change to the tropical fishes is remarkable, if one attends to its importance to people and to the ecosystem services (e.g. fisheries; Morrongiello *et al.,* 2011; Ziv *et al.,* 2012; Winemiller *et al.,* 2016). Thus, key open questions prevail on how variations in the historical climate-driven flood regime will impact tropical fishes and eco-hydrologic interactions on the diversity of habitats supported? How the tropical fisheries will be affected? How the unpredictability of ENSO and tropical cyclones will magnify the drought/flood pattern? How climate change will magnify the threats already posed by hydropower and irrigation scenarios? and what are the likely consequences to food security in under-developed countries?

Projections of climate change impacts on large tropical river basins, regarding future (e.g. 30-50 years) modifications in the long-term flood regime, sediment flux and sea level rise, have increased by applying a significant number of modelling exercises with inputs from different RCPs scenarios (see Chapter 1 for the definition; Palmer *et al.,* 2008; Västilä *et al.,* 2010; Lauri *et al.,* 2012; van Vliet *et al.,* 2013; Aich *et al.,* 2014; Thompson *et al.,* 2014; Van Manh *et al.,* 2015; Darby *et al.,* 2016; Hoang *et al.,* 2016;2019). Therefore, providing a description and comparison of different studies' projections to each basin, would uncover the purpose of this section (yet detailed assessment can be found in Chapter 3 for the Mekong river), which consists in demonstrating the main threats to large tropical river basins from climate change projections and the gaps in studies assessing the consequences to fish diversity and fisheries. Importantly, all studies recognise the uncertainty on the projections, linked to constrains on the historical time-series records of hydrology, sediment flux and atmospheric data, and the urgency to develop further analyses, in light of the already felt modifications in flood regime, sediment flux and retreat in the land area of productive deltas, due to increase in water infrastructure developments, land erosion, sediment starvation and increase in tidal influence (van Vliet *et al.,* 2013; Van Manh *et al.,* 2015; Kondolf *et al.,* 2014;2018; Kondolf *et al.,* 2019). Regarding the likely directions of change, although with intrinsic differences among large tropical basins (for the monsoonal systems and periodicity of tropical cyclones and ENSO events. Also, even within basins, different modelling exercises show different projections of change) studies agree that the mean annual global tropical temperature will become warmer, with projections to Africa being the highest (3-6 ^{0}C increase in the next 30 years; Aich *et al.,* 2014). Also, future precipitation pattern is projected to become extremely variable among and within different sections of the basins, making it difficult to establish a range of change. However, Aich *et al.,* (2014) for four large African river basins (Niger, Upper Blue Nile, Oubangui (main tributary of the Congo river) and Limpopo), van Vliet *et al.,* (2013) for multiple large river basins (e.g. Amazon, Congo and Mekong), and Thompson *et al.,* (2014) and Van Manh *et al.,* (2015) for the Mekong basin, all state that wet seasons will become wetter and dry seasons will become drier.

The studies also argue the likely increase of extreme flood events, not only connected to rainfall, but also it is likely that tropical cyclones and ENSO events (for the La Niña phase), will become more variable and intense, magnifying the uncertainty on the timing and duration of the wet season, and flow magnitude (Delgado *et al.,* 2012; Räsänen and Kummu, 2013; Darby *et al.,* 2016). In the dry season, the warmer climate combined with an intensification of El Niño events, is projected to increase droughts areas, particularly in African and Amazon basins, with Castello and Macedo (2016) stating an alarming increase in the dry season period for the Amazon basin to nearly 1 month over the next 50 years. Moreover, the synergistic effect of temperature rise and habitat clearance (mainly associated to increase in irrigation schemes), with the loss of nearly 698,000 km^2 of vegetated habitats from the surveyed 1 million km^2, was linked to the increase in dry season duration, with losses in vegetated areas (Castello and Macedo, 2016). Additionally, the combined effects of land-use and warmer dry season, have been responsible to an increase in wild fires in all tropical basins (with the effects being less significant in the Mekong basin), which have resulted in a significant decrease in vegetated areas and unquantified losses in the nutrients reaching the aquatic environment by recycling, opening key questions regarding the impacts on primary and secondary production, and to fish production (Castello *et al.,* 2012; Aich *et al.,* 2014; Castello and Macedo, 2016). Furthermore, for the Mekong basin, the WWF (2005) mentioned that during the last 40 years, the glacial extent of the Tibetan Plateau has shrunk by 6,600 km^2 out of a total of 110,000 km^2. Yet, this has not profoundly modified downstream flows in the Mekong river, with the study from Lu and Siew, (2006) stating that over the last 50 years, the dry season flows arriving as downstream as Kratie have not shown significant differences, even after the construction of the Manwan mainstream hydropower dam (see Chapter 3). Also, studies on the likely effects of climate change in the Mekong river, all state the increase variability in the timing and duration of dry and wet seasons and climatic events, which will potentially disrupt the flow pattern and sediment flux reaching the Vietnamese delta (see Chapter 3 for detailed projections; Van Manh *et al.,* 2015; Kondolf *et al.,* 2014;2018).

As previously reported, the levels of flow regulation, habitat clearance and fragmentation, loss of vegetation and shifts on sediment flux, mainly by hydropower and irrigation schemes, have been profoundly shaping large transboundary river basins, with dramatic consequences to the fishes, ecosystem services supported and to people (Kummu *et al.,* 2010; Winemiller *et al.,* 2016; Latrubesse *et al.,* 2017; Kondolf *et al.,* 2014;2018). Thus, predicting the impacts of climate change on the natural river hydrodynamics and effect on fish abundance, migratory dynamics, production and fisheries, should acknowledge the impacts by hydropower and irrigation development. Hydropower's projections to tropical basins (although with differences among scenarios) state the increase in dry season flows and decrease in wet season flows, also shifting the timing, duration and sediments carried. However, climate change projections state the increase in wet season floods and dry season droughts, with the unpredictability of tropical cyclones and ENSO, posing important questions on how these apparent opposite projections on the flood regime might disrupt habitat flood dynamics, productivity and, importantly, how fish abundance and migratory dynamics will be impacted (Kummu *et al.,* 2010; Lauri *et al.,* 2012; Van Manh *et al.,* 2015; Castello and Macedo, 2016; WWF, 2016; Kondolf *et al.,* 2018). Consequently, the more one tries to predict the future modifications in the flood regime, sediment-nutrient bounded flux and the effects on habitats, fishes and fisheries, the challenging the task reveals, because of the already experienced flow and sediment regulation from hydropower and irrigation development, the incompleteness of historical data and the key gaps in knowledge on the importance and interactions of the complex eco-hydrologic drivers to fish dynamics and abundance. Xenopoulos *et al.,* (2005) by applying modelling analysis of climate change and water withdraw impacts on 237 river's discharge and the effect on global freshwater fishes by 2070, provided one of the few global estimations, stating that the highest fish loss (up to 75% of local fish diversity) is expected in the tropics. Freitas *et al.,* (2013), following the projections of Xenopoulos *et al.,* (2005), for the Amazon basin, projected a 7 to 12% loss in fish species by 2070, due to the drought effect on floodplains, with no hydropower and irrigation scenarios. Also, studies from Ficke *et al.,* (2007), Morrongiello *et al.,* (2011), Ziv *et al.,* (2012), Winemiller *et al.,* (2016) and WWF (2016) all claim that fish production and fisheries will likely be severely disrupted by the combined impacts of the multiple drivers of environmental change, magnifying food security issues in the region. Yet, no quantification was performed to support those inferences, mainly linked to the difficulty in distinguish between individual drivers' actions on the system. Moreover, the paucity of knowledge on most fish species diversity and distribution is claimed to make it difficult to effectively project future scenarios (Dudgeon, 2000; Ficke *et al.,* 2007; Campbell *et al.,* 2009). Nevertheless, and corroborating the views on the paucity of data on fish diversity, abundance and migratory behaviour, and the difficulties on effectively distinguish different drivers' impacts, one should acknowledge the natural and social importance fishes and fisheries have in the tropics and develop studies that provide future estimations-attempts on the likely modifications in the fishery resources, driven by hydrologic and habitat flood dynamics change by the individual and combined action of the multiple drivers of environmental change.

2.4 ENVIRONMENTAL DNA: A MOLECULAR-BASED SURVEY TOOL TO ASSESS TROPICAL FISH COMMUNITIES AND MIGRATORY DYNAMICS IN LARGE TROPICAL RIVERS, UNDER ENVIRONMENTAL CHANGE THREAT

The increasing pace in which tropical freshwater environments are being imperialised by anthropogenic-driven disturbances, connected to the uncertainty on the estimations of fish species richness supported and paucity of knowledge on the distribution behaviour, habitats explored and role of the historical variations in the flood regime and sediment flux in driving the patterns of species migrations; make it vital to apply effective studies capable of providing answers to the identified gaps in knowledge (Val *et al.,* 2006; Abell *et al.,* 2008; Winemiller *et al.,* 2008;2016; Thieme *et al.,* 2011). Additionally, the significance of tropical freshwater fishes to the inland productive fisheries and food security to over half of the World's population, make it crucial to understand the diversity of life-forms and ecological dynamics, to them apply effective conservation measures seeking the preservation of the resources, in light of the escalating environmental change threats (Dudgeon, 2000; Thieme *et al.,* 2011; Winemiller *et al.,* 2016; FAO, 2018). Of note, the limitations of traditional survey methods to describe fish communities and patterns of distribution (see Chapter 1), make it vital to establish novel methodologies capable of effectively improve one's knowledge on tropical fish diversity (Fischer, 2013). The recent advances in molecular ecology (e.g. use of Environmental DNA-eDNA as a molecular-based survey approach), with reported success on species detection and assessing distribution patterns in freshwater temperate systems (Taberlet *et al.,* 2012;2018; Deiner *et al.,* 2015;2017; Hänfling *et al.,* 2016; Handley *et al.,* 2018; Harper *et al.,* 2018b), led to the inference on the application of eDNA-based survey approaches in the tropics. This was followed by few studies performed in large tropical freshwater systems, aiming to characterise the tropical fish abundance and distribution, using eDNA molecular approaches (Bellemain *et al.,* 2016; Robson *et al.,* 2016; Sales *et al.,* 2018).

2.4.1 THE REVOLUTIONISING ROLE OF EDNA TO MONITOR FISH COMMUNITIES AND DISTRIBUTION DYNAMICS

eDNA is characterised by a complex mixture of genomic DNA from the many organisms inhabiting a certain environment (e.g. freshwater, marine and terrestrial; Figure 2.5; Taberlet *et al.,* 2012;2018). All organisms (e.g. fishes, invertebrates, terrestrial and aquatic mammals and birds) release their DNA (e.g. in the form of shed cells, gametes, mucus, urine and other excreta; Taberlet *et al.,* 2012;2018; Valentini *et al.,* 2016; Cristescu and Hebert, 2018; Senapati *et al.,* 2018) into the environment where they inhabit and interact, with this shedding DNA providing reliable evidences of their presence, interactions with others (e.g. the DNA of preyed species detected in faeces or stomach content) and dispersion levels (Laramie *et al.,* 2015; Thomsen and Willerslev, 2015; Bista *et al.,* 2017). Also, the released DNA is found in two states, intracellular (DNA from living cells) and extracellular (DNA from cell death and destruction of cell structures, followed by degradation through physical, chemical and/or biological processes; see sub-section 2.4.3; Herder *et al.,* 2014; Pilliod *et al.,* 2014; Taberlet *et al.,* 2018).

Taberlet *et al.,* (2012;2018), Herder *et al.,* (2014) and Deiner *et al.,* (2017), recently reviewed the differences in eDNA detected from different environments, stating that eDNA detected in terrestrial environments, particularly in soils and sediments is generally highly preserved, depending on the chemical and physical properties of the sediment, compared to the eDNA found in aquatic environments. This is associated to the chemical processes established between DNA molecules and sediment components, compared to aquatic environments, where variations in flow dynamics, landscape interactions, biotic and abiotic factors, all contribute to the higher fragmentation and transport of DNA molecules, affecting the persistence and degradation (Figure 2.5; see sub-section 2.4.3).

In 2003, Willerslev *et al.,* (2003) applied the first eDNA metabarcoding study (see below for the definition) focusing on macroorganisms, to show that it was possible to obtain information from ancient organisms (e.g. mammoth, bison, ratite moa and plants) from permafrost and cave sediments. On the other hand, in aquatic environments, Taberlet *et al.,* (2012;2018), Herder *et al.,* (2014), Hänfling *et al.,* (2016), Valentini *et al.,* (2016) and Deiner *et al.,* (2015;2017;2018), all state the relative low persistence of DNA molecules, with studies reporting a maximum of few weeks to a month for eDNA to be effectively detected. Therefore, the DNA retrieved from aquatic samples is able to provide near-real time information of a species or group of species to be effectively present, compared to terrestrial environments, where already extinct species can still be found, increasing the confidence, accuracy and reliability on the identified species to understand species diversity, community structures and patterns of distribution (Thomsen and Willerslev, 2015; Taberlet *et al.,* 2018).

Importantly, and as recently pointed out by Harper *et al.,* (2018b), eDNA is a rapid, non-invasive and cost-efficient survey tool, able to significantly improve the knowledge on species diversity, community structure and distribution, providing vital information for improving conservation measures of threatened aquatic systems, while surveying the entire species community or particularly focusing on a single species, to assess the health status of a system and/or detect early stages of invasion by non-native species (Keskin, 2014; Bellemain *et al.,* 2016).

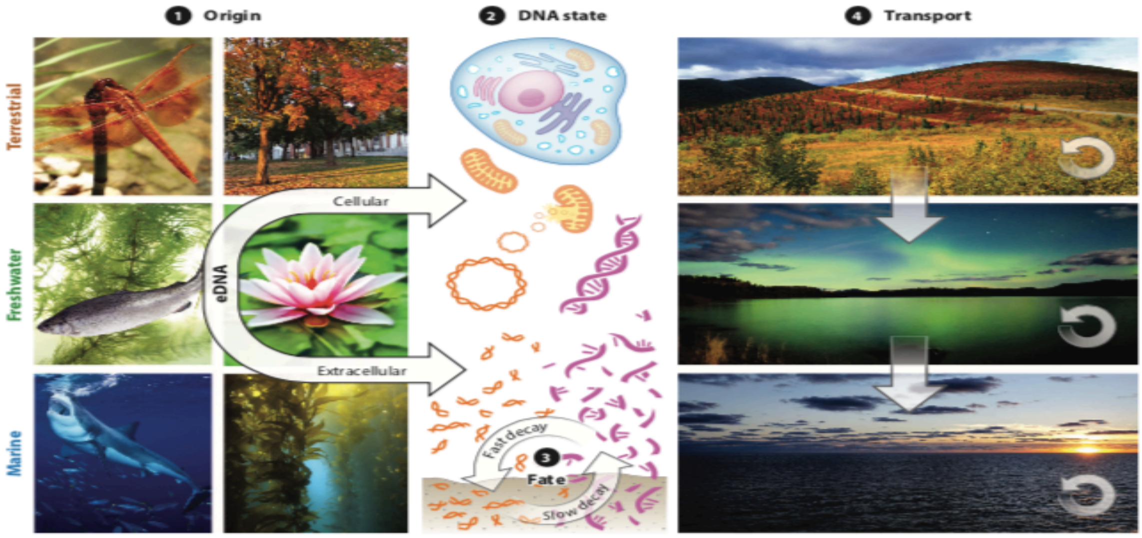

FIGURE 2.5 - DIAGRAM SHOWING THE ORIGINS OF EDNA IN DIFFERENT ENVIRONMENTS, THE STATE AND FATE OF SMALL AND LARGE DNA MOLECULES AND TRANSPORT (SOURCE: CRISTESCU AND HEBERT, 2018).

Two main approaches have emerged from eDNA studies in aquatic systems, mainly for biomonitoring and/or improving management and conservation actions (Keskin, 2014; Rees *et al.,* 2014; Thomsen and Willerslev, 2015; Valentini *et al.,* 2016; Harper *et al.,* 2018b). Single species eDNA detection (i.e. DNA barcoding), aims to identify a target species from an aquatic sample, applying species-specific primers and for example qPCR molecular analyses (see sub-section 2.4.2). Fukumoto *et al.,* (2015), Blackman *et al.,* (2017) and Cowart *et al.,* (2018), have enhanced the knowledge on specific freshwater amphibians and crustacean species (e.g. salamander, shrimp and crayfish, respectively) and target fish species (Ficetola *et al.,* 2008, Bellemain *et al.,* 2016; Harper *et al.,* 2018b), applying DNA barcoding and metabarcoding tools, with the aims being the investigation of species distribution and community composition. Since Giovannoni *et al.,* (1990) pioneering eDNA metabarcoding study on the bacterioplankton diversity in the Sargasso Sea, by using a specific genetic marker (16S rRNA; see sub-section 2.4.2 for the definition and importance of using genetic markers in eDNA studies; Taberlet *et al.,* 2012;2018), an increasing number of studies have been applying eDNA metabarcoding tools to detect the presence/absence of more than one species, assess the community structure of an aquatic system and patterns of spatio-temporal distribution (Kelly *et al.,* 2014; Thomsen and Willerslev, 2015; Creer *et al.,* 2016; Hänfling *et al.,* 2016; Valentini *et al.,* 2016; Handley *et al.,* 2018; Sales *et al.,* 2018). Notably, the focus changed from addressing questions regarding one species known to inhabit a given environment, without isolating the target organism (although studies still apply DNA barcoding tools to provide evidences of population and conservation status of a target species; e.g. Bellemain *et al.,* 2016; Harper *et al.*, 2018b), to a broader surveillance of the diversity of species inhabiting an aquatic system, without the needs to isolate different taxa (Taberlet *et al.,* 2012;2018; Herder *et al.,* 2014; Valentini *et al.,* 2016; Bista *et al.,* 2017; Cristescu and Hebert, 2018).

The developments on eDNA metabarcoding followed advances in high-throughput sequencing platforms (i.e. NGS - next generation sequencing; see sub-section 2.4.2), which allowed rapid, secure and cost-effective processing of a significant number of samples at the same time, combined to increased storage capacity and developments on bioinformatics pipelines and statistical packages, reducing the processing time of computer platforms and enabling the analyses of gigabytes of data and thousands of sequences at the same time, to detect biodiversity (Taberlet *et al.,* 2012;2018; Herder *et al.,* 2014; Szitenber *et al.,* 2015; Thomsen and Willerslev, 2015; Boyer *et al.,* 2016). Thus, the last decade has seen an unprecedent increase in the number of published eDNA studies (Figure 2.6), applied to different environments, organisms and with a diversity of implemented protocols. Remarkably, eDNA is becoming a key component for species surveillance and conservation ecology, with the scientific community acknowledging the reliable information contained on DNA molecules obtained from environmental samples, providing answers to key questions on species diversity, ecological interactions and distribution dynamics (Taberlet *et al.,* 2018).

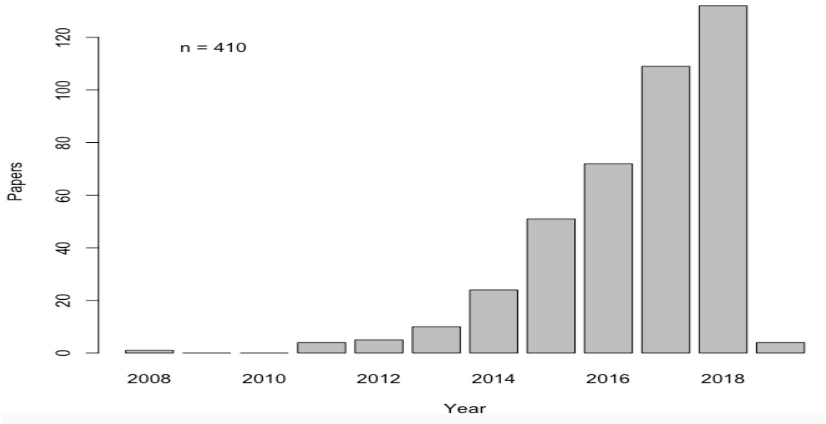

FIGURE 2.6 - COMPILATION OF EDNA STUDIES FOCUSING ON MACROBIAL EDNA FROM AQUATIC ENVIRONMENTS. THIS BIBLIOGRAPHIC ANALYSIS WAS COMPILED BY DR TAYLOR WILCOX (AN EXPERT ON EDNA STUDIES WITH OVER 600 CITATIONS) ON HIS BLOG: HTTPS://TAYLORWILCOX.WEEBLY.COM/ENVIRONMENTAL-DNA.HTML), FOLLOWING CONTRIBUTIONS FROM A NUMBER OF AUTHORS, LITERATURE SEARCH AND WAS LAST UPDATED IN DECEMBER 2018.

2.4.2 THE EDNA WORKFLOW AND IMPORTANCE OF APPLYING STANDARDIZED METHODOLOGIC APPROACHES

The implementation of any survey approach to assess the species diversity of a given system, first focus on the study aims. Questions regarding what species one wants to detect? Single-species or multi-species approach? Where to survey? How many times? What protocols, equipment and procedures to adopt? What are the benefits and challenges to be considered? Are some of the key questions to be addressed for successfully achieve the study aims (Taberlet *et al.,* 2012;2018; Herder *et al.,* 2014; Rees *et al.,* 2014; Hoffmann *et al.,* 2016; Cristescu and Hebert, 2018). Accordingly, and particularly if limited knowledge exists (e.g. based on traditional survey data on the species known or likely to be present in the system, distribution pattern and environmental dynamics) regarding the system to be sampled, pilot studies are advisable to tackle the uncertainties on the protocols and sampling strategy to adopt (Herder *et al.,* 2014; Taberlet *et al.,* 2018). Also, eDNA-based survey methodologies employ knowledge of different disciplines, from molecular biology to ecology and bioinformatic analyses that can be a constraint, if correct training is not acquired (Herder *et al.,* 2014; Hoffmann *et al.,* 2016; Cristescu and Hebert, 2018).

The DNA molecules found in aquatic systems are generally degraded and of short fragment size (see sub-section 2.4.3 for the factors affecting eDNA in aquatic environments). This constrains the choice of the capture and extraction protocols to be implemented (i.e. for maximising the quality and yield of short-fragmented DNA molecules recovered from water samples), the primers to be used for DNA amplification in PCR (Polymerase Chain Reaction) and the quality of the sequencing reads in sequencing platforms, which will further influence the species detected and robustness of the results (Taberlet *et al.,* 2012;2018; Rees *et al.,* 2014; Deiner *et al*., 2015;2017;2018; Cristescu and Hebert, 2018; Harper *et al.,* 2018b). Therefore, from study workflow design, exploratory trial, sampling, DNA capture, preservation, extraction, choice of genetic markers, library preparation, in silico and in vitro analyses, amplification and sequencing to bioinformatic and statistical analyses, all steps are crucial for the reliability and accuracy of the study. For this reason, each individual step needs to be careful considered during the eDNA workflow for the potential risks in detecting species that are not present in the sampled environment (i.e. false positives) and/or not detecting species known to effectively inhabit the target environment (i.e. false negative; Pilliod *et al.,* 2014; Ficetola *et al.,* 2015;2016; Deiner *et al.,* 2015;2017). The remarkable increase in eDNA studies have resulted in the implementation of different methodologies based on laboratory facilities, financial constraints, preference for a certain set of protocols by the researchers, among other reasons (Deiner *et al.,* 2015;2018), making it challenging to effectively define the best approaches to answer the study aims. Deiner *et al.,* (2015;2017;2018) followed by Cristescu and Hebert (2018) addressed the issues from a lack of standardised protocols (e.g. reproducibility of studies, presence of false positives/negatives, low species' read counts and contamination levels), stating the needs to implement standardised procedures. Despite of that, all eDNA studies rely on sampling the target environment to obtain the species DNA that will be further analysed. Importantly, before any sampling is undertaken, a number of crucial steps need to be taken into account (e.g. materials to be use and training on the best practices to adopt in the field and laboratory, to avoid potential sources of contamination), which would significantly influence the downstream results (Herder *et al.,* 2014; Taberlet *et al.,* 2012;2018).

I) SAMPLING, PRESERVATION AND DNA CAPTURE AND EXTRACTION

Different studies have applied distinctive sampling strategies in aquatic environments, regarding the volume of collected water, number of sites sampled, and number of replicates performed, which resulted in variations in species detection and downstream analyses. Crucially, all studies reported the importance of undertaking best practices while sampling to avoid contamination levels (e.g. use and exchange of gloves between sample collections, sterilization of all equipment and appropriate conditioning of the sampled bottles; see Chapter 6 for further information; Herder *et al.,* 2014; Deiner *et al.,* 2015;2017;2018). Deiner *et al.,* (2015), Spens *et al.,* (2016), Li *et al.,* (2018) and Sellers *et al.,* (2018) assessed the technical aspects of eDNA for the quantity and quality of the DNA obtained, by comparing the efficiency of different DNA capture (e.g. filtration, precipitation, filter papers used, filtered volume, among others), preservation and storage methods (e.g. use of buffers, chemical addiction, temperature and time) and extraction protocols (i.e. use of commercial and/or developed kits and modifications in protocols). Noteworthy, all studies described differences for the species detected and downstream inferences, based on the methods or combination of methods applied (e.g. pore size of the filter papers, freezing versus application of buffers and extraction method), stating that the sampled environment (e.g. lotic versus lentic, turbidity, etc.), fieldwork conditions (e.g. access to cold storage containers for samples to be preserved before DNA capture), access to laboratory and sampling time, all influence the results and should be careful considered when implementing an eDNA study.

II) GENETIC MARKERS, PRIMER DEVELOPMENT AND TESTING ANALYSES

The heterogeneity of life-forms inhabiting aquatic environments result in presence of DNA molecules from different organisms. Thus, it is necessary to effectively separate these DNA molecules to detect the aimed taxa in metabarcoding studies. Genetic markers or DNA metabarcodes (e.g. 12S, COI, cyt-b; Taberlet *et al.,* 2012;2018; Kelly *et al.,* 2014; Valentini *et al.,* 2016; Deiner *et al.,* 2015;2017;2018) have been developed and modified according to the taxa to be detected. Fundamentally, the choice of metabarcode rely on the taxonomic group, resolution level and expected level of DNA degradation. Consequently, for highly degraded DNA molecules found in aquatic environments, preference should be given to metabarcodes of short fragment size (<100-150 bp; Taberlet *et al.,* 2018). The DNA metabarcode displays a highly variable sequence, flanked by two conserved regions, where the central variable region is discriminative for all species of the target group (i.e. uniquely associated to a given species and not shared with others) and the two flanked regions are conserved across the target group, but different in non-target taxa. The conserved regions correspond to the place where designed primers (i.e. short DNA fragments used to initiate the DNA synthesis) will anneal assuring the unbiased amplification of DNA sequences (Taberlet *et al.,* 2018). Ficetola *et al.,* (2010), Kelly *et al.,* (2014), Myia *et al.,* (2015) and Elbrecht and Leese (2016) have developed and analysed the efficiency of different primers for the taxa detected, stating the importance of designing in silico and testing the primers to be used in eDNA studies for assuring the specificity on species detection.

III) PCR, NGS SEQUENCING, BIOINFORMATICS AND STATISTICAL ANALYSES

After DNA extraction it is necessary to produce high number of copies of the DNA molecules for downstream analyses. Different amplification methodologies have been applied for eDNA barcoding or eDNA metabarcoding studies, focusing on PCR approaches to amplify (i.e. copy) the DNA molecules (Figure 2.7). Kelly *et al.,* (2014), Deiner *et al.,* (2015;2017;2018), Evans *et al.,* (2016), Hänfling *et al.,* (2016) and Spens *et al.,* (2016), all reported different PCR conditions (i.e. for the number of cycles, temperature and duration of each stage), which significantly influenced the number of DNA copies obtained, sequencing yields and quality for the total read counts obtained in high-throughput NGS sequencing. Also, the efforts applied in developing custom reproducible bioinformatic pipelines, connected to progresses in computer systems and packages to store and analyse large amounts of data, highly improved the rapidity, accuracy and confidence in the eDNA results (Figure 2.7; Boyer *et al.,* 2016; Hänfling *et al.,* 2016). Yet, constrains in bioinformatic knowledge, particularly for computer programming, is a challenge for ecologists lacking on a molecular background, which may result in the non-application of eDNA for ecological assessment. Of note, available bioinformatic courses and tutorials, combined with companies providing sequencing platforms and bioinformatic analyses (e.g. Illumina and Macrogen), have helped non-molecular researchers to understand and apply eDNA in studies to assess species diversity and dynamics (Taberlet *et al.,* 2018).

2.4.3 THE FACTORS INFLUENCING EDNA DYNAMICS IN AQUATIC SYSTEMS AND CHALLENGES IN TROPICAL FRESHWATER ENVIRONMENTS

Environmental DNA has been regarded as a powerful molecular-based survey tool for biodiversity assessment and conservation ecology, outperforming established methodologies, by detecting species that are present in low number and, therefore, are usually not present in for example tagging survey methods. Moreover, the non-invasive nature of the tool, while being able to survey larger catchment areas without requiring much effort, provide evidences of the benefits of applying eDNA-based survey approaches to enhance the knowledge on species diversity, migratory behaviour and interactions with aquatic environments (Taberlet *et al.,* 2012;2018; Thomsen and Willerslev, 2015; Valentini *et al.,* 2016; Senapati *et al.,* 2018).

The previously reported technical challenges for the lack of standardized molecular protocols, combined with gaps in knowledge regarding the factors influencing eDNA release by different organisms, dispersion and interaction with aquatic environments for the persistence and degradability rates (Figure 2.7), all remain largely unassessed, particularly in tropical freshwater systems, where the highest diversity of fish species is found, while experiencing pronounced seasonal variations in the hydrologic regime and habitats conditions (Lucas and Baras, 2001; Val *et al.,* 2006), expected to significantly influence fish ecology (see the previous sections; Winemiller *et al.,* 2008). Thus, abiotic factors as UV-radiation, temperature, pH, turbidity and flow variations, all influence DNA transport, persistence and degradation. Bellemain *et al.,* (2016), Robson *et al.,* (2016) and Sales *et al.,* (2016) argued that these factors are likely to be highlighted in the tropics, since annual sunlight intensity, pronounced climate-driven seasonal flood regimes and variations in the sediment flux influence the aquatic conditions and fish species supported. Moreover, biotic factors as enzymatic and bacterial activity on breaking DNA molecules, combined with species life stage and levels of interaction with the environment, influence the DNA release by the diversity of organisms, the persistence and transport in tropical aquatic environments. Therefore, these studies argue the lower DNA persistence, compared to temperate freshwater environments, linked to the higher temperature, sunlight intensity and turbidity. Moreover, the pronounced variations in the flood regime are expected to significantly dilute and transport the already degraded DNA, making it challenging to effectively detect patterns of species distribution and diversity (Bellemain *et al.,* 2016).

Subsequently, the few assessments made in the tropics reported the needs to apply seasonal eDNA sampling procedures to capture the variations in the flood regime, while applying effective preservation and capture methods (e.g. use of buffers and enclosed capsule filters; Spens *et al.,* 2016; Li *et al.,* 2018), which would decrease the degradation of DNA (see Chapter 6 for further information). Moreover, sampling over sampling points of the catchment and combining the results with available survey data, are argued to promote the understanding of the challenges of eDNA in the tropics, while increasing the knowledge on patterns of species distribution (Bellemain *et al.,* 2016; Sales *et al.,* 2018).

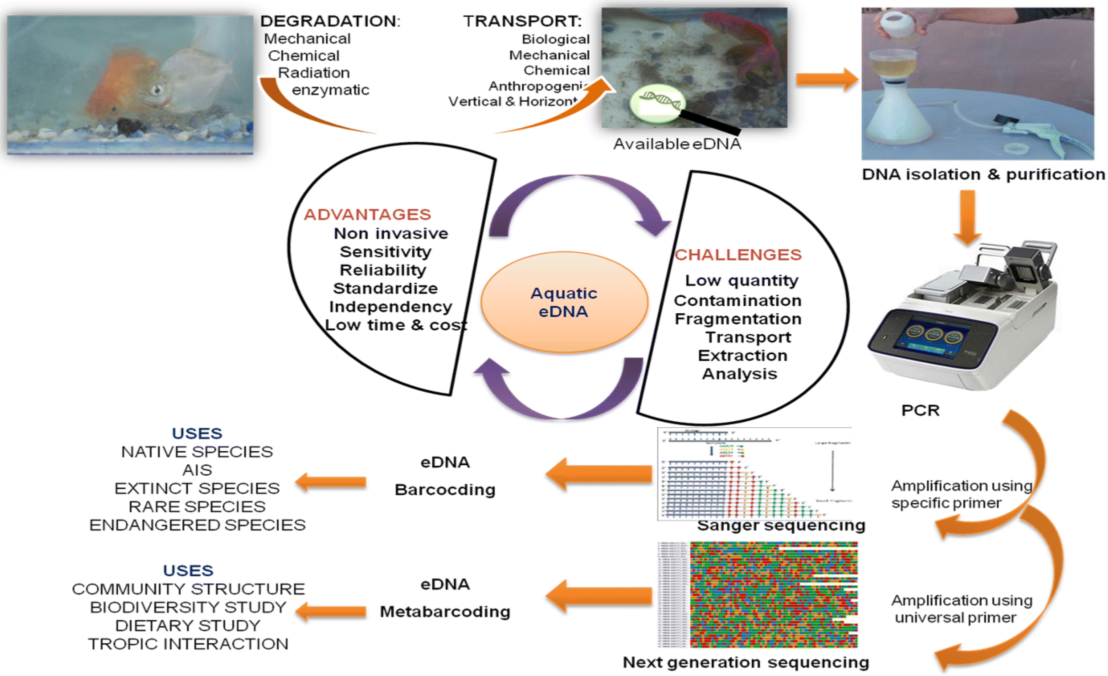

FIGURE 2.7 - OVERVIEW OF THE ADVANTAGES AND CHALLENGES IN AQUATIC EDNA, THE FACTORS AFFECTING IT (I.E. DEGRADATION AND TRANSPORT), TWO APPROACHES AND AIMS (I.E. EDNA BARCODING AND EDNA METABARCODING) AND THE MOLECULAR WORKFLOW (I.E. FROM SAMPLING TO NGS SEQUENCING; SOURCE: SENAPATI *ET AL.,* 2018).

In conclusion, eDNA-based molecular approaches offer a window of opportunity to tackle the level of threats by anthropogenic-driven disturbances on fish dynamics and abundance, by detecting the fish species inhabiting aquatic systems, the levels of adaptation and dispersion pattern, outperforming traditional surveys (Taberlet *et al.,* 2012;2018; Thomsen and Willerslev, 2015; Hänfling *et al.,* 2016; Handley *et al.,* 2018). However, the lack of a standardised methodology on the eDNA workflow, combined with important questions regarding eDNA dispersion, interactions with biotic and abiotic components of aquatic systems and their influence on the persistence and degradation, are expected to be highlighted in tropical freshwater systems (Dejean *et al.*, 2011; Barnes *et al.,* 2014; Ficetola *et al.,* 2015;2016; Strickler *et al.,* 2015; Bellemain *et al.,* 2016; Cristescu and Hebert, 2018; Taberlet *et al.,* 2018). Notwithstanding, it is unquestionable that eDNA is a promising and powerful tool to increase the knowledge on tropical fish diversity and assess levels of spatio-temporal dispersion, evidencing the applicability to establish effective conservation actions for preserving the habitats and hydrologic conditions that fish rely on, in light of the escalating threats to the natural system and fish diversity supported (Winemiller *et al.,* 2016).

Although studies have reported the outperformance of eDNA survey approaches, compared to traditional methodologies, it is also agreed that eDNA should not replace traditional surveys but complement them (Hänfling *et al.,* 2016; Shaw *et al.,* 2016). This is important since traditional fish surveys provide the baseline knowledge of the species inhabiting a system. However, and particularly in large tropical transboundary rivers (e.g. the Mekong), limitations in historical fish survey data are major constrains when comparing eDNA-based identified species and patterns of distribution, for the reduced number of species assessed and spatio-temporal surveillance (Poulsen *et al.,* 2002;2004; Baran, 2006).

Hence, in large tropical transboundary river basins, where pressures on fish resources are high, and where the long-term surveillance exhibits significant gaps in knowledge, eDNA offers a promising approach to answer fundamental questions as, how many fishes are there? What is the distribution pattern? What is the role of the seasonal flood regime and sediment flux in driving the adaptive strategies? What are the consequences of anthropogenic-driven disturbances on fish abundance and dynamics? What are the consequences for fisheries and food security? Thus, further implementation of eDNA-based survey studies in large tropical transboundary freshwater systems, assume a critical step for understanding the threats to fish resources and food security (see Chapter 6).

2.5 SUMMARY OF THE IDENTIFIED GAPS IN KNOWLEDGE

Tropical freshwater environments support the highest freshwater fish diversity (Abell *et al.,* 2008), exhibiting distinctive distribution dynamics, argued to be driven by the long-term predictability and seasonality of the monsoonal climate influence on the flood dynamics, sediment flux, and consequent habitat eco-hydrologic interactions, affecting fish abundance, fisheries and food security (Lucas and Baras, 2001; Baran, 2006; Winemiller *et al.,* 2008; Hortle, 2009).

Hydropower, irrigation and climate change scenarios are expected to threaten this historical flood regime, sediment flux and habitat eco-hydrologic dynamics, opening challenging questions regarding the impacts on fish adaptive strategies, fishery yields and food security (Arias *et al.,* 2012;2013;2014; Hoang *et al.,* 2016;2019; Hecht *et al.,* 2019). However, significant gaps in knowledge regarding tropical fish diversity, migratory behaviour and role of the historical flood regime and sediment flux in driving the levels of dispersion and interaction on the unique diversity of habitats, opened a window of opportunity to the implementation of eDNA-based survey approaches for biomonitoring and improving conservation actions on large tropical freshwater environments, under anthropogenic-driven environmental threat (Herder *et al*., 2014; Bellemain *et al.,* 2016; Deiner *et al.,* 2017; Handley *et al.,* 2018; Sales *et al.,* 2018; Taberlet *et al.,* 2018).

The following interlinked chapters will attempt to provide answers to the identified gaps in knowledge, focussing on the tropical transboundary Mekong river:

- How important are the long-term predictability and seasonality of the monsoonal climate-driven flood regime and sediment flux of the system in driving fish migratory behaviour, habitats and fishery yields? (Chapters 3)
- How important is the long-term Mekong river's flood regime-driven flood pulse on floodplain habitats flood dynamics, *dai* fishery and migratory species guild composition, focussing on the TSGL system? (Chapter 4)
- How future individual and combined hydropower, irrigation and climate change scenarios of the Mekong river will impact upon the historical flood pulse and floodplain habitats flood dynamics in the TSGL system? What are the consequences to the *dai* fishery, species guild composition of the catch and food security to the increasing populations? (Chapter 5)
- Can eDNA metabarcoding detect the Mekong's fish communities, while assessing patterns of seasonal distribution and habitats explored, evidencing the needs to establish environmental conservation actions, in light of the threats by the multiple drivers of environmental change scenarios in the basin on fish abundance and dynamics? (Chapter 6)

CHAPTER 3

Ecosystem services and fish diversity, abundance and migration under environmental change in the Mekong basin

Overview of the chapter: This chapter provides an overview of the hydrological and ecological functioning of the Mekong basin in the recent past, highlighting the significance of the basin to its inhabitants, which rely on agriculture and fisheries supported by the river. By assessing long-term spatio-temporal variations in water level, discharge, water quality and sediment flux, as drivers of the system's productivity, fish diversity, abundance, migratory dynamics and productive fisheries found in the Mekong, are explained. Furthermore, current and projected threats to the system in the form of hydropower, irrigation and climate change scenarios are detailed and explored.

3.1 THE MEKONG RIVER: A TRANSBOUNDARY LARGE RIVER BASIN

The Mekong river, the largest freshwater river in Southeast Asia and one of the World's last pristine rivers for its free-flowing waters for most of its length (i.e. without significant flow regulation from hydropower dams, reservoirs and weirs on the main steam; Adamson *et al.,* 2009; Keskinen *et al.,* 2012; Grill *et al.,* 2019), rises in the Tibetan Plateau (Figure 3.1) on an altitude of over 5200 meters above mean sea level (amsl), flowing through China, Myanmar, Thailand, Lao PDR, Cambodia and Vietnam, where it empties in the South China Sea (van Zalinge *et al.,* 2004; Campbell, 2009; Baran, 2010). Geographically, it can be distinguished in two zones (i.e. Upper and Lower Mekong Basin) with complex geomorphology affecting the flow dynamics and sediment flux (see section 3.2), lateral vegetated habitats and fish diversity (see section 3.4; Carling, 2009; Gupta, 2009; Baran, 2010; Lu *et al.,* 2014a). The Khone Falls in the border of Lao PDR with Cambodia, delimits key hydrodynamics of the river and the fish ecology (see section 3.4; Baran *et al.,* 2005; Valbo-Jorgensen *et al.,* 2009). The total length of the river is estimated as 4800 km, with the Upper and Lower Basins making up 24 and 76%, respectively, of the total area of the basin (MRC, 2005;2010). The Mekong exhibits a drainage area of 795,000 km^2, ranking 21st globally (Piman *et al.,* 2013) and 10th for its mean annual discharge (475 km^3; Thompson *et al.,* 2013). Darby *et al.,* (2016) provide a recent estimation on the annual sediment flux to the delta at 87 million tonnes year^{-1} in Kratie, which is key for landscape morphology and nutrient provision to the ecosystem services supported (see sub-section 3.2.2 and section 3.6) across the delta. The Mekong is ranked 2nd in the World for fish species diversity and richness (estimated as 1200 reported species; Rainboth, 1996; Coates, 2003; Hortle, 2009; Valbo-Jorgensen *et al.,* 2009).

FIGURE 3.1 - THE MEKONG RIVER BASIN, WITH THE SIX SHARING COUNTRIES AND DELIMITATION OF THE UPPER AND LOWER BASINS. THE UPSTREAM SECTION OF THE RIVER NAMED LANCANG RIVER IS ALSO PRESENTED. THE MAINSTREAM RIVER, LATERAL TRIBUTARIES AND THE TONLE SAP LAKE IN CAMBODIA ARE SHOWN, ALONG WITH THE HYDROLOGICAL AND WATER QUALITY MONITORED GAUGING STATIONS IN YELLOW CIRCLES (CHIANG SAEN, LUANG PRABANG, VIENTIANE, MUKDAHAN, PAKSE, STUNG TRENG, KRATIE, PREK KDAM, KAMPONG LUONG, PHNOM PENH PORT, CHAU DOC AND CAN THO). THE RED STAR SHOWS THE KHONE FALLS REGION (ADAPTED FROM: WWW.MEKONGRIVER.INFO/HISTORIC-FLOWS).

The Mekong river has attracted considerable scientific, political, economic and environmental interest over the last few decades (Hortle, 2007;2009; Baran, 2010;

MRC, 2003;2009b; Hirsch, 2010; Kondolf *et al.,* 2018). Historically, the river has supported a succession of human civilizations and empires, from the Khmer civilization centred in Angkor city (in the 1400s), in the margins of the Tonle Sap Great Lake system (what is nowadays known as Siem Reap, Cambodia; Higham, 2001; Coe, 2003), to the 19th century European French colonizers across Vietnam, Cambodia and parts of Thailand (starting in 1862; Hall, 1981; Wyatt, 1991). In fact, throughout the basin's history, people have been directly dependent on the river on an everyday basis, due to the ecosystem services provided for livelihoods (e.g. agriculture, fisheries and navigation options for trade markets; Baran *et al.,* 2007). The basin was also involved as a theatre for severely destructive wars (World War II, USA-Vietnamese War, and political regional conflicts between Thailand, Lao PDR, Cambodia and Vietnam for the delimitation of each countries' boundaries). The *Khmer Rouge* influence (1975-1979) in Cambodia, marked a dark time in the history of the country, where more than 2 million Cambodians were killed, and the country declined from having one of the highest levels of literacy in Southeast Asia, to being among the least, linked to the death or escape to other countries of the best-educated teachers and academics (Campbell, 2009). Consequently, all of these political and social instabilities in the region resulted in low economic development until nearly 1990s, when the first mainstream hydropower dam, Manwan, was built (Lu and Siew, 2006; Adamson *et al.,* 2009; Campbell, 2009; Hirsch, 2010).

After the building of the Manwan dam in 1993, hydropower development has boomed throughout the basin, mainly in the upstream basin and lateral tributaries (Lu and Siew, 2006; Fan *et al.,* 2015), and a high number of future development plans are in place (Keskinen *et al.,* 2012; Lauri *et al.,* 2012; Kondolf *et al.,* 2014;2018; Hecht *et al.,* 2019), to improve energy generation and respond to increasing populations and economic growth (Campbell, 2009). Studies have focussed on the problems of hydropower development in the region, stating the lack of full perception on the environmental, social and economic consequences that might result from the development of these dam infrastructures (see section 3.7; Xue *et al.,* 2011; Dore *et al.,* 2012; Lu *et al.,* 2014a; Schmitt *et al.,* 2017). This reflects the paucity of knowledge surrounding the importance and interactions of the key drivers that influence the Mekong basin's productivity; namely the role of the climate in the seasonal variations on the flood regime and sediment flux (Delgado *et al.,* 2012; Darby *et al.,* 2013;2016), the consequential effects on the available and connected habitats and nutrient flux (Kummu and Sarkkula, 2008; Kummu *et al.,* 2014; Whitehead *et al.,* 2019), the gaps in knowledge on the diversity, abundance and migratory dynamics of fish species (Poulsen *et al.,* 2002;2004; Baran, 2006), and the relation to the flood regime and availability of habitats (Lamberts, 2006;2008; Arias *et al.,* 2012;2013).

The Mekong River Commission (MRC), a joint organisation of the countries sharing the Mekong, provides scientific and advisory information towards the sustainable development of the basin (MRC, 2005;2010). The MRC has been providing important technical and scientific information regarding the Mekong's hydrology, fisheries, agriculture, water quality, among other components influencing the river's productivity (MRC, 2003;2005). However, the fact that the Mekong flows through six riparian countries, each with different views on how the river should be managed, and with different levels of economic power to develop it, has resulted in individual countries exploiting the river's resources without fully comprehending the complex multiple interactions involved (e.g. how changes in the flood regime and sediment flux impact lateral habitats flood dynamics and nutrient recycling and how it influence fish abundance, migratory dynamics, production, fisheries and fish protein provision to people?), which would result in the application of mitigation measures to reduce basinwide environmental impacts (MRC, 2009b; ICEM, 2010; Keskinen *et al.,* 2012). Therefore, it is vital to characterise the historical importance and complex interactions of the drivers that contribute to the Mekong's productivity (i.e. climate, hydrology, sediments, habitats and fish diversity). This will allow the assessment of long-term spatial and temporal variations in the drivers, and the linkage with the ecosystem services provided (e.g. agriculture and fisheries); which is vital for predicting how the system will be impacted by future water infrastructure development (e.g. hydropower and irrigation schemes), under a changing climate.

Hence, the significance of this large transboundary river should be acknowledged for high fish diversity (see section 3.4), fisheries and agriculture activities (see section 3.6), driven by seasonal variations in the flood regime and sediment flux, which are in turn influenced by the climate (see section 3.2). Moreover, variations in water quality that supports the population, fish diversity and economic activities, should be understood (see section 3.3), particularly in light of the high population numbers inhabiting the basin, depending on the river's resource for their sustainability (see section 3.5), and whose livelihoods are expected to be severely disrupted by environmental change projections (see section 3.7; Kummu and Varis, 2007; Ongley, 2009; Baran, 2010; Kummu *et al.,* 2010; Lauri *et al.,* 2012; Darby *et al.,* 2013;2016; Piman *et al.,* 2013;2016; Kondolf *et al.,* 2014;2018; Hecht *et al.,* 2019; Whitehead *et al.,* 2019).

3.2 THE CLIMATE: DRIVING FORCE FOR THE SPATIO-TEMPORAL VARIATIONS IN THE FLOOD REGIME AND SEDIMENT FLUX

The Mekong basin straddles two monsoonal systems, the South West Asian Monsoon and the East Asian Monsoon, which are commonly known together as the Asian Monsoon Climate system (AMCS; Holmes *et al.,* 2009; Delgado *et al.,* 2012). The AMCS is responsible for the predictable variations in rainfall, demarking a defined wet season (May-October; with a mean annual rainfall of 1,000 mm in the Upper Mekong and of 3,000 mm in the Lower Mekong) and a defined dry season (November-April; with a mean annual rainfall less than 1,500 mm for the entire basin; Lu and Siew, 2006; Holmes *et al.,* 2009).

The seasonality in the monsoon pattern and consequent variability in rainfall drive the observed annual variations in discharge values. Mean annual is ~15,000 m^3 s^{-1}, with the highest peak occurring in the wet season (i.e. in October) at approximately 45,000 m^3 s^{-1} (Lu and Siew, 2006), corresponding to more than 80% of the entire total annual discharge, and the lowest flow being observed in the dry season (estimated as 1,500 m^3 s^{-1} in March/April; Lu and Siew, 2006). The annual variation in the discharge pattern, driven by the monsoon climate, exhibits a single monotonal flow peak (i.e. only one annual peak; Lu and Siew, 2006; Kummu and Sarkkula, 2008; Lu *et al.,* 2014a; Kondolf *et al.,* 2014;2018), which is characteristic of tropical productive environments under the Flood Pulse Concept (see Chapter 2; Junk *et al.,* 1989; Junk and Wantzen, 2004).

Lu and Siew (2006) showed the vital contribution of the Upper Mekong Basin's flows, for keeping the Lower Mekong Basin's dry season flows in April (an estimated 75% of the dry season flows observed in Vientiane are provided by the flows originated from the Tibetan Plateau. In the wet season, the Upper Mekong Basin contributes to less than 50% of the observed flows in Vientiane). However, the authors argued that the operation of the Manwan dam might have caused the higher flows during the dry season and reduced flows in the wet season (see further analyses from Figure 3.2). Thus, the snowmelt from the elevated Tibetan Plateau in March/April, is crucial for sustaining the dry season flow and downstream water availability, influencing for example habitat connectivity and migratory corridors, that are key for the migratory fish guilds supported (see sub-section 3.4.2; Poulsen *et al.,* 2002;2004; Baran, 2006; Lu and Siew, 2006; Lu *et al.,* 2014a). In the Lower Mekong Basin, the AMCS' heavy rainfalls cause extensive inundation in the lower floodplains (e.g. nearly 18,000 km^2 of flooded floodplains in the TSGL system; see Chapter 4; Lamberts, 2008; Kummu *et al.,* 2014), with key eco-hydrologic interactions established (e.g. recycling of nutrients) that drive fish migratory patterns and life cycles (e.g. spawning behaviour), contributing to the outstanding fish diversity, abundance, production and inland fisheries (see sub-sections 3.4.2 and 3.6.2; Valbo-Jorgensen *et al.,* 2009; Baran, 2010; Halls *et al.,* 2013b; DHI, 2015). Moreover, later in the wet season, tropical cyclones and periodical ENSO events can influence the lower catchment by magnifying the rainfall in the region and causing higher flow peaks resulting in expanded inundation areas across the floodplain (Räsänen and Kummu, 2013; Darby *et al.,* 2016; Räsänen *et al.,* 2017; Pokhrel *et al.,* 2018).

Consequently, it is important to understand the long-term spatio-temporal flow dynamics and flood level variations throughout the basin for the ecological importance to the habitats and fish diversity supported (see sub-section 3.4.2), and to predict how hydrologic changes might bring negative consequences to the basin's productivity and sustainability (Lu and Siew, 2006; Piman *et al.,* 2013;2016). Importantly, these changes have been related to the current upstream cascade dams and tributaries' dams, and future projections under a changing climate and irrigation expansion (see section 3.7; Lauri *et al.,* 2012; Darby *et.*, 2013;2016; Piman *et al.,* 2013;2016; Hoang *et al.,* 2016;2019; Hecht *et al.,* 2019), with studies quantifying disruptions on the long-term predictability and seasonality of the flood regime of the Mekong and consequent decrease in sediment loads (Kondolf *et al.,* 2014;2018; Lu *et al.,* 2014b; Darby *et al.,* 2016) and nutrient fluxes (see sub-section 3.6.1; Whitehead *et al.,* 2019), and disruptions on the timing, duration and inundation magnitude of floodplain habitats (see section 3.7; Arias *et al.,* 2012;2013;2014; Pokhrel *et al.,* 2018). Yet, these assessments contrast with gaps in understanding and quantifying how those changes will impact upon fish abundance, migratory dynamics and triggers (Poulsen *et al.,* 2002;2004; Baran, 2006; Valbo-Jorgensen *et al.,* 2009), the ecosystem services supported (e.g. fisheries; see section 3.6; Baran, 2010; DHI, 2015) and food security to people (see section 3.5; Hortle 2007;2009; So, 2010;

DHI, 2015). Thus, assessing the current spatio-temporal hydrologic dynamics in the basin (see sub-section 3.2.1), particularly in light of the potential effect felt after the operation of the first upstream cascade dam (i.e. Manwan dam; Lu and Siew, 2006), helps to build a crucial understanding of the likely current and future consequences to the habitats and fish dynamics and abundance supported.

3.2.1 THE BASIN'S LONG-TERM SPATIO-TEMPORAL VARIATIONS IN THE FLOOD REGIME UNDER DAM DEVELOPMENT

Figure 3.2 shows a long-term (1960-2017) regression analysis assessing the influence of the pre and post-dam period (i.e. based on the 1993 operation start of the Manwan dam; Lu and Siew, 2006; Räsänen *et al.,* 2012;2017) to the mean annual water level **(A)** and discharge **(B)** variations, measured from upstream (Chiang Saen, Thailand) areas to the border of the Mekong delta (i.e. Phnom Penh Port). The data was collected from the historical daily measurements of the MRC[4] and the same analysis was applied to the mean annual maximum and minimum water level (Appendix 1; **A** and **B**, respectively) and mean annual maximum and minimum discharge (Appendix 2; **A** and **B**, respectively). From Figure 3.2 **(A)** it is possible to see mean annual water level increases in the post-dam period for all stations, with statistically significant differences in the mean annual water level of the pre and post-dam period detected for Chiang Saen (pre-dam: 2.83m; post-dam: 3.61m; Mann-Whitney U Test; *p*=0.000); Vientiane (pre-dam: 3.64m; post-dam: 4.12m; Mann-Whitney U Test; *p*=0.035); Pakse (pre-dam: 3.71m; post-dam: 4.49m; Mann-Whitney U Test; *p*=0.010); Stung Treng (pre-dam: 4.33m; post-dam: 4.68m; Mann-Whitney U Test; *p*=0.006) and Kratie (pre-dam: 10.50m; post-dam: 11.21m; Mann-Whitney U Test; *p*=0.001). Non-statistically significant differences were detected for Luang Prabang (pre-dam: 6.86m; post-dam: 7.33m; Mann-Whitney U Test; *p*=0.079); Mukdahan (pre-dam: 4.21m; post-dam: 4.71m; Mann-Whitney U Test; *p*=0.067); Prek Kdam (pre-dam: 4.02m; post-dam: 4.30m; Mann-Whitney U Test; *p*=0.116) and Phnom Penh Port (pre-dam: 4.09m; post-dam: 4.15m; Mann-Whitney U Test; *p*=0.654). Notably, Chiang Saen and Pakse showed the highest mean annual water level increase from the pre-

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[4] http://portal.mrcmekong.org/
Chiang Saen station ID: 010501; Luang Prabang station ID: 011201; Vientiane station ID: 011901; Mukdahan gauging station ID: 013402; Pakse gauging ID: 013901; Stung Treng gauging ID: 014501; Kratie gauging ID: 014901; Prek Kdam gauging ID: 020102 and Phnom Penh Port gauging ID: 020101.

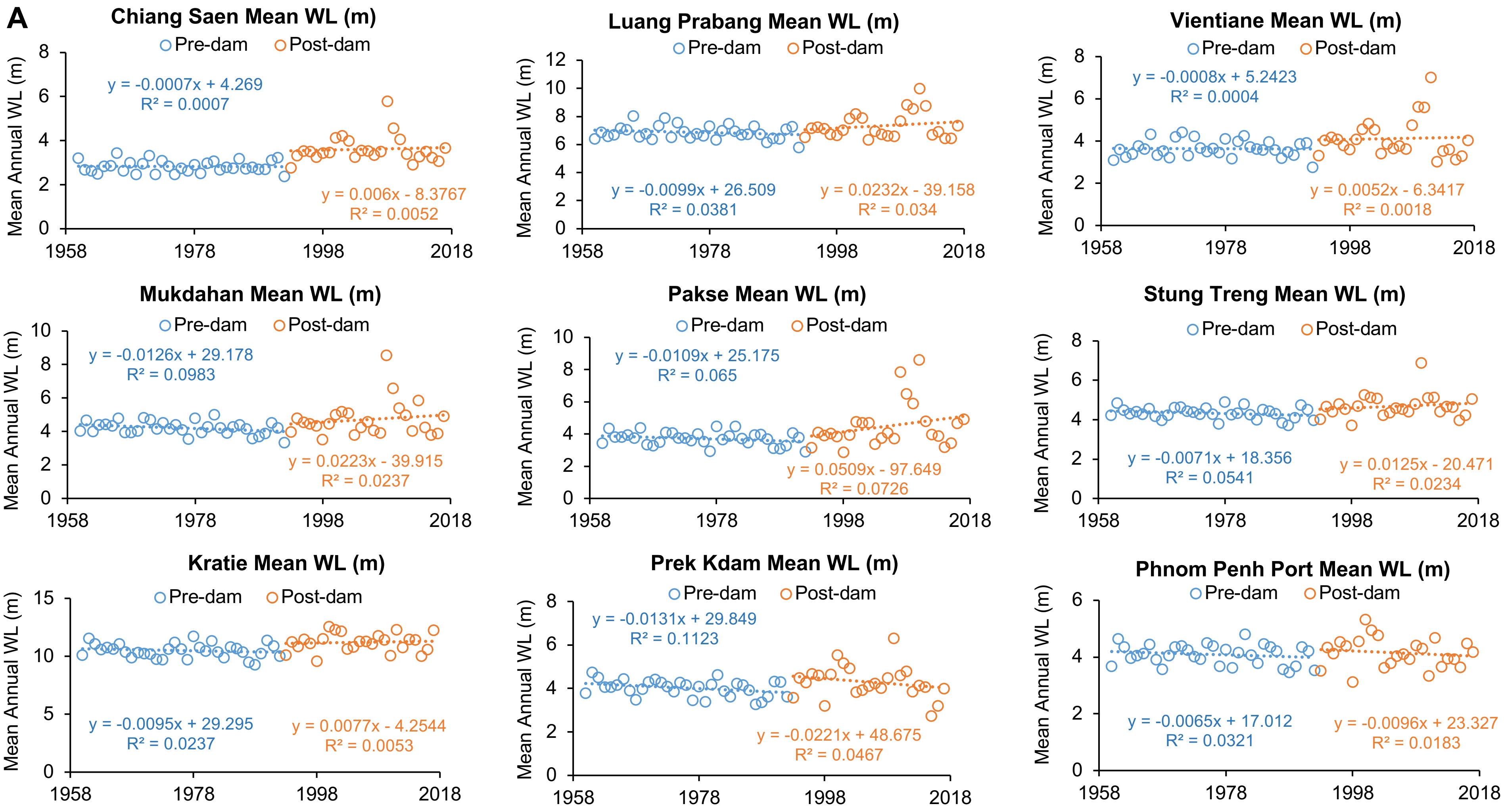
dam to the post-dam period (i.e. +0.78m for both stations), with Kratie showing the second highest increase (i.e. +0.71m) and Prek Kdam and Phnom Penh Port showing the lowest increase (+0.28m and +0.06m, respectively). Moreover, regarding the mean annual maximum water level variations for the pre and post-dam period (see Appendix 1; **A**), a 0.10m decrease was observed on the post-dam period in Prek Kdam (pre-dam: 8.80m; post-dam: 8.70m) and Phnom Penh Port (pre-dam: 8.86m; post-dam: 8.75m). Also, although statistically significant differences were not detected, Luang Prabang (pre-dam: 15.81m; post-dam: 15.49m; Mann-Whitney U Test; *p*=0.221) and Pakse (pre-dam: 11.58m; post-dam: 11.31m; Mann-Whitney U Test; *p*=0.362) had a 0.31m and 0.27m decrease in the mean annual maximum water level for the post-dam period, respectively. Regarding the mean annual minimum water level variations for the pre and post-dam period (see Appendix 1; **B**), statistically significant differences were detected for all stations, except for Luang Prabang (Mann-Whitney U Test; *p*=0.484). An averaged of all stations mean annual minimum water level increase of 0.43m (pre-dam: 1.63m; post-dam: 2.05m) was observed for the post-dam period. Also, Kratie (pre-dam: 5.32m; post-dam: 6.33m; Mann-Whitney U Test; *p*=0.000), Chiang Saen (pre-dam: 0.70m; post-dam: 1.49m; Mann-Whitney U Test; *p*=0.000) and Pakse (pre-dam: 0.59m; post-dam: 1.11m; Mann-Whitney U Test; *p*=0.000) had, respectively, 1.01m; 0.78m and 0.52m highest increase in the mean annual minimum water level, compared to the remaining stations, for the post-dam period. These results corroborate the findings by Lu and Siew (2006), although the authors reported their results regarding the seasonal (i.e. dry and wet season) operation of the Manwan dam (i.e. storage of water in the wet season and release in the dry season). Consequently, it is likely that the higher mean annual water level increase observed in Chiang Saen (the closest hydrologic station to the Manwan dam; Lu and Siew, 2006) reflect the higher dry season water level (i.e. near 2m for the dry season mean water level according to Lu and Siew, 2006), with the previously reported decrease in the mean annual maximum water level (see Appendix 1; **A**), reflecting the wet season storage of water. This pattern is further corroborated by the detected second highest increase in the minimum water levels (i.e. +0.78m; see Appendix 1; **B**), with the operation of the dam likely to rise the post-dam minimum flood level (i.e. dry season period) values observed in Chiang Saen. For the remaining stations although overall mean annual water level increases were detected for the post-dam period (see Figure 3.2), these were lower (i.e. <+0.40m) compared to Pakse (i.e. +0.78m) and Kratie (i.e. +0.71m). The same was observed in the two stations for the mean annual minimum water level (see earlier and Appendix 1; **B**), while a decrease in the mean annual maximum water level was observed, except for Kratie (+0.35m; pre-dam: 20.80m; post-dam: 21.15m; see Appendix 1; **A**). Notably, for Pakse it is likely that the seasonal operation of the Manwan dam, combined with the later developments of five more mainstream cascade dams and the lateral tributary dams, particularly in Thailand (over 20 reservoir and irrigation dams; MRC, 2009b; Räsänen *et al.,* 2012;2017; Lu *et al.,* 2014a), have implications on the seasonal provision of water, resulting in the observed variations in the mean annual water level and mean annual maximum (Appendix 1; **A**) and minimum (Appendix 1; **B**) water level for the post-dam period (Lu and Siew, 2006; Lu *et al.,* 2014a).

This has recently been quantified by Räsänen *et al.,* (2012), for the role of the six upstream dams (i.e. Gonguoqia, Xiaowan, Manwan, Dachaoshan, Nuozhadu and Jianghong, with an active storage of 23.19 km^3; Räsänen *et al.,* 2012) in providing waters (i.e. for the flow dynamics) to the downstream catchment, with the combined action of the upstream dams likely to result in a nearly 10% decrease in the flows reaching Pakse in September and in a nearly 60% increase in the flows reaching Pakse in April. Further downstream, for Kratie, it is likely that the combined 20-meter elevation drop in the Khone Falls region (with consequent widening of the channel and the downstream catchment being mainly characterised by shallow waters - maximum of 25 meters observed in Kratie; see Appendix 1; **A**), the higher monsoonal rainfall and periodic tropical cyclone and ENSO events, and the 20% flow contribution from the 3S system (i.e. Se San, Se Kong and Se Prok) as reported by Piman *et al.,* (2016), have higher influence in the increased mean annual water level (i.e. +0.71m; Figure 3.2), increased mean annual maximum water level (i.e. +0.35m; see Appendix 1; **A**) and the highest observed increased in the mean annual minimum water level (i.e. +1.01m; see Appendix 1; **B**). The same point was argued by Adamson *et al.,* (2009), saying that the over 1,000 km distance of the upstream dams to the location of this gauging station, connected to the natural elevation decrease in the Khone Falls region and the lateral flow inputs from the extensive network of tributaries, are likely to drive the flood level variations. Yet, Räsänen *et al.,* (2017) recently quantified the dry season flows reaching Kratie (i.e. 41-68%), with the upper cascade dam development, only explaining half of the dry season variation.

Nevertheless, how this apparent half influence on the dry season water flow influence the flood levels in the region, was not explored in the study.

The long-term assessment of the pre and post-dam influence on the mean annual discharge conditions (Figure 3.2; **B**) corroborates the findings from Lu and Siew (2006), particularly for the Chiang Saen station, the closest to the Manwan dam, where the smallest, non-statistically significant, increase in the mean annual discharge was observed (+8.95 $m^3 s^{-1}$; pre-dam: 2,696 $m^3 s^{-1}$; post-dam: 2,706 $m^3 s^{-1}$; Mann-Whitney U Test; *p*=0.735). On the other hand, although non-statistically significant differences were detected on the mean annual maximum discharge in this station, a 372 $m^3 s^{-1}$ increase was detected (pre-dam: 10,293 $m^3 s^{-1}$; post-dam: 10,665 $m^3 s^{-1}$; Mann-Whitney U Test; *p*=0.3010; see Appendix 2; **A**), while a statistically significant 118 $m^3 s^{-1}$ mean annual minimum discharge decrease was identified (pre-dam: 762 $m^3 s^{-1}$; post-dam: 644 $m^3 s^{-1}$; Mann-Whitney U Test; *p*=0.012; see Appendix 2; **B**). Lu and Siew (2006) argued the reservoir filling and further dam's operation for the releases of water in the dry season and storage during the wet season, as the reasons behind the lowest mean annual discharge increases from the pre-dam conditions and the observed variations in the mean annual maximum and minimum discharges. Importantly, further downstream, an increase in the mean annual discharge conditions is observed for all stations (from 43.09 $m^3 s^{-1}$ in Luang Prabang to 1,401 $m^3 s^{-1}$ in Stung Treng, compared to the pre-dam conditions – Luang Prabang: pre-dam: 3,935 $m^3 s^{-1}$; post-dam: 3,978 $m^3 s^{-1}$ and Stung Treng: pre-dam: 12,771 $m^3 s^{-1}$; post-dam: 14,174 $m^3 s^{-1}$), with statistically significant differences identified to Mukdahan (pre-dam: 7,534 $m^3 s^{-1}$; post-dam: 8,602 $m^3 s^{-1}$; Mann-Whitney U Test; *p*=0.009) and Stung Treng (Mann-Whitney U Test; *p*=0.015) gauging stations. Regarding the mean annual maximum discharge (see Appendix 2; **A**), statistically significant differences were only detected to Kratie, reflecting the highest decrease in the mean annual maximum discharge (-6,378 $m^3 s^{-1}$; pre-dam: 52,098 $m^3 s^{-1}$; post-dam: 45,720 $m^3 s^{-1}$; Mann-Whitney U Test; *p*=0.019), regarding the post-dam period. Also, for the mean annual minimum discharge (see Appendix 2; **B**), only Vientiane (Mann-Whitney U Test; *p*=0.594) and Pakse (Mann-Whitney U Test; *p*=0.197) showed non-statistically significant differences. These results contrast with reports from Lu and Siew (2006) that stated for all stations, that the mean annual maximum discharge increased from the pre-dam conditions, with significant differences only detected for Nakhom Phanom (a station not assessed in this long-term analysis), yet in the current analysis not only the statistical significance was only detected for Kratie, but also decreases in the mean annual maximum discharge were observed for Pakse (pre-dam: 37,836 $m^3 s^{-1}$; post-dam: 35,983 $m^3 s^{-1}$) and Kratie. The reasons behind these contrasting views are likely to be associated to temporal differences in the period of dataset analysis (i.e. Lu and Siew, 2006: 1960-2000) and the influence of the further five upstream cascade dams, connected to the lateral tributary dams. Consequently, the findings here reported follow the assessments made of the same dataset (i.e. from the MRC dataset portal) by later studies (Lu *et al.,* 2014a, although the authors did not assess the downstream effect of the cascade dams and Räsänen *et al.,* 2012;2017), which demonstrated the higher impact of the further five upstream cascade dams to the lower reaches of the catchment. Notably, Räsänen *et al.,* (2017) argued at Chiang Saen for the 2014 year, increases (121-187%) in the discharge measured in March-May and decreases (32-46%) in the discharge measured at July-August. Thus, it is likely that the previously reported increase in the mean annual maximum discharge (i.e. +372 $m^3 s^{-1}$; see Appendix 2; **A**) and decrease in the mean annual minimum discharge (i.e. -118 $m^3 s^{-1}$; see Appendix 2; **B**) reflect the seasonal operation of the combined dams. Further downstream in Stung Treng, the highest increase in the mean annual discharge (i.e. +1,402 $m^3 s^{-1}$; see Figure 3.2; **B**) for the post-dam period is likely to reflect the increase in flow velocity due to the elevation decrease in the Khone Falls region (since this station is the closest to the fall), but it is also likely to be associated to the 20% flow contribution from the 3S system, as earlier reported for the effect on water level (Piman *et al.,* 2016). Thus, Räsänen *et al.,* (2012;2017) have demonstrated that further downstream, particularly at Kratie, the current upstream dams have not significantly affected the historical flow conditions and water level (see earlier analysis) at the lower catchment areas. Piman *et al.,* (2013;2016), for the lateral tributaries, where historical dam development has evolved, have reported that reduced storage capacity of these dams have not significantly changed the historical flood regime in the mainstream river, nor the inundation dynamics in lateral habitats, which are key to fish abundance (see section 3.4) for the eco-hydrological interactions established.

These analyses show the relatively stable downstream flood regime. Yet future basin's development plans are likely to impact upon this pattern (see section 3.7; Baran and Myschowoda, 2009; Hortle, 2009; Baran, 2010; Hecht *et al.,* 2019).


A
Chiang Saen Mean WL (m)
Pre-dam
Post-dam
Mean Annual WL (m)
y = -0.0007x + 4.269
R² = 0.0007
y = 0.006x - 8.3767
R² = 0.0052
Luang Prabang Mean WL (m)
y = -0.0099x + 26.509
R² = 0.0381
y = 0.0232x - 39.158
R² = 0.034
Vientiane Mean WL (m)
y = -0.0008x + 5.2423
R² = 0.0004
y = 0.0052x - 6.3417
R² = 0.0018
Mukdahan Mean WL (m)
y = -0.0126x + 29.178
R² = 0.0983
y = 0.0223x - 39.915
R² = 0.0237
Pakse Mean WL (m)
y = -0.0109x + 25.175
R² = 0.065
y = 0.0509x - 97.649
R² = 0.0726
Stung Treng Mean WL (m)
y = -0.0071x + 18.356
R² = 0.0541
y = 0.0125x - 20.471
R² = 0.0234
Kratie Mean WL (m)
y = -0.0095x + 29.295
R² = 0.0237
y = 0.0077x - 4.2544
R² = 0.0053
Prek Kdam Mean WL (m)
y = -0.0131x + 29.849
R² = 0.1123
y = -0.0221x + 48.675
R² = 0.0467
Phnom Penh Port Mean WL (m)
y = -0.0065x + 17.012
R² = 0.0321
y = -0.0096x + 23.327
R² = 0.0183
1958
1978
1998
2018
0
2
4
5
6
8
10
12
15

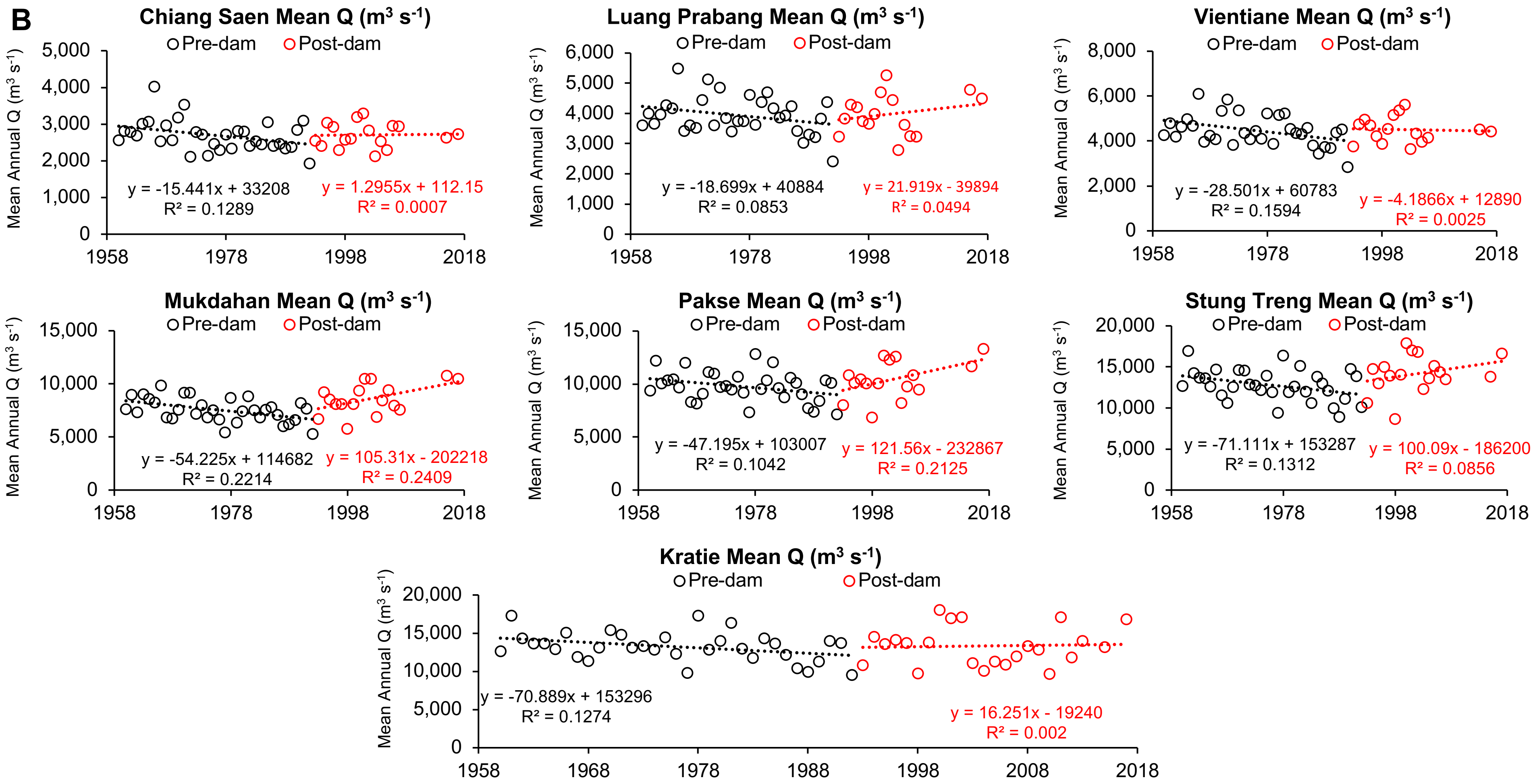


FIGURE 3.2 - LONG-TERM (1960-2017) REGRESSION ANALYSIS (R^2 VALUES PRESENTED WITH THE STATISTICAL SIGNIFICANCES REPORTED AT 95% CONFIDENCE LEVEL) OF THE MEAN ANNUAL (A) WATER LEVEL (M) AND (B) DISCHARGE (M^3 S^{-1}) VARIATIONS, FOR THE PRE AND POST-DAM DEVELOPMENT (I.E. BASED ON THE 1993 MANWAN DAM OPERATION START), ALONG THE MEKONG BASIN'S GAUGING STATIONS. NOTE THAT THE DISCHARGE RECORDS FROM THE MRC ONLY HAVE AVAILABLE DATASETS UNTIL KRATIE.

3.2.2 THE SEDIMENT FLUX DYNAMICS ACROSS THE BASIN

The sediment loads of the Mekong basin have been much debated due to the lack of long-term records from sediment gauging stations and identified bias for the methodology applied in different gauging stations (Walling, 2005;2008;2009; Lu *et al.,* 2014b). This has made it difficult to effectively assess long-term trends in the sediments transported by the flow (e.g. assess a potential decrease in the spatio-temporal upstream-downstream sediment flux and the causes; Lu and Siew, 2006; Kummu *et al.,* 2010; Kondolf *et al.,* 2014;2018; Darby *et al.,* 2016), the accuracy of the quantifications made for the sediment volumes reaching downstream areas (e.g. in the delta) and the sediments trapped not only in tributary reservoirs, but also in upper basin dam cascade (Lu and Siew, 2006; Kondolf *et al.,* 2014;2018; Fan *et al.,* 2015), and the sediments stored in floodplains (Kummu and Sarkkula, 2008; Dang *et al.,* 2018). Yet, it is important to characterise the spatio-temporal sediment dynamics across the basin, for the role in maintaining landscape morphology, by providing sediments that replace that lost by erosion (particularly in the Mekong delta for the tidal influence of the South China Sea; Kondolf *et al.,* 2018). Additionally, sediments bring nutrients that boost the productivity of soils for agriculture (e.g. nitrates and phosphates; see sub-section 3.6.1; Whitehead *et al.,* 2019) and the primary and secondary production, with critical increase in food sources (see Chapter 2; Davies *et al.,* 2008), influencing fish production and migratory dynamics (see sub-section 3.4.2; Baran *et al.,* 2015), which are then reflected in the annual productive fisheries (see sub-section 3.6.2; Poulsen *et al.,* 2002;2004; Baran, 2010; DHI, 2015).

The origin of sediment loads is due to the combined action of the Tibetan ice melting, hillslope denudation, and river bank erosion, with the resulting sediments being carried by the flows (Milliman and Meade, 1983; Milliman and Syvitski, 1992; Gupta and Liew, 2007; Walling, 2008;2009; Carling, 2009). The range of values reported in the literature for the mean annual sediment flux measured along the basin or at different locations, reflect the application of different methods to measure the sediment concentration (e.g. Acoustic Doppler Current Profiler – ADCP vs point and depth integrated sampling; Darby *et al.,* 2016), differences in sampling locations, and different temporal scales used, while contemplating the seasonal variability in sediment transport linked to the climatic influence on the discharge pattern (see sub-section 3.2.1; Kondolf *et al.,* 2018).

Early attempts (i.e. in the 1950s) to characterise the mean annual sediment flux reaching the South China Sea, were based on a one-year sampling point measurement (i.e. in Mukdahan; see Figure 3.1), reporting that nearly 160 million tonnes of sediments reached the South China Sea, with this value being acknowledged by several studies (Milliman and Meade, 1983; Lu and Siew, 2006; Carling, 2009; Walling, 2009; Kondolf *et al.,* 2018). On the other hand, recent estimations have pointed out that this historically acceptable value is likely to be an over-estimation from the less reliable available datasets at the time (Darby *et al.,* 2016). Thus, Manh *et al.,* (2014) reported 106 million tonnes per year of sediments reaching Kratie for the 2010-2011 years, based on recalculations of the historical sediment loads (i.e. from the 160 million tonnes). The authors also stated that a third of the proposed load was deposited in the floodplains downstream from Cambodia, with the remaining two thirds reaching the delta. Upstream of the confluence of the Mekong river with the Tonle Sap River, Lu *et al.,* (2014b) corroborated these views by calculating a sediment load of 50-91 million tonnes per year at Chroy Changvar gauging station, from field measurements made covering the 2008-2010 period. The authors estimated a 50-54 million tonnes of sediments reaching the delta, assessing the importance of the complex flow dynamics and fluxes of sediments transported in (i.e. mean 6.3 million tonnes for the 2008-2010 period) and out (i.e. mean 8.5 million tonnes for the 2008-2010 period) of the TSGL system to this estimation. Notably, although annually the sediment load from the TSGL system contributed less than 15% of the total flux into the delta, its seasonal share is highlighted, if compared with the monthly sediment load from the Mekong river, reaching 58-88% of the total sediment load to the delta during December-April. Also, the authors argued that 6-10% of the total sediment loads are stored in floodplains from Cambodia to the delta, which are likely to influence fish production and fisheries for the nutrient recycling and boost in primary and secondary productivity, providing food sources (see Chapter 2; Davies *et al.,* 2008). Yet, the lack of long-term assessment of the nutrients' importance to fish diversity, constrain further analyses of the role on fish production, fisheries and fish protein provision to people (see section 3.3 and sub-section 3.6.1; Baran *et al.,* 2015; DHI, 2015).

Darby *et al.,* (2016) recently assessed the role of tropical cyclones in the basin in influencing the sediment load's transportation along the basin and through the delta, by variations of the run-off pattern, acknowledging the importance of assessing those hydrogeomorphologic dynamics for the crucial role on land replacement and sustainability of the ecosystem services supported (e.g. agriculture) and to people. Darby *et al.,* (2016) reported a mean annual sediment load at Kratie (the station closer to the border of the Mekong delta region) of 87.4±28.7 million tonnes, which is lower than the estimations from Manh *et al.,* (2014) and nearly 55% lower than the historical annual sediment load of 160 million tonnes. Notably, the authors stated that the suspended sediment loads reaching the delta have declined by 52.6±10.2 megatonnes over 1981-2005, with 33.0±7.1 megatonnes linked to shifts on tropical cyclone events (i.e. timing and frequency), and the remaining linked to upstream and tributary dam development and sand mining. Likewise, future projections on hydropower, irrigation and climate change are expected to magnify this declining trend (see section 3.7), with likely, yet unassessed, consequences to fish production, migratory dynamics, fishery yields and to the highest protein consumption in the region (see section 3.5), for the losses in nutrient fluxes (see sub-section 3.6.1 for the estimates by Whitehead *et al.,* 2019), which urge to be tackled (Hortle, 2007;2009; Baran, 2010; Kondolf *et al.,* 2014;2018; DHI, 2015, Darby *et al.,* 2016; Whitehead *et al.,* 2019).

3.3 THE WATER QUALITY OF THE MEKONG BASIN

The water quality of the basin is key to human health (e.g. drinking water), agriculture (e.g. the salinity intrusions from the delta and variations in pH and nutrients, influence soils' productivity for agriculture; Kondolf *et al.,* 2014;2018; Whitehead *et al.,* 2019), fisheries and fish migratory behaviour (e.g. differences in small and large body-size species' tolerance to variations in dissolved oxygen, temperature and turbidity; Poulsen *et al.,* 2002;2004; Baran, 2006).

The MRC Water Quality Database[5] is the only long-term (1985-2011) available dataset for the entire Mekong basin (Ongley, 2006;2009; MRC, 2007; Kongmeng and Larsen, 2014). The monitoring programme started in 1985 for Thailand, Lao PDR and Vietnam, with Cambodia joining the programme in 1993. The programme consisted in monthly sampling and measurements of temperature (^{0}C), dissolved oxygen (DO; mg l^{-1}), conductivity (mSm^{-1}), pH, total suspended solids (TSS; mg l^{-1}), sodium (mg l^{-1}), potassium (mg l^{-1}), calcium (mg l^{-1}),

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[5] http://portal.mrcmekong.org/search/search?q=water+quality&format=&max=5&offset=0&sort=title&order=ASC&h=Dataset

magnesium (mg l^{-1}), chloride (mg l^{-1}), sulphate (mg l^{-1}), alkalinity (mg l^{-1}), nitrates (mg l^{-1}), ammonia (mg l^{-1}), aluminium (mg l^{-1}), silica (mg l^{-1}), total phosphorous (mg l^{-1}), iron (mg l^{-1}), chlorophyll-a (added in 2007; mg l^{-1}) and faecal coliforms (added in 2007; mg l^{-1}). Yet, limitations are observed in the data, with gaps in the monthly measurements across stations and years. Also, the fact that sampling is made only once a month and constrains in transportation and preservation (i.e. samples are preserved in ice coolers and transported to the laboratories of each country national agencies. However, obtaining ice during fieldwork and sampling at remote long-distance locations limit the preservation of samples; Ongley, 2009) influence the reliability of the results. Nevertheless, the MRC Water Quality Database is the only one available to the Mekong and should be acknowledged more as an indicator of the general status of water quality, than reflecting the exact conditions (MRC, 2007; Ongley, 2009; Kongmeng and Larsen, 2014).

3.3.1 THE BASIN'S LONG-TERM SPATIO-TEMPORAL VARIATIONS IN WATER QUALITY AND ROLE ON FISH DIVERSITY AND ECOSYSTEM SERVICES SUPPORTED

Figure 3.3 presents the long-term analysis (1985-2011) from the MRC Water Quality Database for the parameters that influence fishes and the ecosystem services supported (i.e. fisheries and agriculture). The results are shown for the mainstream (except for Kampong Luong - TSGL system) monitored stations from Chiang Saen (Thailand), Luang Prabang (Lao PDR), Vientiane (Lao PDR), Pakse (Lao PDR), Stung Treng (Cambodia), Kratie (Cambodia), Phnom Penh Port (Cambodia) and Can Tho (Vietnam), present in Figure 3.1.

The mean annual temperature for the entire basin is 27 ºC (with 31 ºC and 20 ºC maximum and minimum temperature variations, respectively). From Figure 3.3, it is possible to see that between stations mean annual temperature variations are observed, potentially reflecting the Upper and Lower Mekong climatic conditions (see section 3.2). Thus, from Chiang Saen until Stung Treng (see Figure 3.1), the mean annual temperature increased from 23 ºC to 27 ºC, respectively, while in Cambodia the assessed stations showed a mean annual temperature of 30 ºC, with a 2 ºC decrease (i.e. 28 ºC) for the Vietnamese station, potentially reflecting tidal influence from the Mekong delta (Ongley, 2006;2009). These affect fish distribution and production (see sub-section 3.4.2), with the lower basin supporting higher fish abundance, for the optimal temperature conditions for physiologic functions (e.g. spawning; Valbo-Jorgensen *et al.,* 2009). For an average temperature of 27 ^{0}C, this corresponds to a DO of approximately 7 mg l^{-1} (Ongley, 2009). This is corroborated in Figure 3.3, with a 7 mg l^{-1} mean annual DO quantified for the entire basin (11-5 mg l^{-1} maximum and minimum DO variations). Also, between stations low (i.e. from 8 mg l^{-1} in Chiang Saen to 7 mg l^{-1} in Can Tho) mean annual DO variations were observed. Yet, for both Kampong Luong and Pnhom Penh Port below the TSGL system, the lowest mean annual DO levels were observed (average of 6 mg l^{-1}). According to Ongley (2006;2009), the fact that these stations joined the monitoring program later and an increase in urban waste loads, with increased concentrations in total phosphates and nitrates for the same stations (see below) from the densely populated areas (see Table 3.3), are likely to be associated to the quantified lower DO for the stations. Notably, the Mekong fish species are highly tolerant to low DO with Poulsen *et al.,* (2002;2004), Baran, (2006) and Valbo-Jorgensen *et al.,* (2009) stating that small body-size fish species (e.g. cyprinids of the *Henycorhynchus* sp.) are seen in the TSGL system, particularly in floodplains in the dry season, when DO levels can be below 4 mg l^{-1} (Sarkkula *et al.,* 2003). On the other hand, large body-size species (e.g. *Labeo chrysophekadion*; see Chapter 4 for the body-size) require DO levels higher than 5 mg l^{-1}, with a reduction in this value triggering the migratory behaviour (Poulsen *et al.,* 2002;2004; Baran, 2006; Valbo-Jorgensen *et al.,* 2009). Consequently, it is likely that seasonal variations in the DO levels in certain sections of the basin, affect differently small and large body-size species, with species lacking on migratory behaviour (blackfish; see Table 3.1) exhibiting key adaptations to the lower DO conditions in the dry season (see sub-section 3.4.2), displaying the diversity of life and adaptive strategies of the Mekong fishes (Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009; Halls, 2010). Likewise, the productive inland fisheries of the basin, relying heavily on migratory fishes (see sub-section 3.6.2; Baran, 2010) are likely to benefit from the seasonal DO variations along the basin, for the influence on different fish species migratory behaviour (Poulsen *et al.,* 2002;2004; Baran, 2006; Valbo-Jorgensen *et al.,* 2009). The pH of the Mekong basin showed a decreasing pattern from upstream to downstream stations (Figure 3.3), where the highest mean annual pH is observed in Chiang Saen (i.e. 7.80) and the minimum is observed in Kampong Luong (i.e. 6.97), followed by a marginal increase to 7.25 observed in Can Tho, potentially linked to salinity intrusions from the delta. Thus, it is likely that salinity intrusions and nutrient content (i.e. phosphorous, nitrates and ammonia) resulted in variations in the pH levels, with expected consequences to the productivity of soils for agriculture and fisheries (FAO, 1985; Schmitt *et al.,* 2017; Kondolf *et al.,* 2018). Yet, for the Mekong no threshold values exist regarding the acceptable pH conditions to sustain fishes, fisheries and agriculture (Ongley, 2006;2009).

The nutrient analysis in Figure 3.3 corroborates the findings from Whitehead *et al.,* (2019) for the importance that both total phosphorous and nitrates (ammonia was not assessed in the study) place to soil productivity for agriculture practices, particularly in the Mekong delta. From Figure 3.3 it is possible to see that both total phosphorous and nitrates are highest in the downstream stations Kampong Luong (i.e. mean annual total phosphorous of 0.11 mg l^{-1} and mean annual nitrates of 0.23 mg l^{-1}) and Can Tho (i.e. mean annual total phosphorous of 0.15 mg l^{-1} and mean annual nitrates of 0.31 mg l^{-1}). According to Whitehead *et al.,* (2019) the higher agriculture activity, particularly in the Mekong delta (where the Can Tho station is the closest station) and the densely populations, directly releasing their effluents to the river, result in this higher nutrient content. Importantly, the authors argued that future climate change projections and socioeconomic developments in the region will severely impact upon the flow dynamics and nutrient fluxes (see sub-section 3.6.1 for the detailed findings), with expected negative consequences to the land for agriculture (with increase of tidal influence likely to threatens the available land for agriculture; Kondolf *et al.,* 2018) and to fisheries. Yet, although it is known that nutrients are key to fish for improving food sources (see sub-section 3.2.2; Davies *et al.,* 2008; Baran *et al.,* 2015), gaps in knowledge exist on the quantified assessment of the nutrients' importance to different fish species and the influence on fisheries. Moreover, no research exists on the levels of ammonia that are toxic to the Mekong fish species. Additionally, Whitehead *et al.,* (2019) argued that increased nutrients in the water would result in lower DO levels, with expected consequences to fisheries. Yet, and as earlier mentioned, the Mekong fish species are highly tolerant to low DO levels. Consequently, not only is vital to characterise the nutrient flux dynamics in the Mekong river for the role on agriculture productivity, but also the role on fish abundance and dynamics should be pursued, for the expected influence on different fish species production, fisheries and food security to people.

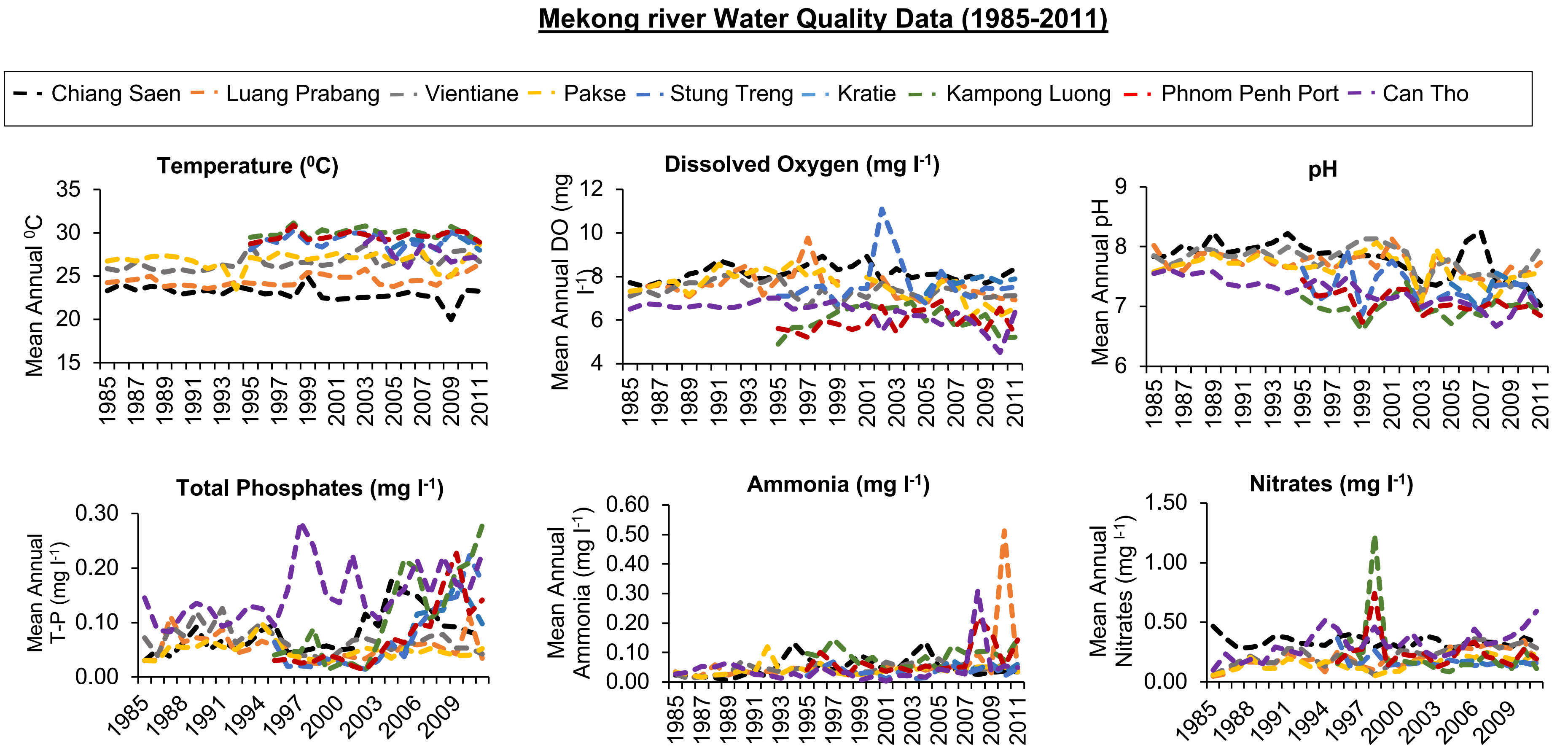

FIGURE 3.3 - MRC WATER QUALITY DATA FOR THE DIFFERENT GAUGING STATION IN THE MEKONG BASIN (FIGURE 3.1). THE VALUES ARE PRESENTED AS MEAN ANNUAL VARIATIONS FOR THE PERIOD 1985-2011. BLACK: CHIANG SAEN (THAILAND), ORANGE: LUANG PRABANG (LAO PDR), GREY: VIENTIANE (LAO PDR, YELLOW: PAKSE (LAO PDR), DARK BLUE: STUNG TRENG (CAMBODIA), LIGHT BLUE: KRATIE (CAMBODIA), GREEN: KAMPONG LUONG (TSGL; CAMBODIA), RED: PHNOM PENH PORT (CAMBODIA) AND PURPLE: CAN THO (VIETNAM). NOTE THE Y-AXIS CHANGES IN EACH PANEL.

3.4 THE MEKONG FISH DIVERSITY AND MIGRATORY BEHAVIOUR

3.4.1 FISH DIVERSITY

The Mekong river supports the second highest fish species richness in the World, behind only the Amazon basin (Figure 3.4). It is estimated that the total number of fish species is close to 1200 species (Poulsen *et al.,* 2002;2004; Coates, 2003; Baran *et al.,* 2007). In Cambodia alone, it is thought that nearly 500 species occur (Rainboth, 1996). The Mekong Fish Database (MFD), developed by the MRC (MRC, 2003), provides a comprehensive data source on the Mekong fish diversity, although it does not cover species from upstream areas of Yunnan Province (China) and Myanmar. The MFD reports 924 species, with 815 being under the status of “confirmed” and 45 species under the status of “expected” (Baran, 2010). Hortle (2009) mentioned that there are about 850 freshwater fish species recorded, with the number possibly increasing to 1100 species if coastal or marine species are included. The global reference database for the worldwide fish species, FishBase, only reported 781 species for the Mekong (Figure 3.4) in 2009 (with recent increase to 801 species in 2017, Fishbase website). Although the number is lower than previous predictions, it is supported by scientific studies, providing a rigorous assessment so far (Baran, 2010). Nevertheless, with the complexity of the Mekong basin flood regime and diversity of habitats, it is possible that many more species remain to be identified, as argued for other tropical basins (see Chapter 2). The WWF (2009) highlighted that in the last decade more than 279 new species of fish were discovered in the basin.

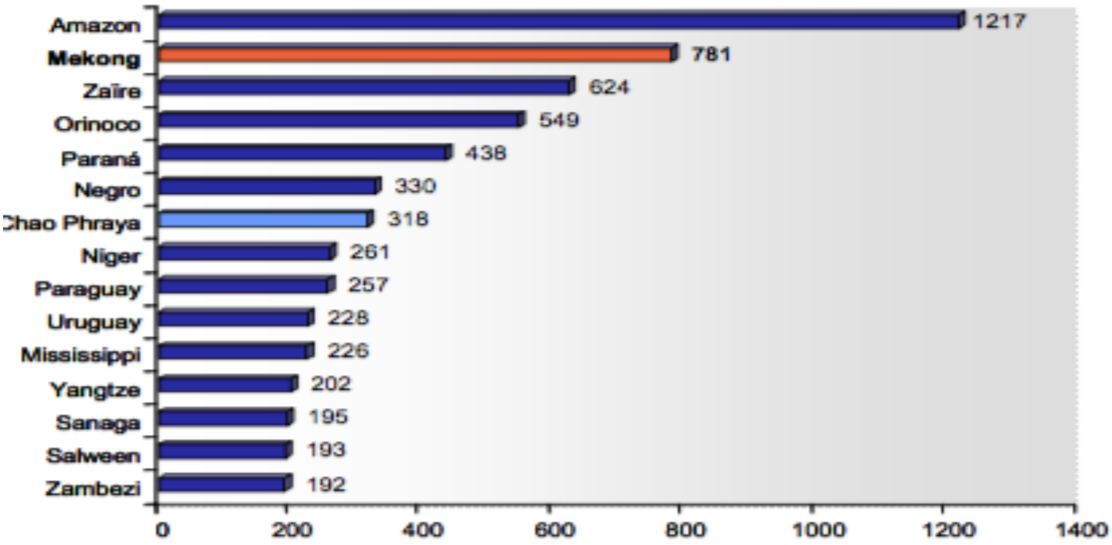

FIGURE 3.4 - FISH SPECIES RICHNESS FOR DIFFERENT RIVERS OF THE WORLD (SOURCE: FISHBASE, 2009).

3.4.2 THE MIGRATORY BEHAVIOUR AND HABITATS EXPLORED: IMPORTANCE OF THE SEASONAL FLOOD REGIME AND SEDIMENT FLUX

More than 50% of the Mekong fish species are known to perform migrations along the basin (Baran *et al.,* 2001;2007; Poulsen *et al.,* 2002;2004; Valbo-Jorgensen *et al.,* 2009). Poulsen *et al.,* (2002;2004), supported by Baran (2006) and Valbo-Jorgensen *et al.,* (2009), provide an overall comprehensive review of the migratory patterns, by first grouping different fish species into migratory guilds (white, black and greyfishes; Table 3.1). These migratory guilds (for detailed information see Chapter 4) connect the ability of the three groups of fishes in performing seasonal medium to long distance migrations along the basin (i.e. grey and whitefishes, respectively) or local movements (i.e. black and some greyfishes), with the different habitats explored for spawning, feeding and shelter purposes, recognising the fundamental role of the flood regime and sediment flux in driving the life cycle of fishes and habitats' available (Figure 3.5).

TABLE 3.1 - FEATURES OF THE THREE MIGRATORY FISH GUILDS (ADAPTED FROM: BARAN, 2010).

	Whitefishes	Blackfishes	Greyfishes
Migrations	Long distance longitudinal and lateral migrations	Local movements	Medium longitudinal and lateral migrations
Body form	Round or fusiform	Vertically compressed; no scales	Laterally compressed, spiny, usually with strong scales
Colour	Silvery or light	Very dark, often black	Rather dark, usually ornamented and coloured
Reproduction guild	Non-guarders; open substrate spawners	Guarders; build complex nests	Guarders; nest builders or open substrate spawners
Examples	Pangasiidae family	Channiidae family	*Mystus albolineatus*

This review, although providing important information regarding the migratory life history of more than 50 species inhabiting the Mekong, leaves more than 700 species without a full assessment. Nevertheless, the findings of the study provide an important description of the role of the system's flood regime and carrying sediments containing vital nutrients, in driving the life cycle of a number of fish species, while describing the seasonal habitats explored. Thus, for **blackfish** that live in floodplains, swamps and other lateral habitats, the increase in the flood level in the onset of the wet season (May-June), represents the spawning time, with the larvae being protected by adults to avoid being moved by the flow.

Moreover, the increase in the flood level promotes food source availability (e.g. phytoplankton) through the recycling of nutrients from the aquatic-terrestrial environment and the incoming sediments carried by the flow, which are vital for larvae and juveniles to grow and adults to build fat layers. Additionally, both adults, larvae and juveniles remain in the inundated floodplains for feeding and sheltering purpose during the wet season. In the dry season (December-April), both adults, larvae and juveniles remain in floodplain ponds, exhibiting adaptive characteristics (e.g. burying in the mud) to survive the period of low water levels, low DO and high turbidity (Baran, 2006; Valbo-Jorgensen *et al.,* 2009). Poulsen *et al.,* (2002;2004), stated that **white** and **greyfishes** do not stay year-round in the mainstream river, but also seasonally occupy floodplains, rice-fields, lakes, reservoirs, estuary and deep pools. In the onset of the wet season (Figure 3.5), when the waters of the mainstream river start to rise, white and greyfishes concentrated in the mainstream river perform upstream/downstream migrations (longitudinal pattern) and lateral migrations (lateral pattern) to recently inundated spawning grounds (e.g. floodplains), with the Mekong's complex geomorphology delimiting distinctive fish populations and fish production (Figure 3.6). The larvae are then washed downstream with the flow, reaching lowland flooded floodplains, rice-fields and canals, to feed and grow, associated with the boost in food sources from nutrient recycling of the recently inundated floodplains and income of nutrients bounded to sediments carried by the flow. Adults also migrate to flooded floodplains to feed and build fat layers for the dry season, where significant reduction in the food availability prevails. In the onset of the dry season, when the waters start to decrease (Figure 3.5), white and greyfishes leave the floodplains and migrate to the tributaries (greyfish species) or mainstream river (white and greyfishes), where deep pools (see below) are key habitats for species that perform medium to long distance migrations (Valbo-Jorgensen *et al.,* 2009; Halls *et al.,* 2013c). Consequently, for the three groups of fishes, floodplains are highly appreciated for food, nursery and shelter grounds (Figure 3.5).

Viravong *et al.,* (2004) and Chan *et al.,* (2005) define a deep pool as a deeper canyon, fissure or crack in the river bed (up to 79 meters depth), which in the dry season may become disconnected from the mainstream river. Moreover, these vital dry season habitats are mainly concentrated in southearn Lao PDR and northeastern Cambodia, being identified 97 pools in Cambodia and 70 in Lao PDR. The ecological role of deep pools has been recognised for supporting the only available mainstream habitats during the dry season, which large body-size fish species rely on for their survival (e.g. Pangasiidae family; Halls, 2010). Moreover, deep pools concentrate IUCN Red List threatened species like the Mekong giant catfish (*Pangasianodon gigas*), Irrawaddy dolphins (*Orcaella brevirostris*) and Jullien's golden carp (*Probarbus jullieni*). Notably, the future sustainability of deep pools has been under intense debate by studies, for the possible disruption in the water availability, sediment deposition and consequent decrease in the inhabiting species, due to environmental change scenarios expected to affect the basin (Poulsen and Valbo-Jorgensen, 2001). The general assumption that dams and climate change will increase dry season flood levels and reduce wet season floods, connected with unpredictable pattern of the timing of the flood, threatens the migratory behaviour of fishes to the dry season pools and the required connectivity of the migratory corridors (e.g. tributaries to the mainstream river) to reach these habitats (Baran and Ratner, 2007; Baird, 2011).

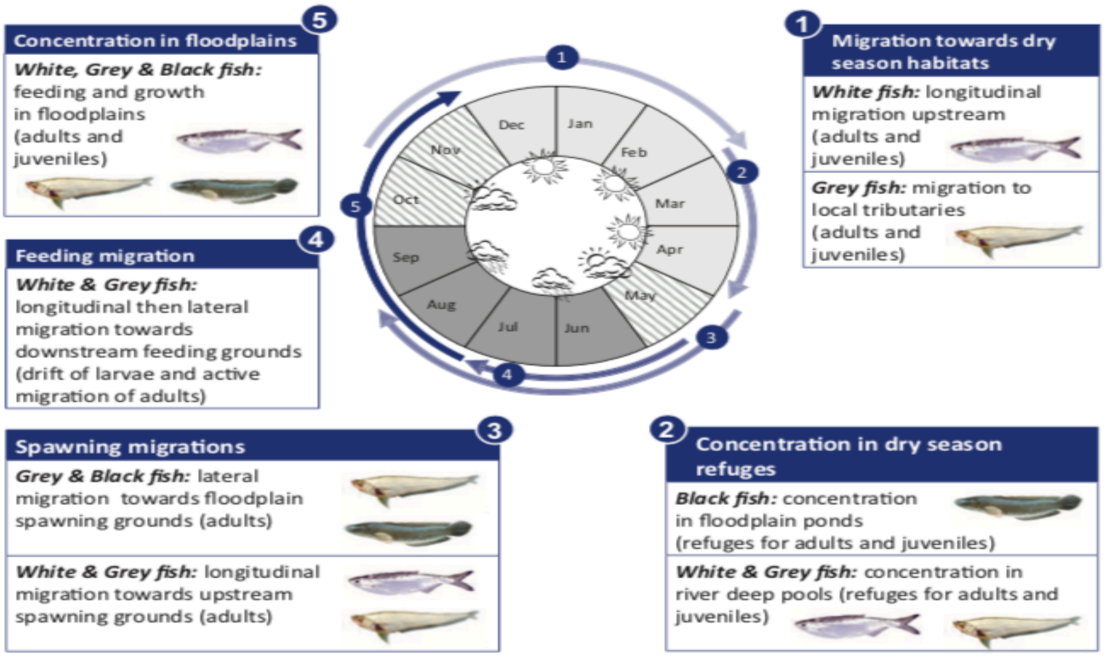

FIGURE 3.5 - INFLUENCE OF THE SEASONAL FLOOD REGIME OF THE MEKONG RIVER IN THE MIGRATION PATTERN OF BLACK, WHITE AND GREYFISH, AND THE HABITATS EXPLORED (SOURCE: BARAN AND UN, 2012).

The diversity of habitats along the basin and role of the seasonal flood dynamics and sediment flux in providing the connectivity, availability and inundation pattern of habitats explored by different groups of fishes and food sources, connected to the complex geomorphology of the river, delimits three distinctive migratory system, resulting in differences on fish populations and production (Figure 3.6; Poulsen *et al.,* 2002;2004; Baran, 2010).

The **Upper Migration system** is characterised by high elevation (5000 meters amsl), narrow channels and rocky substrate. Therefore, the fish production (number of fishes per km^2) is the lowest observed for the entire basin, marked by mainly 57 endemic species (Baran, 2010), due to the few habitats available (mainly in the mainstream river). Also, and linking with the analyses made in sub-section 3.2.1, it is likely that the upstream cascade dams contribute to the lower species number and production, for the impacts upon the seasonal flood regime and consequent habitats' flood dynamics affecting species' spawning grounds and life cycles (Valbo-Jorgensen *et al.,* 2009).

The **Middle Migration system** is marked by the widening of the Mekong mainstream channel, where a complex network of more than 30 tributaries sustain floodplains, swamps and other lateral habitats, facilitating the dispersion of fishes and resulting in an increase in fish production. Yet, this system is delimited upstream by an elevation of nearly 5000 meters and a downstream fall of more than 20 meters (Khone Falls region), making it difficult for species to disperse through the upper and lower migration systems. Also, the observed changing conditions on the long-term flood regime seasonal dynamics driven by upstream and tributary dams (see sub-section 3.2.1), is likely to cause important variations in the habitats' flood dynamics and availability to fish. Therefore, this system is mainly characterised by endemic fish populations argued to be genetically different than the same species populations found in the lower system (112 endemic species; Baran, 2010; Halls, 2010).

The **Lower Migration system** exhibits the highest fish production of the basin. This system is characterised by an extensive lowland area of productive floodplains, rice paddies and the delta, which linked to the relatively stable seasonal hydrodynamics of the basin (see the analyses in sub-section 3.2.1), facilitates habitat connectivity and dispersion of species. This system harbours the highest fish species in the basin (669 species; Baran, 2010), combining fresh, brackish and marine species that inhabit year-round or seasonally enter the delta (Valbo-Jorgensen *et al.,* 2009). Furthermore, the Lower system does not possess falls and the tributaries connected to the mainstream river, although supporting reservoir dams with low storage capacity (see sub-section 3.2.1), maintain year-round the flood levels that keep the migratory corridors for fish to disperse from as south as the delta to as north as the deep pools in Cambodia, below the 20 meters waterfalls separating Cambodia from Lao PDR (Valbo-Jorgensen *et al.,* 2009; Baran, 2010). Yet, the fact that this area is marked by shallow waters, as already mentioned, means that the minimum flood level that sustain the migratory corridors should be kept, particularly in the dry season (Kummu and Sarkkula, 2008), to allow fish to disperse. Thus, future modifications in the flood magnitude, driven by hydropower development, irrigation schemes and climate change scenarios in the basin, will threaten the sustainability and fish production of the Lower Mekong system and the productive ecosystem services provided, that human populations rely on (see section 3.7; Hoang *et al.,* 2016;2019; Pokhrel *et al.,* 2018; Hecht *et al.,* 2019).

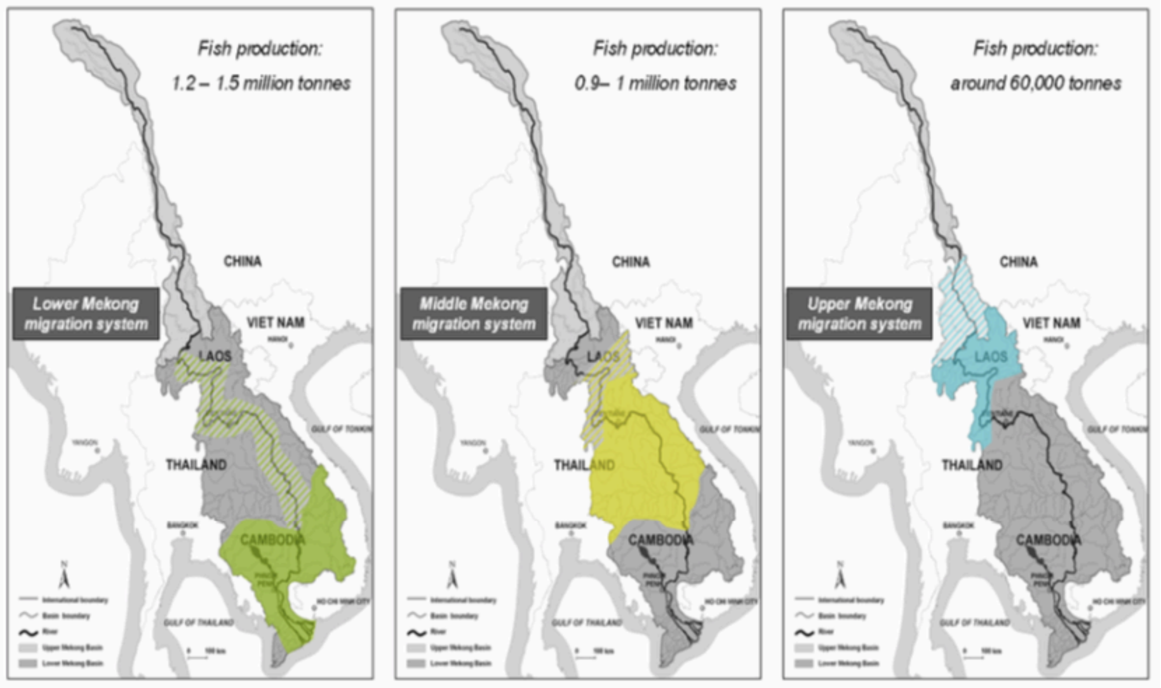

FIGURE 3.6 - THE THREE MIGRATION SYSTEMS OF THE MEKONG RIVER AND FISH PRODUCTION (SOURCE: BARAN, 2010).

Although efforts have been made to increase the knowledge on the migratory behaviour of fishes inhabiting the Mekong river, and the link to the systems' flood regime and sediment flux in driving those migrations, for only 24% (165 species) the migratory behaviour was assessed, according to a later study from Baran (2006), compared to the assessments made by Poulsen *et al.,* (2002;2004). This means that for the remaining 76% (over 600 species), no knowledge exists on the migratory behaviour and habitats explored, and it is likely that the suitable habitats for these species have already been modified by water abstraction, conversion of floodplains for rice paddies and impoundment by dams, with unknown consequences to these fishes (Baran *et al.,* 2007; Baran, 2010; Kondolf *et al.,* 2018). Moreover, the fact that high uncertainty surrounds the exact number of species inhabiting the river, makes it challenging to effectively understand how current and future environmental change in the basin have already impacted or will impact these "unknown species" (Valbo-Jorgensen *et al.,* 2009).

Baran (2006) in his study mentioned that of the species whose migration status are known (165 species), 135 species (87%) are migrants (performing long, medium or short distance longitudinal and lateral migrations) and 30 (13%) are non-migrant species (blackfishes). Also, the author effectively quantified the role of the seasonal flood regime and sediment flux in triggering the migration for only 30 species (18% of the 165 species; Table 3.2). The findings revealed that water level and discharge triggered the migration of 90% of the species assessed, with 26 species responding to increases/decreases in water level and discharge. Moreover, the timing of the first rains, which consequently affects the variations in the hydrodynamics and changes in turbidity, reflecting the sediments carried by the flow, affected in 30% the migratory behaviour with 9 species responding to these changes for both triggers. Importantly, the influence of the hydrologic regime and sediments carried in triggering the migratory behaviour of the species assessed, was related to the significant effect on lateral floodplain flood dynamics in providing key spawning, feeding and sheltering grounds that these species seek, as previously mentioned. Lastly, only 3 species showed connection with the appearance of insects. The Pangasiidae family was found to be the most sensitive to environmental triggers with 11 of the 19 species of this family (58%) responding to one or more environmental triggers. Furthermore, the Siluridae and Cyprinidae families are the second and third most sensitive species to migration triggers, with 3 and 9 species responding to environmental clues, respectively.

TABLE 3.2 - MIGRATORY TRIGGERS FOR 30 OF THE 165 SPECIES WITH KNOWN MIGRATORY STATUS AND % RESPONSE TO THE CLUES (ADAPTED FROM BARAN, 2006).

Clues	Number of fish species responding	% from the 30 species
Water level and discharge	26	90
First rains	9	30
Changes in turbidity	9	30
Appearance of insects	3	10

The detailed analysis in this section showed that high uncertainty surrounds the number of fish species inhabiting the basin and migratory behaviour, the habitats explored and the role of the river's seasonal flood regime and sediment flux in triggering the migrations. Notably, even though studies report that over 50% of the Mekong fish diversity exhibit migratory behaviour (Rainboth, 1996; Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009), this has only been described for 165 species, with the role of the seasonal hydrodynamics and sediment flux being quantified to 30 species (Baran, 2006). Thus, these gaps in knowledge set the target for the importance of applying studies and effective methodologies (see Chapters 4-6) aiming to characterise the diversity of species inhabiting the basin, the migratory behaviour and habitats explored, and role of the seasonal flood regime and sediment flux in triggering the migratory dynamics. These are crucial issues in light of the current and future basin water infrastructure development and climate change projections, which are expected to severely impact upon the long-term predictability and seasonality of the flood regime and sediment dynamics, with consequences to the habitats eco-hydrologic interactions (i.e. flood dynamics and food sources established; see section 3.7), migratory pattern, by disruptions on the migratory triggers and barrier effect by dams, ultimately influencing fish abundance, production and the ecosystem services provided (e.g. fisheries; see section 3.6) that people rely heavily for livelihoods and food security (see section 3.5; Baran, 2010; Hortle, 2007;2009; DHI, 2015; Hecht *et al.,* 2019).

3.5 THE MEKONG BASIN POPULATION

The Mekong basin hosts over 70 million people distributed along the six riparian countries (MRC, 2005;2010; DHI, 2015; Kondolf *et al.,* 2018). Table 3.3 shows that 60 million people live along the four Lower Mekong Basin countries, while over 10 million people live in the Upper Basin (see Figure 3.1; MRC, 2005;2010).

TABLE 3.3 - ESTIMATED TOTAL POPULATION NUMBER (MILLION INHABITANTS) LIVING IN THE FOUR LOWER MEKONG BASIN COUNTRIES (SOURCE: MRC, 2010).

Countries	Population number (million inhabitants)
Thailand	23
Lao PDR	6
Cambodia	13
Vietnam	16 in the Delta and 2 in the Central Highlands

Although urbanization is occurring in all Lower Mekong countries, an estimated 85% of the total basin’s population still live in rural areas. This trend is mainly linked with activities related to the river, with over 60% being dependent on agriculture and fisheries (see section 3.6; Baran *et al.,* 2007). The gender participation in the fisheries and agriculture activities is disproportional, with men usually being responsible for the fishery and agriculture activities, and women and children often more associated with fish processing, market, gear-making and fish growth in integrated agriculture practices (e.g. rice fields; Hatha *et al.,* 1995; So *et al.,* 1998). Food security and malnutrition pose great challenges across the basin. For this reason, fishes represent an easy and major source of protein intake (DHI, 2015). Table 3.4 summarises the estimated annual per capita consumption of fish protein from the four Lower Mekong countries.

TABLE 3.4 - ESTIMATED ANNUAL CONSUMPTION OF FISH PROTEIN FROM THE FOUR LOWER MEKONG COUNTRIES (ADAPTED FROM: HORTLE, 2007;2009).

Countries	Annual averaged fish protein consumption (kg capita^{-1} year^{-1})
Thailand	46.9
Lao PDR	43
Cambodia	52.4
Vietnam	49.5

The recent FAO (2018) report places the remarkable importance of the Mekong fish to people, both for consumption and protein source, stating that in 2016 the global average fish consumption was 20.2 kg capita^{-1} year^{-1}, providing 20% of protein. Yet, in the Mekong basin this scenario is more than double, with an averaged nearly 48 kg capita^{-1} year^{-1} of fish consumption for the four countries (Table 3.4), providing 47-80% of protein consumed (Hortle, 2007;2009; ICEM, 2010; DHI, 2015). Moreover, in certain regions (e.g. the TSGL system) the importance of fish to sustain the populations is highlighted by an annual fish consumption of 70 kg capita^{-1} year^{-1}, with migratory fish species comprising over 50% and the consumption being the highest for the entire Mekong basin and SE Asia (see Chapter 4; Hortle, 2007;2009; So, 2010). The fish importance to people is associated to the provision of high levels of essential minerals (i.e. calcium, iron and zinc) and vitamins, particularly vitamin A, which are essential to human health (Hansen *et al.,* 1998; Kawarazuka and Béné, 2011). Also, different types of vitamin B and trace minerals (e.g. selenium, which is important for proper immune function; Rayman, 2000), can be found in fishes and are beneficial for human health. Small body-size fishes possess higher mineral content than large body-size fishes, making them more appreciated, while large species are sold for economic income (Roos, 2003). This is observed along the Mekong, particularly in the TSGL system, where over 50% of the fish captured comes from small body-size species (e.g. *Henycorhynchus* sp.; see Chapter 4; So, 2010; Halls *et al.,* 2013a). The importance of small body-size fish species should, therefore, be appreciated for the provision of important minerals and vitamins, particularly in children for the prevention of diseases (e.g. rickets). Moreover, small cyprinid species (one of the most eaten across the basin) contribute to improved bone health and neuromuscular function, due to the provision of vitamin D, which is essential for the correct absorption of calcium by the human body (Pettifor, 2004). Hence, the preservation of the annual Mekong fish production should be pursued for the key role on food security to people, living under low economic income (i.e. one-third of the basin's population live with less than US$ 2 per day; Baran *et al.,* 2007; MRC, 2010) and malnourishment. Yet, this can only be achieved by improving the knowledge on the drivers influencing fish abundance and dynamics (Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009; Halls, 2010; DHI, 2015).

3.6 THE BASIN'S ECONOMIC ACTIVITIES: AGRICULTURE AND FISHERIES

3.6.1 AGRICULTURE

Agriculture has been pursued in the basin for more than a thousand years. This was predominantly for subsistence, although some level of trade market and exportation to other regions of the basin occurred, favoured by the easy access to the river for transportation of goods (Baran *et al.,* 2007). Nowadays, agriculture is an established activity throughout the basin, with particular incidence in the delta for the land area available and easy access to water (Kondolf *et al.,* 2018). Also, an estimated 60% of the total basin's population engage in full or part-time agriculture practices, with 80% of the agriculture production directly used for subsistence, while the remaining being for export markets (mostly done by Thailand and Vietnam), and generating a revenue value of nearly US$ 262 million annually, mostly by rice crop (estimated production of 16 million tonnes annually in the Mekong delta; Baran *et al.,* 2007; CDRI, 2008; MRC, 2010). Notably, rice farming integrated with aquaculture practices for rearing shrimps and pangasiid fish species are a well-established technology, particularly in the Mekong delta, highlighting the key role of crops and aquatic resources for food security and livelihoods to people (see sub-section 3.6.2 for the production yield; DHI, 2015).

The monsoonal climate plays an important role on agriculture, for the seasonal land area available in the basin (i.e. 6.6 million hectares in 2010; Hoanh *et al.,* 2010), directly dependent on the flow of water and sediments for irrigation purpose and soil productivity. Consequently, dams for irrigation purpose have boomed for sustaining the flood levels, particularly in the dry season, where the estimated 1.2 million hectares of available irrigated area in 2010 for the Lower Mekong Basin is projected to increase to 1.8 million hectares until 2030, driven by population growth (i.e. 100-145 million people by 2050; MRC, 2009b;2010; Hoanh *et al.,* 2010; Varis *et al.,* 2012).

Kondolf *et al.,* (2014;2018) and Whitehead *et al.,* (2019) applied numerical modelling approaches to project the expected changes in the basin's flow regime, sediment flux and nutrient dynamics, driven by the basin's future dams, irrigation schemes and climate change scenarios, with a particularly focus on the consequences to the agriculture productivity in the Mekong delta. Likewise, both studies highlight expected variations in the seasonal flow conditions measured along the basin (i.e. wet season decrease, and dry season increase), 96% decrease in the sediment flux reaching the delta and 8.3% and 0.7% decrease in nitrogen and phosphorous concentrations, respectively, linked to flow dilution, however a projected flux increase by 5% of these nutrients is expected, primarily driven by increase in agriculture activities and population growth.

These changes were argued to impact upon soil productivity and agriculture land, with Whitehead *et al.,* (2019) arguing that the likely 1 month earlier monsoonal flow peak, would cause land flooding, impacting upon the crops. Notably, Whitehead *et al.,* (2019) states that the increase in agriculture activities under population growth scenario will result in higher nitrates and phosphates content in the water, potentially resulting in eutrophication issues, likely to affect negatively the fisheries by the reduction in dissolved oxygen and people's livelihoods. Although this is certainly true to large body-size species, small body-size species (e.g. small carps of the *Henycorhynchus* sp.) that contribute over 50% to the species composition found in inland fisheries (particularly in the TSGL system; see Chapter 4), are highly adaptable to low DO concentrations (see sub-section 3.3.1; Poulsen *et al*., 2002;2004; Baran, 2006;2010; Halls, 2010). Consequently, for the Mekong fishes it is likely that increase in land change for agriculture practices, will result in loss of suitable spawning, feeding and sheltering grounds, which connected to disruptions on the flood regime affecting both the migratory triggers and habitat flood dynamics (see sub-section 3.4.2) and a decrease in nutrient concentrations in other areas (e.g. in the TSGL system), driven by the multiple drivers of environmental change, will impact the most the abundance and production of medium to long distance migratory species, with fisheries and fish protein loss to people (Baran and Myschowoda, 2009; Halls, 2010; DHI, 2015). Yet, changes in suitable habitats (either by agriculture activities and/or flood regime) and the impacts of flow, sediments and nutrient flux variations in fish abundance and migratory dynamics are largely unassessed in the Mekong, which urge to be tackled for the consequences to fisheries and fish protein to people, highlighting the environmental threats of the Mekong river's water development plans, under changing climate (see section 3.7; DHI, 2015; Hoang *et al.,* 2016;2019; Whitehead *et al.,* 2019).

3.6.2 THE FISHERIES IN THE BASIN: IMPORTANCE, TRENDS AND LIMITATIONS

The inland fisheries along the basin is another important practice for food security and economic income in the Mekong basin (Baran and Mychowoda, 2009; Baran, 2010; Ziv *et al.,* 2012; DHI, 2015). The high fish diversity found along the basin, plays an important role on the annual fisheries yield reported in official fisheries statistics (i.e. from the MRC Fisheries Monitoring Programme; MRC, 2003; Halls *et al.,* 2013b; DHI, 2015), with 47-80% of the catch comprising migratory fish species (DHI, 2015). Furthermore, the seasonal migratory behaviour of the majority of the Mekong species (see sub-section 3.4.2), connected to the variations in the flood regime and sediment flux (see section 3.2), contribute to different timing in operation of the fisheries throughout the basin (Baran *et al.,* 2007; Baran, 2010; Ziv *et al.,* 2012; Halls *et al.,* 2013b; DHI, 2015). Thus, the profitable fisheries (including other aquatic animals mainly shrimps, snakes, frogs and snails, contributing one-fifth to the annual catch) translate to an estimated selling value of US$ 1-1.80 kg^{-1}, a reported annual catch of 2.8-3.2 million tonnes and a total revenue value of US$ 3.7-7 billion (Hortle, 2007;2009; MRC, 2010; DHI, 2015). Yet, throughout the basin important differences exist regarding the annual yields reported by the different fishery statistics, constraining the overall assessment of the value and importance of the Mekong inland fisheries (Baran, 2010; ICEM, 2010). This is associated with under reporting of the total value of the annual catch (e.g. as observed in the different fisheries occurring in the TSGL system; see Chapter 4) and differentiation between catches from the freshwater environment and from brackish or marine environments (e.g. the Mekong delta fisheries; Hortle, 2009; Halls *et al.,* 2013b; DHI, 2015).

Difficulties also exist regarding the differentiation of inland captures and aquaculture production, which has been growing in the basin, particularly in the Vietnamese delta (see sub-section 3.6.2), with an estimated contribution to the inland fisheries of 1.2-1.9 million tonnes (DHI, 2015). Therefore, if combining the total annual estimated yields from inland captures (without differentiating between freshwater and marine fisheries) with aquaculture production, it is likely that the total annual yields reach 4.0-5.1 million tonnes. For this reason, the inland fisheries of the Mekong are regarded as the most productive in the World (Table 3.5; Hortle 2007;2009; Baran and Myschowoda, 2009; ICEM, 2010; DHI, 2015).

TABLE 3.5 - FISHERIES PRODUCTION IN THE LMB COUNTRIES. CATCH VALUES ARE DERIVED FROM SUBTRACTING AQUACULTURE PRODUCTION FROM PHILLIPS (2002) AND TOTAL CATCH ESTIMATED BY HORTLE (2009; ADAPTED FROM: KONDOLF *ET AL.,* 2018).

Countries	All fisheries (Mkg/yr)	OAA (Mkg/yr)	Calculated Catch fisheries
	Hortle (2009)		Phillips, (2002) and Hortle (2009)
Thailand	721	191	653
Lao PDR	168	41	163
Cambodia	482	105	468
Vietnam	692	161	521
Total	2,063	498	1,357

The MRC Fisheries Monitoring Programme collects and reports the annual catch yield and species composition statistics from the different fisheries developed throughout the basin. Yet, the unstandardized methodology (e.g. the timing, frequency, gears used, catch effort, number of boats, among others; Hall *et al.,* 2013b) that each country apply to survey and report the catches, connected to the seasonality of the operations and the types of fisheries (see following sub-sections), make it difficult to identify basin-wide trends in the fisheries (Hortle, 2009; ICEM, 2010; Halls *et al.,* 2013b). For this reason, to characterise and assess long-term dynamics in the inland captures, an individual analysis of each countries' fisheries should be followed. Moreover, due to the different sources of available publications (e.g. National Agencies, MRC Fisheries Programme, International funding to study the Mekong fisheries (e.g. from the Danish Government) and scientific publications; Baran *et al.,* 2001; van Zaling *et al.,* 2004; Mahasarakarm, 2007; Baran, 2010; Halls *et al.,* 2013b; Phonvisay, 2013; DHI, 2015), each reporting different yields, a critical analysis of the available information should be implemented, to evaluate the state of the Mekong fisheries.

I) UPPER MEKONG BASIN FISHERIES

Compared to the Lower Mekong Basin, long-term analyses of the trends in species diversity and inland fisheries in the Upper Mekong Basin are constrained to a few studies (Sjorslev, 2000; Hortle, 2009; Kang *et al.,* 2009). The main reasons for this are associated to the difficulty in accessing most of the Upper Basin's environment (see section 3.1; Carling, 2009). Also, the high elevation from where the river starts (approximately 5200 meters amsl), combined with presence of glacier areas and reduced water temperature, make it difficult for fishes to inhabit this area, for fisheries to develop and for populations to establish (Gupta and Liew, 2007; Kang *et al.,* 2009; DHI, 2015; Kondolf *et al.,* 2018). Thus, the Upper Mekong Basin exhibits the lowest population number found for the entire Mekong basin (estimated as over 10 million people; Table 3.3).

Few studies have reported the fisheries in the Upper Mekong Basin. Yet, for the northern area of Lao PDR, an extensive survey was conducted to access the trends in the fishery and catch composition of Luang Prabang, a rugged mountainous province (247-1600 meters amsl) in northern Lao PDR (Figure 3.1; Sjorslev, 2000). Although rice and livestock assume a higher importance than fisheries, regarding human participation, an estimated 83% of households (42% of people inhabiting the Luang Prabang area, although total population number for this area is not provided by Sjorslev, 2000) engaged in capture fisheries, with the majority of the fishery taking place in the mainstream river and small streams, producing nearly 90% of the total reported catch for the Luang Prabang region (with 167,922 tonnes estimated for the entire Lao PDR inland fisheries by Phonvisay, 2013). This report, compared to Table 3.5, reflects a over-estimation than the one from Kondolf *et al.,* (2018). The remaining catch is associated with rice fields (7%) and ponds (3%). The composition of the catch was found to possess 73 taxa, mainly whitefishes and two invasive species (Nile tilapia and common carp), which are assumed to be a result of escapes from aquaculture ponds. Furthermore, in Luang Prabang, fishes are estimated to provide around 20% of total animal protein intake (Sjorslev, 2000; Hortle, 2009). Further north, in the Yunnan Chinese province, reports of the total annual catches, are scarce (estimated to be about 20,000 tonnes per year; Xie and Li, 2003; Hortle, 2007;2009). Also, due to the presence of the cascade dams (either in operation and/or construction phase; Lu and Siew, 2006; Fan *et al.,* 2015), the fishery activities are highly concentrated to few areas, mainly in Lake Erhai, where the Mekong giant catfish is thought to spawn by observations of Smith (1945). Yet, the presence of the cascade dams makes it physically unreliable to find nowadays populations of the Mekong giant catfish in the lake (Hortle, 2009).

Aquaculture, along with rice-farming, assume a higher importance in this area, than fisheries. Aquaculture in the Yunnan province is estimated to provide 12,000 tonnes per year, being mainly developed in rice fields and ponds. Moreover, and because the upstream section of the Mekong presents a relatively low number and diversity of fish species compared to the Lower Mekong Basin (Kang *et al.,* 2009 estimated the presence of 173 species inhabiting this section, with 87 species being endemic), this activity focused in culturing species highly adaptable to low water temperature and high presence of sediments (associated with natural landscape erosion and sediment accumulation from the dams in operation; Fu and He, 2007), mainly introduced species (e.g. African tilapias, *Oreochromis mossambicus* and *Oreochromis niloticus*).

II) THAILAND FISHERIES

Official fishery statistics and scientific field surveys to document the Thai fisheries activities (e.g. types of gears used, seasonality, fishing yields and species composition) are highly constrained by the lack of English language studies (Mahasarakarm, 2007). Moreover, due to the high tributary dam development and reservoir impoundment throughout Thailand, Baran *et al.,* (2007) reported the general perception that the Thailand's inland fishery is mainly restricted to reservoir fisheries, with over 2,000 reservoirs available, a total surface area of more than 2,000 km^2 and an estimate annual total catch of approximately 30,000 tonnes. Yet, and as reported by Coates (2002), not all inland captures develop in reservoirs, and captures in swamps, rice-fields, floodplains and canals are also significant, although exact estimations are not available in the literature, apart from the reported trend that these inland captures are mainly concentrated in the Mekong's tributaries and are highly used for domestic consumption (Baran *et al.,* 2007; Mahasarakarm, 2007; Hortle, 2009).

Aquaculture and marine fisheries assume higher importance for fish supply, than freshwater captures (estimated to contribute only 6% to the total fisheries production of Thailand; Baran *et al.,* 2007). Moreover, both aquaculture and marine fisheries are regarded as artisanal and industrial activities, with an economic value of over US$1 billion annually, mainly for local consumption and trade (Mahasarakarm, 2007). The main species used for aquaculture are exotic Nile and African tilapias, pangasiid species, shrimps and prawns. These species are cultured in high densities (unfortunately exact values could not be found in the literature; Mahasarakarm, 2007; Baran, 2010; DHI, 2015), because of their high resilience to low water conditions, in earth ponds and rice-fields. These have extensively grown in terms of hectare's areas (estimated in 2007 to be nearly 600,000 hectares; Mahasarakarm, 2007), linked to modifications in land for agriculture, construction of roads and establishment of industrial factories, which resulted in a significant decline in water quality (Mahasarakarm, 2007; Hortle, 2009; Baran, 2010). Consequently, the fact that Table 3.5 shows that Thailand possesses the highest total annual inland capture of the Lower Mekong basin, should be careful analysed as it is likely that the estimations made by both Phillips (2002) and Hortle (2009) did not differentiate between freshwater inland captures and marine fisheries. The same issue was observed by Baran *et al.,* (2007) when analysing Phillips (2002) estimations. Regarding the importance of Thailand's inland fisheries for the livelihoods, in the northeast region, an estimated 825,000 households earn their living from both agriculture and fisheries, and an additional 47,000 households earn their living from inland fisheries alone. Furthermore, an estimated 80% of the total households that rely on both agriculture and fisheries (i.e. 825,000), are concentrated near the Mekong (Baran *et al.,* 2007).

As mentioned, most of the inland captures in Thailand are associated with reservoir fisheries, due to the large number of dams built in the Mekong's tributaries (Baran *et al.,* 2007, Mahasarakarm, 2007; ICEM, 2010). Moreover, the reservoir impoundment by dams significantly changed the water availability and quality, flow conditions and act as a migratory barrier for most species. In Thailand, the building of the Pak Mun dam resulted in failed plans to improve inland fisheries' catch (SEARIN, 2004; Baran *et al.,* 2007). Environmental impact assessments made by the Thai Government stated that the construction of this dam would provide a reservoir fishery, which would yield annually 100 kg per hectare per year if no fish stocking was in place (with fish stock the yields would increase to 220 kg per hectare per year), with a value of 19.7 million Baht (approximately US$ 620,000). After the construction of the dam, the fishery yields were much lower than expected (approximately 19 to 38 kg per hectare per year) and the value of the fishery was estimated at 0.9 million Baht (approximately US$ 28,500; Baran *et al.,* 2007). Also, the species diversity decreased 50-80% both upstream and downstream the dam and the number of households that were dependent exclusively on the fishery decreased from 96% to 67%. By 2000, 488.5 million Baht (nearly US$ 15.5 million) had been paid to 6,202 households to compensate for the loss of fisheries and livelihoods by the Thai Government (SEARIN, 2004; Baran *et al.,* 2007). The main reasons for the problems raised by the construction of this dam were linked to dam's location (a migratory pathway and natural habitat for 77 and 35 species, respectively, of the 265 species known to inhabit the Mun-Chi watershed), and changes in water quality (low dissolved oxygen levels associated to increase in turbidity from high sediment content) and flow conditions (modification from lotic to lentic environment; Baran *et al.,* 2007).

III) LAO PDR FISHERIES

The Lao PDR fisheries are mainly characterised by small-scale subsistence fisheries, contributing to food security, economic income and livelihoods (Phonvisay, 2013). The fisheries mainly develop in rivers, reservoirs, floodplains and rice fields, with an estimated total area of water resources for captures fisheries estimated as being more than 1.2 million hectares. According to Phonvisay (2013) the total annual inland captures is 167,922 tonnes, which is close to the reported value by Hortle (2009; Table 3.5). Moreover, the consumption of other animals is estimated at nearly 41,000 tonnes per year. The inland captures values almost US$ 150 million per year, with the importance of fish protein to local people, present in Table 3.4, being the lowest of the four Lower Mekong countries (43 kg per capita per year; Hortle, 2007;2009).

The *lee* trap fishery is one of the most assessed and important fisheries (other fisheries are gill-net, reservoir, mainstream and tributary fisheries, particularly in Nam Ou, Vientiane and Pakse; Phonvisay, 2013) taking place in the Khone Falls, Champassak Province in southern Lao PDR, with an estimated over 20,000 tonnes of fish being caught every year, in which 70% is consumed locally as fresh or preserved, and 30% is sold in markets. The fishery generates approximately US$100 of annual income per family (Halls *et al.,* 2013b; Phonvisay, 2013). The Khone Falls zone is characterised by a network of 18 channels from where the Mekong river braids, before it falls nearly 20 meters reaching downstream lowland areas (Halls *et al.,* 2013b). This zone is also characterised by many islands (4,000 islands in Siphandone and Khone Falls areas; Carling, 2009), where an estimated 60,000 people live in and are directly dependent on the fisheries resources (Halls *et al.,* 2013b; Phonvisay, 2013). According to Baran *et al.,* (2005), fishers utilize over 40 different gear types, with the semi-submerged *lee* trap being one of the most used. The *lee* trap fishery (Figure 3.7), a large immovable semi-submerged trap with long wings made usually of bamboo fences, is set in rapids (nearly 400 *lee* traps are annually distributed in the 18 channels of the Khone Falls area; Warren *et al.,* 2005) to target upstream spawning migrations (mainly Pangasiidae, Bagridae and Siluridae), mostly at night. In the wet season (May-October), *lee* traps also target species migrating downstream (mainly Cyprinidae). The most important captured species by landing weight are *Pangasius larnaudii* and *Pangasius conchophilus*, which are long body-size species performing upstream/downstream migrations and contributing over 50% to the annual fisheries (Roberts and Baird, 1995; Halls *et al.,* 2013b; Phonvisay, 2013). The *lee* trap has been monitored since 1994, due to financial support from the Government of Canada (Halls *et al.,* 2013b). The monitoring takes place in the Hoo Som Yai (HSY) channel (Figure 3.7) below the Khone, where the HSY diverges above the Khonephageng Falls, flowing in a narrow vegetated channel between 10 and 20 meters wide for almost 800 meters, before re-joining the main channel, 600 meters downstream of the falls in Northern Cambodia.

Halls *et al.,* (2013b) in their assessment of the daily role of water level variations from Pakse in the observed daily catch per unit effort (CPUE, kg per *lee* trap per day) in 2008 for the HSY channel, found a significant influence of the water level variations in the reported CPUE ($p<0.001$). However, on a long-term analysis (1994-2009), variations in Pakse water level did not significantly influenced the CPUE values. Thus, although the water level and flow conditions are important in this area, particular for upstream and downstream migrations of *Pangasius* species, the extensive network of rapids and channels and the fast-swimming capacity of long body-size fish species, resulted in fishes being able to reach upstream and downstream areas, regardless of the flow conditions, with variations in water level acting more as a migration trigger. Of note, modifications in water level and flow would decrease the migration clues, particularly for *Pangasius* species, which would significantly influence the annual catches (Halls *et al.,* 2013b). This issue is particularly important, in light of the projected Don Sahong dam (Baird, 2011) to be built in the Hoo Sahong channel (Figure 3.7). This dam has been argued to block the only deep channel that allows fish to migrate through the falls year-round, and, therefore, disrupt the migration pattern of over 200 species (Baran and Ratner, 2007).

FIGURE 3.7 - *LEE* TRAP FISHERY TAKING PLACE IN THE RAPIDS (LEFT) AND LOCATION OF THE HOO SOM YAI (HSY) CHANNEL WHERE THE LONG-TERM MONITORING (1994-2009) TAKES PLACE (RIGHT; FROM: HALLS *ET AL.,* 2013B).

IV) CAMBODIA FISHERIES

Cambodia possesses the longest (estimated as 140 years) monitored fishery for the entire basin, the *dai* fishery (Baran *et al.,* 2001; van Zalinge *et al.,* 2004; ICEM, 2010; Halls *et al.,* 2013a; DHI, 2015). This fishery is of vital importance to more than 1.2 million people who live in floating villages and the riparian areas of the TSGL system, and to whom both the *dai* fishery and the other fisheries that develop (see Chapter 4) are the primary economic activity and protein source, with an annual fish protein consumption estimated as 70 kg per person (So, 2010). The *dai* fishery occurs in the onset of the dry season (October-March), when the fishes are leaving the lake to find shelter in upstream pools, mainly near the border with Lao PDR (Poulsen *et al.,* 2002;2004; Halls *et al.,* 2013a). Furthermore, the fishery contributes approximately 7% (estimated as 7.5-46 million tonnes annually; Halls *et al.,* 2013a) to Cambodia's total annual landings (Table 3.5), and targets mainly migratory species of the *Henycorhynchus*, *Pangasius* and *Labeo* species, which together make up over 50% of the annual species composition of the catch (Baran *et al.,* 2001; van Zalinge *et al.,* 2004; Halls *et al.,* 2013a; DHI, 2015). The *dai* fishery is highly dependent on the seasonal flood dynamics of the lake (Kummu *et al.,* 2014), promoting food availability, spawning and sheltering grounds in the seasonally inundated extensive floodplain habitats, resulting in high fish production (Lamberts, 2006;2008). Chapter 4 quantifies the influence of long-term variations in the lake's flood regime to the *dai* fishery and species composition, with important results for the needs to preserve the lake's flood pulse pattern for the sustainability of the fishery and the species supported.

The fisheries of the lake are responsible to nearly 70% of Cambodia's total annual catch (Table 3.5). The remaining 30% comprises the fisheries that operate year-round in the mainstream river and particularly in the 3S system (although exact statistics are not available in the literature), from Stung Treng to Phnom Penh areas (Hortle, 2009; Halls *et al.,* 2013a). These fisheries are locally consumed, although in Phnom Penh trade market is also seen (Baran *et al.,* 2007).

V) VIETNAM FISHERIES

The Vietnamese fisheries provide the second highest yield of the total Mekong basin's capture fisheries (~692,000 tonnes; Table 3.5; Kondolf *et al.,* 2018). The

Mekong delta is of vital importance, by providing over 40% of the total fish production (i.e. the remaining less than 60% are attributed to the whole country, which comprise the fisheries that happen in rice-fields in floodplains and central highland areas, and the fisheries that develop from mountainous highlands in streams and canals, to downstream areas, in both mainstream and Bassac distributary), with an estimated increase from 552,240 tonnes (1995) to over 1 million tonnes (early 2015; Figure 3.8; Baran, 2010; DHI, 2015). Moreover, regarding protein intake, the Mekong delta contributes over 60% to the country's inland production for both fish and other aquatic products (Baran, 2010; DHI, 2015). However, caution needs to be taken when analysing the annual inland captures from the delta. This is mainly due to the reported misconception from the General Statistics Office (GSO) of Vietnam (from where annual inland capture and aquaculture statistics are provided for the whole country and regional zones) in differentiating between the freshwater and the estuarine fisheries (which relates to fishing activities in coastal inland waters, including estuaries, rivers and canals; DHI, 2015). Moreover, marine production usually means marine catch in off-shore and near-shore waters. Unfortunately, there is no clear discrimination or definition of these categories, and, therefore, marine catches are also included in the delta's inland capture (Baran, 2010; DHI, 2015).

According to the DHI (2015) report, which attempts to distinguish between freshwater inland captures and marine captures (although the data reported is provided from the GSO and from the Ministry of Agriculture and Rural Development of Vietnam - MARD), there has been a significant reduction in freshwater captures from 2000 to 2011 (188,873 tonnes to 124,626 tonnes), and a steady increase in marine captures, with in 2011, 88% of the country's captures being from marine origin and the remaining 12% from freshwater origin. The marine production has, therefore, increased from 465,732 tonnes in 2000 to 691,700 tonnes in 2011. The reasons behind it are linked to an increase in fishing effort in coastal and off-shore areas along the delta, from an increase in population. However, caution needs to be taken when analysing the role of coastal fishery in providing fish for the entire country, because on a long-term basis, coastal fisheries might not be sustainable, since this fishery relies on just a few species (usually less than 10 species make more than 60% of the annual catch; Valbo-Jorgensen *et al.,* 2009; Hortle, 2009; DHI, 2015). Also, both in the Vietnamese delta and in the northern and central highland areas, fishes are consumed locally, based in small-scale fisheries, but also exported to international markets. These exportations are possible due to the increasing role of aquaculture in the region (DHI, 2015; Figure 3.9).

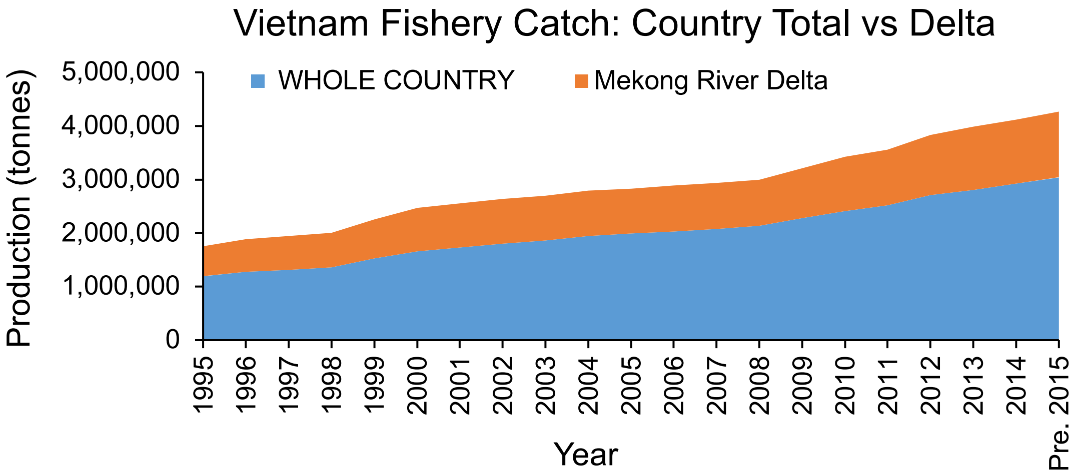

FIGURE 3.8 - ANNUAL INLAND CAPTURE PRODUCTION (TONNES) FROM THE MEKONG DELTA AND VIETNAMESE WHOLE COUNTRY (1995-2015; SOURCE: GSO, 2016).

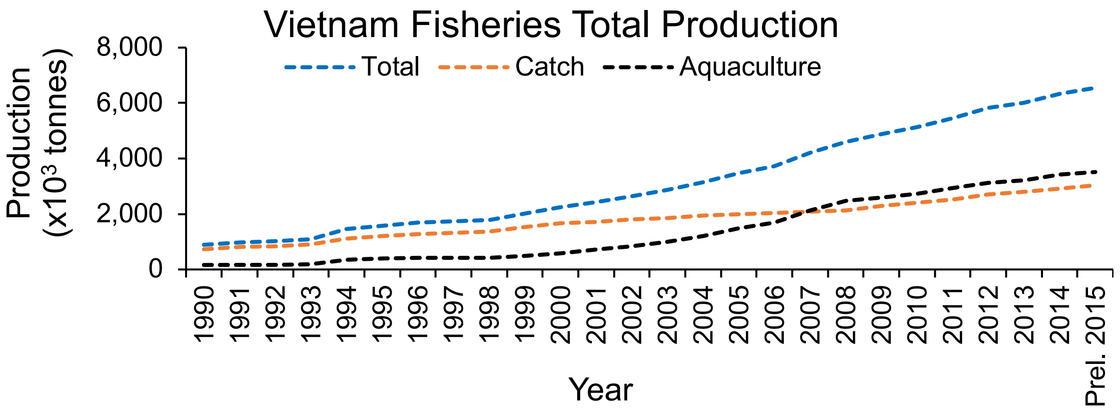

FIGURE 3.9 - ANNUAL VIETNAMESE FISHERY PRODUCTION FROM FISHERY CATCH (NO DISTINCTION IS MADE BETWEEN FRESHWATER, ESTUARINE AND MARINE CAPTURES) AND AQUACULTURE PRODUCTION (1990-2015; SOURCE: GSO, 2016).

The aquaculture production in Vietnam is a common activity from the northern highland areas down to the shallow floodplain waters of the delta, with an estimated increase from nearly 300,000 tonnes in 1990 to almost 4,000,000 tonnes in 2015 (ICEM, 2010; DHI, 2015). Furthermore, since 2007 the annual aquaculture production has suppressed the annual inland catch (Figure 3.9). The turning point from inland captures to aquaculture production is linked to the fact that aquaculture production is integrated with agriculture practices (mainly in rice fields) for the production of shrimps (78% of annual aquaculture production, DHI, 2015) and pangasiid fish species (22% - the low percentage is linked to the fact that it takes at least 6 months to grow pangasiid fishes to the commercial market.

Consequently, the profit margin is low, only 0.1-0.15 US$ per kg, although this species can be grown in high densities, 40-50 fish per m^2, with yields of 50-60 tonnes per hectare; Poulsen *et al.,* 2008; Phan *et al.,* 2009).

The integrated aquaculture provides annual high yields connected to the low technology employed for rearing these animals, and the fact that they can be reared in high densities. Also, an estimated 750,000 hectares of surface area is used for aquaculture production (i.e. almost 72% of the entire land area of Vietnam), although clear distinction between the amount of land used for shrimp and pangasiid culture is not available in the literature. A further estimated 80,000 ha is also used for rice-fish culture, with a mean annual production of 370 kg per hectare (DHI, 2015). Moreover, the exportation market of these species yields over US$ 3 billion annually, contributing to the current 3rd position of Vietnam for inland fish production (Baran, 2010; ICEM, 2010; DHI, 2015). With the observed increases in human population, particularly in the delta, which already possesses the highest densely populated area (Table 3.3), and the continuous clearing of wetland habitats for extending the area for agriculture, it is likely that the current nearly 40,000 km^2 of available land for agriculture (within only 3.6 million hectares are currently being used for rice-fields; MRC, 2010) will substantially increase in the near future (also contributing to the continuous diversion of floods and consequent environmental modifications to the riparian habitats), and the aquaculture production will continue to rise (Hortle, 2009; Baran, 2010).

However, the fact that current aquaculture farming practices rely on capturing wild fish (both adults and juveniles) for rearing in ponds, and the continuous harvest of what is known as “trash fish” (small non-commercial fishes) for feeding the fishes, make it unlikely that aquaculture can effectively replace inland captures (Hortle, 2009; Baran, 2010; DHI, 2015). Thus, the future of the Vietnamese delta in providing fish protein to the increasing population is highly uncertainty with potential food security issues.

3.7 MAIN THREATS TO THE BASIN: HYDROPOWER DEVELOPMENT, IRRIGATION SCHEMES AND CLIMATE CHANGE SCENARIOS

Hydropower (see Appendix 1) is threatening the pristine flow conditions of the mainstream river since 1990-93 when the building and operation of the Manwan dam in the Upper Mekong started (Lu and Siew, 2006). Following the Manwan dam, plans for the construction of seven mainstream dams in the Upper Mekong started, with five of these infrastructures in operation (Fan *et al.,* 2015; Kondolf *et al.,* 2018). Additionally, over 120 dams are expected to be built in both mainstream and tributaries according to Lauri *et al.,* (2012), DHI, (2015), Kondolf *et al.,* (2014;2018), Hoang *et al.,* (2016;2019) and Hecht *et al.,* (2019).

Hydropower development is not the only threat to the sustainability and resilience of the Mekong, but climate change projections have also been regarded as bringing high uncertainty to the rainfall and tropical cyclone events, which provide the majority of the flow and sediments flux downstream, while defining the predictable dry and wet seasons' hydrologic regime (Darby *et al.,* 2013;2016). Therefore, a rising number of studies have been focusing on the impacts of hydropower, irrigation schemes and climate change scenarios (both individual and cumulative effects) on the flood regime and sediment flux of the river (Hoanh *et al.,* 2010; Keskinen *et al.,* 2010; Lauri *et al.,* 2012; Van Manh *et al.,* 2015; Kondolf *et al.,* 2014;2018; Hoang *et al.,* 2016;2019; Pokhrel *et al*., 2018; Hecht *et al.,* 2019). On the other hand, different models and scenarios used have provided different outcomes across the studies. Moreover, for climate change, the models applied and RCP's, possess inherent uncertainty, highlighting the uncertainty on the future projections of hydrologic and sediment flux in the basin (Lauri *et al.,* 2012; Thompson *et al.,* 2014). Lauri *et al.,* (2012), by applying modelling scenarios, assessed the individual and cumulative future impacts (2032-2042) of upstream dam development, future development of 126 dams in both mainstream and tributaries and climate change, on the discharge pattern felt in Kratie. The authors found that between the baseline (1982-1992) and future (2032-2042), dams would cause 25-160% higher dry season flows and 5-24% lower flood peaks during the wet season in Kratie. Moreover, for climate change scenarios, the changes in discharge were -11% to +15% for the wet season and -10% to +13% for the dry season. The cumulative impact of damming and climate change resulted in the higher effect of damming in the dry season's flows and uncertainty regarding the climatic consequences, with an overall cumulative effect of increase in dry season flows by 2-20% and decrease in wet season discharge by -15% to +7%. Hoang *et al.,* (2019), following previous modelling exercises from Lauri *et al.,* (2012), Darby *et al.,* (2016) and Hoang *et al.,* (2016), reported the same directions of change, by assessing the cumulative future impacts (2050s) of hydropower, climate change and irrigation schemes on the basin's run-off, with a projected increase by up to 160% of the dry season flows and a decrease in wet season's flows by up to 25%. Räsänen *et al.,* (2017) assessed the role of the upstream cascade dams in the discharge level measured downstream in Kratie and once again reached the same direction of change, with an increase in the dry season discharge measured at Chiang Saen of 121-187%, resulting in an increase in the flow measured in Kratie of 41-74%. In the wet season for Chiang Saen a decrease of 32-46% in the flow, resulted in a decrease in the wet season discharge measured in Kratie of 0-6%.

Consequently, the scientific consensus is that hydropower development provides the most disruptive impact on the Mekong's discharge pattern, increasing dry season flows and decreasing wet season flows downstream (Lu and Siew, 2006; Lauri *et al.,* 2012; Räsänen *et al.,* 2017; Hecht *et al.,* 2019; Hoang *et al.,* 2019). Yet, when combined with irrigation schemes, Hoang *et al.,* (2019) noted a low level of increase in the projected dry season flow than when assessing the individual hydropower effect. The climate change influences in the basin and its broader function have high uncertainty, depending on a number of factors used, and with a general conclusion that both annual wet and dry season flows would increase associated with an increase in the rainfall pattern. Moreover, the increase (decrease) in the dry (wet) season's flow pattern are likely to modify the TSGL's floodplain habitat seasonal inundation pattern (nearly 30% increase and 22% decrease in the dry and wet seasons, respectively; this issue will be further explored in Chapter 5; Arias *et al.,* 2014), which would potentially disrupt fish migratory behaviour, habitat connectivity and inundation dynamics for spawning, sheltering and food purposes, while threatening the land available for agriculture and aquaculture, and the possible re-allocation of people to avoid extreme flood events (see Chapter 2; Keskinen *et al.,* 2010; Olson and Morton, 2018).

Sediment transport, particularly the amount reaching the delta, has been assessed in studies focusing on the role of hydropower development in trapping the sediment transport downstream, and also the role of climate change in increasing sea level, which threatens the sustainability of the delta (Kummu *et al.,* 2010; Van Manh *et al.,* 2015; Kondolf *et al.,* 2014;2018). Kummu *et al.,* (2010) predicted a 60% reduction in the sediments reaching the delta, due to upstream dam development. Kondolf *et al.,* (2014) predicted a much higher sediment trap based on a definitive scenario of dam building (130 dams), with a consequent 96% decrease in sediments reaching de delta. Darby *et al.,* (2016) also stated the

clearance in sediments along the basin, although this was assessed regarding a modification in the frequency of tropical cyclones' events, which resulted in a sediment transportation decrease (for revising information in the reduction value, see sub-section 3.2.2). Piman *et al.,* (2013) showed that due to modifications in water level and discharge, connected to the sediment clearing from dams, salinity intrusions in the delta would increase in 15-17% until 2030 in the wet season, because of a 20% reduction in the hydrologic pattern. Yet, in the dry season, salinity intrusions would reduce by 5-10 km as a result of over 40% increase in the dry season flows. Thus, alterations in sediment flux reaching the delta, along with variations in salinity intrusions from sea level rise, will likely affect agriculture and fisheries activities, while threatening the natural building and sustainability of the delta's landform, posing challenges to the ecosystem services provided and resilience of over 17 million people inhabiting this region (Kondolf *et al.,* 2018).
Notably, an increasing number of studies have been predicting the future impacts of hydropower, irrigation and climate change on the flood regime and sediment transportation along the basin. Yet, studies quantifying the consequences of those alterations to the fisheries and fish dynamics are a major gap. The DHI (2015) study shows key projections in fishery loss in an attempt to fill this gap, yet the assessment only focussed on the impacts by hydropower development (see Chapter 5 for detailed findings). Hence, predictions on the impacts to the flood regime, habitat flood dynamics and consequences to fisheries catch, species composition (particularly for migratory species, which compose over 50% of the Mekong fisheries) and fish protein provision, associated with anthropogenic-driven environmental threats should be prioritise, for the importance the biological resources place to the natural sustainability of the system and to people inhabiting the region for food security (see Chapter 5).

3.8 CONCLUSIONS

This chapter demonstrates the vital role of the Mekong river in supporting high human populations, strictly dependent on the agriculture and fishery resources for their livelihoods, poverty alleviation and food security. Moreover, the profitable fisheries that develop throughout the basin are dependent on the migratory behaviour of over 50% of the known fish species, whose life cycle is argued to be influenced by the predictable seasonal variations in the flood regime and sediment flux in the basin.

The Mekong's seasonal hydrologic regime, driven by the monsoon climate, tropical cyclones and ice melting, mark the long-term observed single peak flood pulse, which drive water availability and quality, the sediment flux and carrying nutrients, and influence habitat inundation pattern and connectivity that are essential for fishes and for the ecosystem services.

The Mekong has remained pristine in terms of its flow conditions until recently, when the first upstream mainstream hydropower dam was built, and a rising number of future developments (estimated as over 120 dams) are likely to disrupt the sediments reaching the delta bringing high uncertainty on the sustainability and resilience of the people inhabiting this area. Furthermore, the increase (decrease) in the dry (wet) season flows and flood level are likely to disrupt the discharge pattern, habitat connectivity and the migratory clues for fishes, which will impact upon the fishery yields that develop along the basin. Moreover, climate change projections, although possessing high uncertainty, are due to affect the basin, mainly by sea level rise and salinity intrusion, which will likely affect agriculture, fish migrations and fisheries. Ultimately, food security issues of the region will magnify from the alterations in the fisheries' yields and composition.

The lack of studies assessing the role of the seasonal flood regime on the fisheries and species composition and predicting the impacts of hydropower, irrigation and climate change scenarios on the fisheries resources, are a major gap in the literature, that urgently needs to be be addressed.

The next chapter will focus on the analysis of historical (1996-2014) variations in the flood pulse of the TSGL system (hotspot of fish species and only long-term monitored fishery) and the importance to the *dai* fishery and species composition (Chapter 4). Also, based on the Chapter 4 relationships established (baseline), the future impacts to this fishery and species composition will be assessed in Chapter 5, by applying individual and combined modelling scenarios of hydropower development, irrigation schemes and climate change influence on the flood pulse and floodplain flood dynamics. Finally, by applying eDNA metabarcoding tools (Chapter 6), it will be possible to detect the fish communities, estimate relative abundance, seasonal distribution, and predict the species likely to be threaten by the multiple drivers of environmental change, and the importance those species place to the *dai* fishery and food security of the basin.

CHAPTER 4

Applying Earth Observation to assess historical variations in the flood pulse of the Tonle Sap Great Lake system: importance to the *dai* fishery and species composition

Overview of the chapter: This chapter focuses on the importance of applying remote earth observation tools and long-term time-series of hydrologic data to assess historical variations in the flood pulse, a driver of ecosystem productivity, where complex eco-hydrologic interactions happen between the aquatic environment and riparian floodplains. Focussing on the Tonle Sap Great Lake (TSGL) system in Cambodia, a hotspot of fish diversity and inland fisheries, the chapter demonstrates the vital role of the flood pulse of the lake, supporting the *dai* fishery (bagnet and only long-term monitored fishery record for the lake), species composition of the catch and water reservoir for the entire Lower Mekong Basin (LMB).

4.1 INTRODUCTION

Tropical floodplains are some of the most productive wetland habitats on Earth (Campbell *et al.,* 2009; Arias *et al.,* 2012;2013;2014; Castello *et al.,* 2015). The seasonal variable inundation dynamics established across many tropical zone floodplains have been acknowledged as providing important functions that underpin success of fisheries, such as nutrient recycling, nursing, feeding and sheltering grounds, influencing fish dynamics and abundance (see later; Lamberts, 2006;2008; Lamberts and Koponen, 2008; Junk *et al.,* 2013). Importantly, the seasonality and magnitude of the inundation pattern reflects the hydrologic regime of the parental body (e.g. river and/or lake), which is strongly influenced by the historical predictable climatic conditions in place (see Chapter 3; Amarasekera *et al.,* 1997; Delgado *et al.,* 2012; Räsänen and Kummu, 2013). Across much of the tropics, El Niño Southern Oscillation (ENSO) events, an ocean-atmosphere coupled phenomenon occurring in the Tropical Pacific Ocean, exacerbates the seasonality of the precipitation patterns in many areas. In the Mekong basin this has been linked to severe low floods and flows (El Niño phase) and extreme high floods and flows (La Niña phase; Darby *et al.,* 2013; Räsänen and Kummu, 2013). Thus, the predictable climate-driven variations in timing, duration, amplitude, smoothness and continuity of the flood are defined under the Flood Pulse Concept (FPC), to explain the historical seasonal inundation patterns and consequent eco-hydrologic interactions, established between the parental body and the floodplain habitats that result in life-long adaptive strategies of fish species (for revising information on the FPC see Chapter 2; Junk *et al.,* 1989; Lamberts, 2006;2008; Kolding and van Zwienten, 2012).

These seasonal variations and cycles in the flood pulse result in a period of low flood level (during the dry season), causing habitat shrinkage, decrease in lateral connectivity between floodplains and the parental body, and modifications in water quality (e.g low dissolved oxygen; <4 mg l^{-1}). The period of high flood level (during the wet season), results in an increase in the lateral connectivity between the parental body and floodplain habitats, with consequent floodplain inundation. Also, the water quality is marked by an increase in the dissolved oxygen levels (>4 mg l^{-1}) and sediment content (see section 3.3 of Chapter 3 for the historical water quality analysis for the Mekong basin; Lamberts, 2008; Campbell *et al.,* 2009; Ongley, 2009; Arias *et al.,* 2013; Castello *et al.,* 2015).

Tropical fishes exhibit an evolutionary history of adapting their life cycles (e.g. spawning) to the variations caused by the flood pulse. Many functions are triggered by the pulse, resulting in the seasonal migratory behaviour of different fish guilds (i.e. black, grey and whitefish) to the recently inundated habitats (see Chapter 2; Lucas and Baras, 2001; Valbo-Jorgensen *et al.,* 2009), with distinctive tolerances to variations in water quality parameters (e.g. dissolved oxygen and sediment loads; for revising information on the Mekong fish diversity, tolerance to shifts in water quality and migratory clues see Chapter 3; Ongley, 2009). Furthermore, the variability in the duration and extent of inundated floodplains, are argued to influence the production and abundance of fishes that seek the newly flooded floodplains to spawn (adults) or feed (larvae, juveniles and adults; Baran *et al.,* 2001; Poulsen *et al*., 2004; Baran, 2006; Campbell *et al.,* 2006).

The influence of the flood pulse variables on the Mekong river's fishes (Figure 4.1), shows that the start of the flood season triggers different fish species to perform local movements (blackfish; e.g. Channidae family), medium (greyfish; e.g. *Botia modesta*) and long distance migrations (whitefish; e.g. Pangasiidae family) from upstream/downstream areas of the basin, for spawning, feeding and shelter purposes (Baran *et al.,* 2001; Poulsen *et al.,* 2002;2004; Baran, 2006; Valbo-Jorgensen, 2009). In the TSGL system, blackfishes that live inside the lake annually, leave the permanent flood area of the lake (dry season refuge), to spawn and feed in the newly inundated floodplains. Furthermore, grey and whitefishes perform migrations from upstream/downstream areas of the Mekong basin, into the lake to spawn and feed in the inundated floodplain areas. These habitats also function as refuges to larvae and juveniles, which migrate out at the onset of the dry season, when the waters start flowing out of the lake (Poulsen *et al.,* 2002;2004; Campbell *et al.,* 2006;2009; Halls *et al.,* 2013a). Consequently, the predictable balance on dry and wet seasons' flood pattern, the resulting variability in habitat inundation extent and the exchange of nutrients (carried by sediments in the flow and from the inundated riparian environment), strictly influence the life cycle and migratory behaviour of different fishes, which are then reflected in the fisheries that develop and human populations, to whom fish are vital as a source of protein (Baran, 2005;2010).

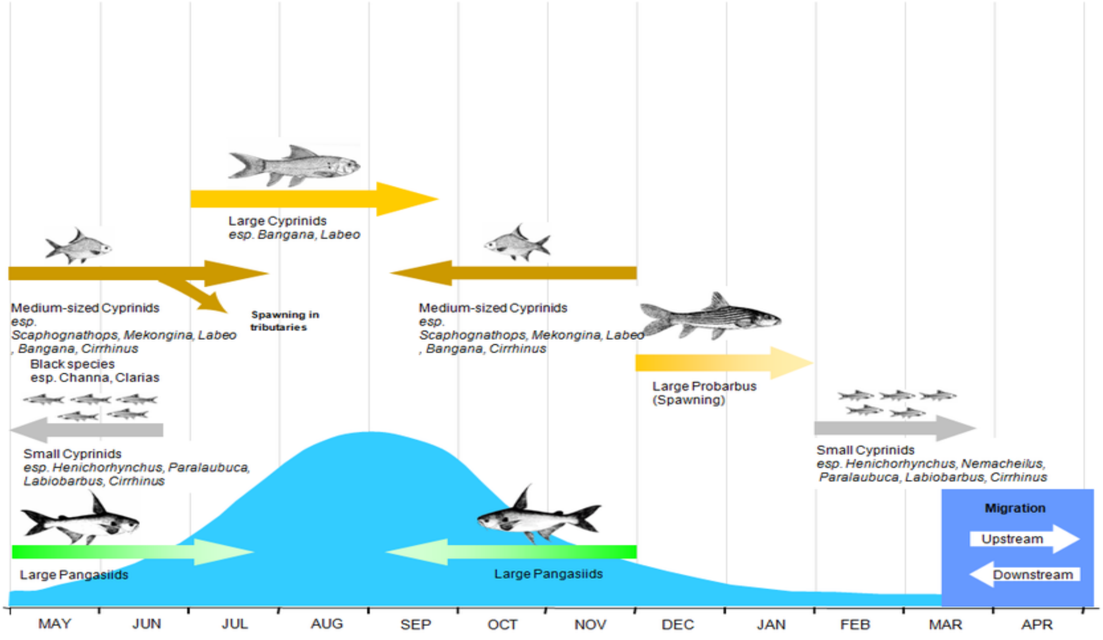

FIGURE 4.1 - INFLUENCE OF SEASONAL VARIATIONS IN THE FLOOD PULSE TO THE ADAPTIVE STRATEGIES OF THE MEKONG FISH SPECIES (SOURCE: BARAN, 2006).

The guild concept (Table 4.1) provides an important classification of fish species according to their ability to behave and exploit different resources (e.g. habitat, spawning behaviour, food preferences, among other characteristics). It shows clearly that fish performing medium to long distance migrations (guilds 2,3 and 4) to complete their life cycles are the ones most affected by hydropower development and modifications in the flood regime, with implications for fisheries (e.g. decrease in the percentage contribution to the annual catch) and food security (Baran and Myschowoda, 2008;2009). Thus, this concept should be acknowledged for its contribution in understanding how fish behave according to modifications in the flood dynamics and preferred habitats. Moreover, it also incorporates the general classification of white, black and grey fish, while providing more information regarding their distribution dynamics and the likely effects of anthropogenic built structures in their migratory pathway (Simberloff and Dayan, 1991; Welcomme *et al.,* 2006; Halls and Kshatryia, 2009).

Fish diversity and tropical floodplain habitats are under severe threat, due to future scenarios of modifications in the flood pulse, associated to hydropower development, irrigation expansion and climate change (Abell *et al.,* 2008; Winemiller *et al.,* 2016; Hoang *et al.,* 2019). Yet, projections on the likely impacts to the fishery resources remain largely unknown. Therefore, to be able to predict future variations in the flood pulse and quantify the consequent impacts on fish abundance, dynamics and fisheries (an issue that will be covered in more detail in Chapter 5), it is important to first assess the historical spatio-temporal flood patchiness in freshwater catchments under the flood pulse concept. Moreover, the influence of climatic events (e.g. ENSO), should also be studied for the variability in the flood provided across the catchment (Frappart *et al.,* 2018). How the fisheries respond to the variability in the flood pulse and how it influences the migratory guild species' behaviour and production should be careful assessed to understand how future modifications in fishery yields and composition, will manifest, which ultimately will affect people through impacts on food security (Baran, 2010; So, 2010; Ziv *et al.,* 2012; DHI, 2015).

The use of remote sensing tools and Geographic Information Systems (GIS) have proliferated in environmental management and impact assessment research. In the TSGL system, Fujii *et al.,* (2010) used RADARSAT satellite images, historical water level observations at 20 locations and discharge measurements by ADCP (Acoustic Doppler Current Profiler) in the main channel and floodplains from Kompong Cham (Cambodia) to the Vietnamese border, to estimate the inundation areas and storage volumes from the lake downstream. Moreover, the analyses also applied a numerical model (i.e. MIKE11), to establish a tool for assistance in understanding the dynamics of recurrent floods as well as the hydrological roles of the floodplains in Cambodia. McAlister and Mahaxay (2009) performed field surveys and collected Landsat-TM images for five sites in the

Mekong. The aim was to improve the available wetland maps' database from the MRC, with the highest accuracy possible. This aim was achieved with accuracy ranging from 77-94%. Dang *et al.,* (2018) recently applied Landsat-TM images to assess spatio-temporal variations in the suspended sediment flux dynamics in floodplains and reaching the Mekong delta, reporting high accuracy on the correlations and explanatory percentage (R^2 from 0.66 to 0.92) for the sediments rating curves driven by the Landsat-TM analysis. On the other hand, these applications of earth observation constrast with the lack of assessment of the influence of variations in the flood pulse-driven floodplain flood dynamics to the fish dynamics, abundance and fisheries supported.

This chapter aims to understand, by applying remote earth observation tools, the long-term (1996-2014) variations in the flood pulse of the TSGL system and the influence on floodplain habitats flood dynamics. Importantly, how the predictable and seasonal variations in the flood pulse-driven floodplain flood dynamics influenced the *dai* fishery and migratory guild species composition is assessed. Also, the role of ENSO events in contributing to the flood level and *dai* catch variability is also assessed. Individually, the aims of this chapter are:

1. To develop a simple model of the lake's flood area based on the Landsat-TM images (1996-2014);
2. To assess how the seasonal flood pulse of the TSGL system has changed over a 19-year period of Landsat-TM image collection (1996-2014);
3. To assess long-term annual variations in the *dai* fishery catch (1994-2014), effort (1997-2009) and species composition (1997-2013);
4. To assess the importance of the historical flood pulse on the fishery yields and migratory guild species composition of the catch (1996-2014);
5. To assess historical variations in the complex flow dynamics (1996-2014);
6. To assess the contribution of ENSO events (1996-2014) to the flood level and fishery yields variability.

TABLE 4.1 - MAJOR GUILD TYPES IN THE LOWER MEKONG BASIN, STATING THE NUMBER, MIGRATORY GUILD NAME, POTENTIAL HABITAT RANGE, MAIN CHARACTERISTICS, DAM AND CHANGES IN THE FLOOD REGIME'S EFFECT ON FISHES AND SPECIES EXAMPLES (ADAPTED FROM: RAINBOTH, 1996; HALLS AND KSHATRIYA, 2009; HALLS, 2010; DHI, 2015).

Guild Nº	**Migratory guild name**	**Potential habitat range**	**Main characteristics**	**Dam and changes in the flood regime's effect**	**Species examples**
1	Rhithron*	Rhithron	Resident in rapid torrents, rocky areas and pools	Little or no impact	*Clupeichthys aesarnensis*
2	Migratory main channel and tributaries resident (Whitefish)	Marine to rhithron	Long distant migrants spawning in the main channel. Adults do not enter floodplains. Migrate to deep pools in the dry season	Very high	*Aaptosyax gryptus, Poropuntius bolovenensis*
3	Migratory main channel spawner (Whitefish)	Floodplain to rhithron	Spawn in main channel, tributaries or floodplains. Long distant migrations. Adults and juveniles feed in floodplains. Migrate to deep pools in the dry season	Very high	*Hemibagrus wyckioides, Henicorhynchus sp. Pangasius sp.*
4	Migratory main channel refuge seeker (white and greyfish)	Floodplain to potomon#	Migrate from floodplain feeding and spawning areas to deep pools in the dry season. Spawning occurs in the floodplains	Medium-high	*Syncrossus helodes, Puntius brevis*
5	Generalist/Residents	Floodplain and potomon	Highly adaptable. Limited migratory behaviour	Little or no impact	*Barbonymus altus, Cyclocheilichthys apogon*
6	Floodplain resident (blackfish)	Floodplains	Local movements. Tolerant to low oxygen concentrations or complete anoxia	Little or no impact	*Channa striata, Chitala lopis, Clarias sp*.
7	Estuarine resident	Estuary	Limited migration in response to seasonal variations in salinity	Little or no impact	*Leptosynanceia asteroblepa*
8	Anadromous	Estuary and lower potomon	Enters fresh/brackish waters to breed. Enters freshwater as larvae and juveniles for growth	Little or no impact	*Megalops cyprinoides, Muraenesox cinereus*
9	Catadromous	Marine to rhithron	Reproduction, feeding and growth in the sea. Enters freshwater to spawn	Very high	*Anguilla bicolor, Anguilla marmorata*
10	Marine visitor	Estuary	Enters estuaries opportunistically	Little or no impact	*Acanthopagrus pacificus*
11	Exotic			Little or no impact	*Cyprinus carpio*

*Rhithron - used for the upper reaches of a river catchment characterised by well-oxygenated water (>4 mg l^{-1}), low temperatures (<20°C), fast and turbulent currents and clear water.
#Potomon - part of a river in which the water is typically slow-moving and relatively warm (>20 ^{0}C), with higher depths (3-15 meters) and different dissolved oxygen concentrations, according to zonation.

4.2 THE TONLE SAP GREAT LAKE SYSTEM

Considered as the pulsing “heart” of the LMB, the TSGL system (Figure 4.2) has attracted a number of studies over the decades, mainly due to its unique bi-directional flow reversals (Kummu *et al.,* 2006; Kummu and Sarkkula, 2008; ICEM, 2010; Arias *et al.,* 2012;2013;2014; Kummu *et al.,* 2014). The TSGL system comprises the permanent lake and an extensive lateral area of seasonally inundated floodplains connecting to Tonle Sap River (Campbell *et al.,* 2009).

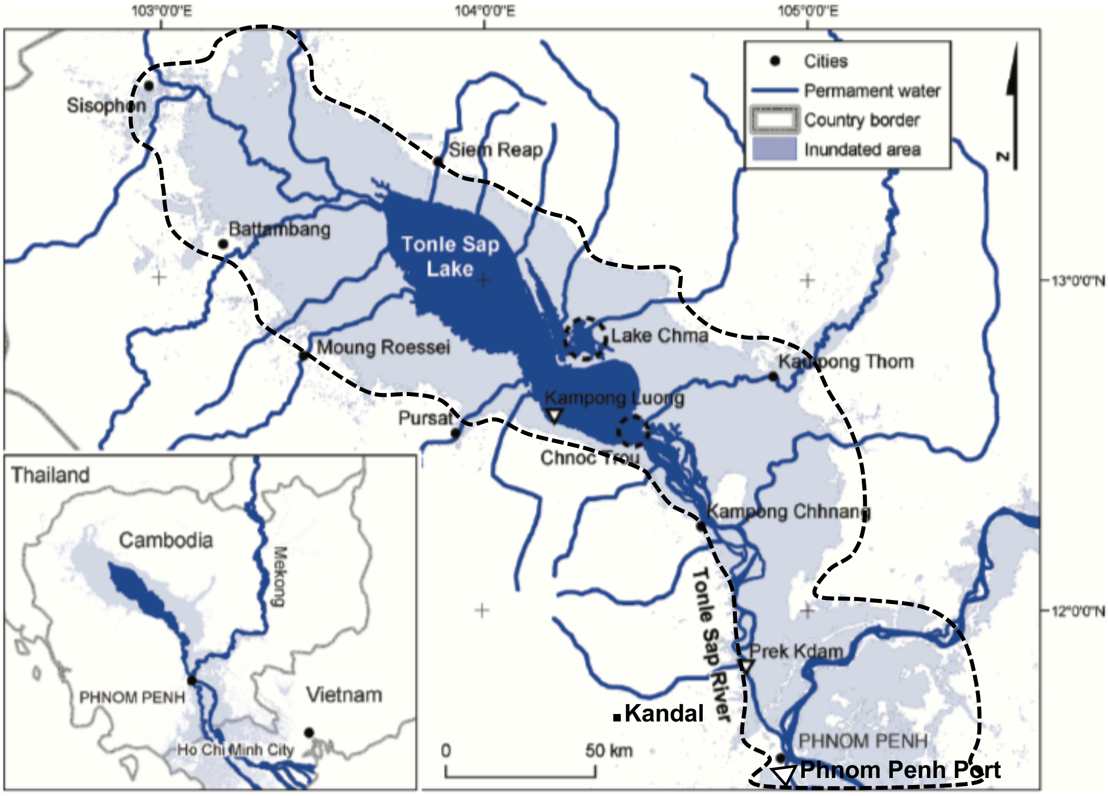

FIGURE 4.2 - THE TSGL SYSTEM WITH THE PERMANENT LAKE (TONLE SAP LAKE) AND EXTENSIVE LATERAL SEASONAL INUNDATED FLOODPLAIN AREA, DELIMITED BY A DARK DASHLINE (INUNDATED AREA), CONNECTING TO TONLE SAP RIVER. THE GEOGRAPHICAL LOCATION OF THE LAKE IN THE SOUTHEAST ASIA REGION IS ALSO PRESENT, ALONG WITH THE MEKONG RIVER. KAMPONG LUONG, PREK KDAM AND PHNOM PENH PORT GAUGING STATIONS ARE MARKED BY A WHITE TRIANGLE (ADAPTED FROM: KUMMU *ET AL.,* 2008B).

The TSGL system is of remarkable importance for the entire Mekong basin. Locally, it supports a population of more than 1.2 million people, who live in floating villages and rely on the lake for its livelihoods, economic income and food security (Koponen *et al.,* 2003; Hortle, 2007; Campbell *et al.,* 2009; Hortle and Bamrungrach, 2015). The fisheries are the most developed activity in the system, generating high economic income, seasonal employment, and with annual catches of 250 - 400 million tonnes (ICEM, 2010; DHI, 2015). The different fishery activities in the system are characterised by the seasonality in its operation and catch reported (Table 4.2). Yet, these fisheries (apart from the *dai* catch) have not been regularly monitored. Therefore, long-term estimations in the annual catches come with high level of uncertainty and completeness (van Zalinge *et al.,* 2000;2003; Baran, 2005; So, 2010; Halls *et al.,* 2013a).

TABLE 4.2 - ESTIMATIONS IN TOTAL ANNUAL CATCH (TONNES) AND SEASONALITY (PERIOD) OF THE DIFFERENT FISHERIES DEVELOPED IN THE TSGL SYSTEM, BASED ON ESTIMATIONS MADE (1994-1997) FROM VAN ZALINGE *ET AL.,* (2000), APART FROM THE *DAI* FISHERY, WHICH REFLECTS LONG-TERM ANALYSES (1994-2014) MADE BY HALLS *ET AL.,* (2013A) AND DHI (2015).

Type	Period	Estimated Catch (tonnes)
Lot fisheries	January – June	25,000 - 75,000
Medium-scale (artisanal) fisheries employing more than 40 gear types	All year	More than 75,000
***Dai* fishery**	October - March	7,500 - 46,000
Small-scale (subsistence) fisheries	All year	115,000 - 140,00

The annual contribution of all fisheries to Cambodia's total annual catches (estimated as 380-520 million tonnes per year, based on catch surveys covering the 2000-2013 fishing seasons; Hortle, 2007;2009; ICEM, 2010; DHI, 2015) is estimated as over 60% (with the individual contribution of the *dai* fishery to the TSGL and Cambodia's annual reported catches estimated as 14% and 7%, respectively[6]; Campbell *et al.,* 2009; Halls *et al.,* 2013a). It also provides an estimated 70 kg person^{-1} year^{-1} of fish protein consumed by the TSGL population, the highest for the entire Southeast Asia region (So, 2010), with over 50% comprising medium to long distance white and greyfishes (guilds 3 and 4; Table 4.1), while the remaining is shared between blackfish (guild 6; Table 4.1) and aquaculture production (Hortle, 2007;2009; Campbell *et al.,* 2009; Halls *et al.,* 2013a). Regionally, the lake is vital for the entire Mekong basin, acting as water and sediment regulator to the delta (Kummu *et al.,* 2005b). Additionally, it is a fish nursery, feeding and shelter area, ranking 4th globally for lake's fish richness (Campbell *et al.,* 2006;2009; Lamberts, 2006;2008; ICEM, 2010).

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[6] Caution should be taken when making assumptions on the low percentage contribution of the *dai* fishery to the TSGL system and Cambodia's annual total captures, since it is likely that high under-reported catch values is present, associated to household consumption and misleading from other fishery activities (e.g. the *dai* fishery is also under the lot system; Lieng *et al.,* 1995; Lamberts, 2006).

The TSGL is the largest freshwater lake in Southeast Asia (dry season average area: 2,500 km^2; wet season average area: 15,000 km^2; Lamberts, 2008; Kummu *et al.,* 2014) and has achieved the highest ecological recognition, with some zones of the lake marked as UNESCO Biosphere Reserves (UNESCO, 2010).

The lake´s flood pulse dynamics is dependent on the Mekong's hydrologic conditioning, under the influence of the Asian Monsoon Climate System (AMCS; see Chapter 3). During the wet season (May-October), when the waters from the Mekong river reach a higher level than the observed water levels in the lake, a unique flow reverse phenomena occurs, and the mainstream river's waters flow back into the lake through the connecting Tonle Sap River, causing an extensive inundation area (i.e. herein referred as flood area) of over 15,000 km^2 and a maximum depth of 11 meters. The decreasing water levels in the Mekong river, mark the end of the wet season and the start of the dry season (November-April), this is followed by a second flow reversal, with the waters flowing out of lake into the Tonle Sap River and down to the Mekong delta, with a reported maximum discharge of 15,000 m^3 s^{-1}. At the end of the dry season period, the lake reaches its minimum depth of approximately 1.5 meters and a permanent inundation area (i.e. herein referred as lake area) of 2,500 km^2 (Lamberts, 2006;2008; Kummu and Sarkkula, 2008; Campbell *et al.,* 2009; Kummu *et al.,* 2014).

4.3 METHODS

4.3.1 *DAI* FISHERY DATA COLLECTION AND ANALYSES

I) *DESCRIPTION AND LOCATION OF THE DAI FISHERY*

The *dai* fishery, established nearly 140 years ago, represents the longest and most accurate record of Cambodian fisheries and the entire inland fisheries activity of the Mekong basin (van Zalinge *et al.,* 2003; Sarkkula *et al.,* 2004; Halls *et al.*, 2013a)[7]. For this reason, in this chapter the focus will only be in the *dai*

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[7] Although the *dai* fishery has been regarded as the longest and most accurate fishery record for Cambodia and the entire basin, it is important to notice that the long-term data was under different methodologic approaches (i.e. for the sampling strategy), influencing the reported catch and reliability of data. Thus, earliest surveys (1938-1939) by Chevey and Le Poulain (1940) reported the location of the fishery, abundance of fish species and estimated the total catch. Political and social instability in Cambodia (e.g. *Khmer Rouge* period) dramatically influenced the total catch reports in the following years. Only after 1980 onwards the *dai* fishery started to be fully monitored by the Department of Fisheries

fishery. Yet, an improvement in the long-term monitoring and report of total annual catch, should be recommended for the remaining fisheries (Table 4.2).

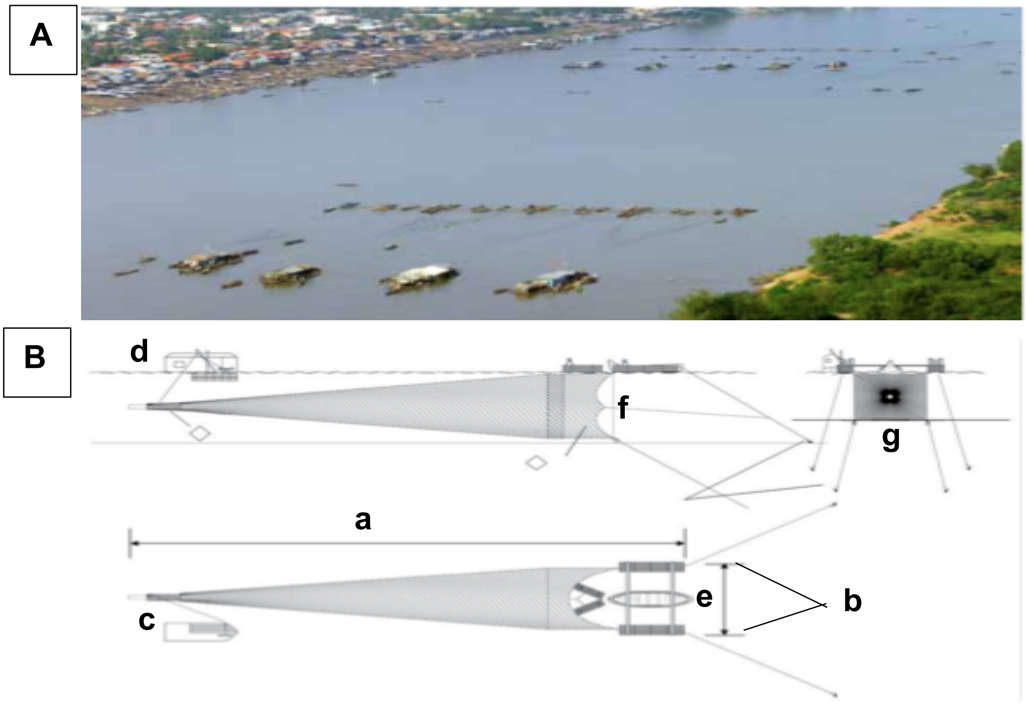

FIGURE 4.3 - A) ROWS OF *DAI* NETS OPERATING IN THE TONLE SAP RIVER. B) EXAMPLE OF A *DAI* NET, COMPRISING A 100-120 METERS LONG CONE-SHAPED NET **(A)** SUSPENDED FROM TWO ANCHORED BAMBOO RAFTS **(B)** AND A FLOATING WORK PLATFORM **(C)** OR A MOVABLE FLOATING HOUSE **(D)**, POSITIONED AT THE END OF THE NET. TO KEEP THE MOUTH OF THE NET OPEN, A SMALL BOAT **(E)** OR FLOATING PLATFORM **(F)** IS SECURED BETWEEN THE TWO ANCHORED RAFTS BY BAMBOO POLES THAT SERVE AS A GANGWAY. THE DISTANCE BETWEEN THE TWO ANCHORED RAFTS (25-27 METERS) AND THE DEPTH OF THE WATER DETERMINES THE NET MOUTH AREA (**G**; SOURCE: HALLS *ET AL.,* 2013A).

The *dai* fishery provides an annual catch of 7.5-46 million tonnes, which represents 7% of Cambodia's total annual landings and generates an economic value of more than US$ 6 million (Hortle *et al.,* 2007;2009; Halls *et al.,* 2013a; DHI, 2015). Additionally, the fishery generates seasonal employment to more than 1.2 million people living in the lake's margin (van Zalinge *et al.,* 2003; Halls *et al.,* 2013a). The fishery consists of artisanal *dai* nets (stationary trawls; Figure 4.3) operating singly or joined with up to six other *dais* in a row, anchored within the river channel, and targeting migratory fish heading out of the lake during the receding of floodwaters on the onset of the dry season (Halls *et al.,* 2013a).

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in Cambodia, before joining the official fisheries monitoring programme of the MRC in 1994, where a standard sampling methodology was implemented (i.e. daily reports of all *dais* in operations), remaining the same until nowadays (Baran *et al.,* 2001; Halls *et al.,* 2013a). Consequently, it is not surprising that such a long-term monitored fishery possesses data limitations for the under-reported values. Nevertheless, it still provides the only reliable long-term information on annual catch, effort and species composition.

The *dai* fishery operates in the Tonle Sap River (spread over 130 km across Kandal and Phnom Penh provinces; see Figure 4.2), from October – March, with a peak in catch occurring in January. Furthermore, the fishery provides valuable source of information on the migratory dynamics of over 50% of the fish species that seasonally utilise the lake for completing their life cycles (Poulsen *et al.,* 2002;2004; van Zaling *et al.,* 2003; Baran, 2010; Halls *et al.,* 2013a).

II) TOTAL ANNUAL CATCH AND EFFORT ANALYSES

As mentioned, during the long-term monitoring of the fishery, a non-standardised sampling methodology was applied until 1994, when the *dai* fishery was incorporated in the monitoring programme of the Mekong River Commission (MRC) and reported in the Official Fishery Logbook by Halls *et al.,* (2013a). The Logbook reported total annual catch (tonnes year^{-1}; 1994-2009), total annual catch effort (kg *dai*$^{-1}$ day^{-1}; 1997-2009 due to absent information regarding 1994-1996. Catch effort is also known as Catch per Unit Effort – CPUE - in fishery studies and it has been used to assess spatio-temporal fishery trends, influenced by the flood pulse in, for example, the Amazon fisheries; Castello *et al.,* 2015;2017; Craig, 2016; Pinaya *et al.,* 2016) and the top five annual species composition of the catch (tonnes year^{-1} for 1997-2009; due to absent information regarding 1994-1996). For this study, the *dai* fishery data information on total annual catch, effort and top five species composition was collated for the period 1994-2014, from Halls *et al.,* (2013a) Official Fisheries Logbook, covering the 1994 – 2009 period. The 2010-2014 data was gathered from the DHI (2015) study. Three main reasons justify this decision:

1) The difficulty in assessing reliable (i.e. without annual gaps and non-standardised survey methods) historical *dai* data from other sources (e.g. Baran *et al.,* 2001; van Zaling *et al.,* 2003);
2) Halls *et al.,* (2013a) is the only available Logbook providing an extended period of *dai* fishery data acquisition (1994-2009), using a standard sampling methodology and reporting total annual catch, effort and species composition, while providing detailed information on the historical fishery trend and the methodology applied for data collection. Moreover, since this Logbook takes part of the MRC extended period of standardised fishery

data collection, it has been under data quality assessment, providing the most reliable available information on the historical fishery trend;

3) The DHI (2015) study focus on predicting future shifts in the fisheries and fish production of the Mekong basin, by applying modelling scenarios of hydropower development. Besides the TSGL system's *dai* fishery, the study also covers other fisheries in the basin, including the *lee trap* fishery in Lao PDR and Vietnam General Statistical Office (GSO) fisheries and aquaculture production (see Chapter 3). The total annual catch from the 2010-2014 *dai* fishing season was a result of the previous findings from Halls *et al.,* (2013a). For this reason, and since the DHI (2015) also checked the reliability of its data sources, it was chosen to cover the 2010-2014 fishing periods of this study for total catch and species composition (only until 2013). Unfortunately, after 2014 no *dai* fishery data has yet been reported, constraining this study to the period 1994-2014.

The analyses are described under the *dai* fishery open season (October – March) from which data is available (Halls *et al.,* 2013a). The April – September period has no fishery data available, due to a closed fishing season.

III) SPECIES COMPOSITION AND GUILD ANALYSES

The top five most common species occurring in the fishery are reported from 1997-2013 and evaluated for its percentage contribution to the annual and overall (1997-2013) total catch. Also, the guilds (see the Introduction section) from each species were also documented and the historical (1997-2013) influence of variations in the flood pulse on the observed top five species composition of each year's catch was assessed (see sub-section 4.3.4).

4.3.2 LANDSAT-TM IMAGES COLLECTION, PROCESSING AND FLOOD MODEL

The Landsat-TM (Thematic Mapper) programme represents the World's most extended (more than 40 years of daily data collection) continuously acquired collection of space-based moderate-resolution land imagery. The programme consists of a series of satellites from Landsat 1-8 (Landsat 9 Programme is due to be launched in 2020) to collect advanced, multispectral real-time images of the

Earth and its resources. TM data are sensed in seven spectral bands simultaneously, with a spatial resolution of 30 meters.

For this study, Landsat images were collected every two months, over a 19 years period (1996-2014) during the dry (November – April) and wet (May – October) seasons. The 19-year period was chosen to match it with the observed water level records of Kampong Luong gauging station (Figure 4.2; only 1996-2014 data available), for assessing annual variations in the lake area and inundation pattern. The sampling period, every 2 months, is in accordance with extended literature, stating that the lake area remains relatively unchanged during the dry season (average area of 2,500 km^2), while in the wet season significant changes can be found, when comparing the lake area in early May (nearly 3,000 km^2) to late August (nearly 12,000 km^2) and a peak occurring in late September/October (more than 15,000 km^2; Kummu and Sarkkula, 2008; Kummu *et al.,* 2014).

Images were selected to account for a <20% cloud coverage whenever possible (Rebelo *et al.,* 2009; Fragal *et al.,* 2016). Although, during the wet season, it was not possible to collect images every two months, since cloud coverage would make it impossible to correctly measure the flood area. Thus, for some years, only late May/early June and peak time (usually October) of the wet season are covered (see Appendix 4). The images were then processed in ArcGIS 10.3 to determine the lake area and a time series constructed over a 19-year period. The errors made in the area measurements were assumed to be at the band resolution of each collected image and was set at 30 m. Following this procedure, a measured area of, for example 4,000 km^2, accounted for an error of 120 km^2 (4,000 km^2 x 0.03 km^2).

The Landsat flood model (1996-2014) was then inferred based on the measured area and plotting it with the observed water levels at Kampong Luong gauging station on the same date of each image collection. Also, using the Landsat flood model equations (see sub-section 4.4.1) and the daily water levels of Kampong Luong gauging station, the historical variations in the lake's mean annual area and maximum and minimum mean annual areas were estimated.

4.3.3 HYDROLOGY AND ENSO DATA COLLECTION AND ANALYSES

I) DATA COLLECTION

Daily water level (m) records were collected from the three gauging stations in the TSGL system: Kampong Luong (KL), Prek Kdam (PK) and Phnom Penh Port (PP Port; Figure 4.2). The water level records were corrected according to the Ha Tien (Vietnam) gauging station, following the standard procedure adopted by the MRC (2009b) and Kummu *et al.,* (2008b;2014), to correct each water level observations from the gauging stations of the Mekong basin, according to the above mean sea level observation of the delta region, where the Ha Tien gauging station is located. A detailed visual inspection of the raw data obtained from the MRC Data Portal revealed inconsistencies in the observed daily water level in the Kampong Luong gauging station for the 1995 year. Therefore, the year 1995 was not included and only records from 1996-2014 were used for the subsequent analyses. The same procedure was applied for Prek Kdam and Phnom Penh Port gauging stations to keep the consistency in the analyses.

The El Niño Southern Oscillation (ENSO) data was collected from the NOAA National Center for Environmental Information[8] covering the period 1996-2014 (see Appendix 5). The data was gathered as a Oceanic Niño Index (ONI; the information and methodology for calculating the ONI is described in the NOAA website and will not be included here, due to the complexity of the same and relatively little purpose for the overall aim of this study). Studies have suggested the role of ENSO events on the flood regime of tropical environments (Räsänen and Kummu, 2013; Frappart *et al.,* 2018). Yet, no study has assessed the long-term influence of ENSO events on the flood level and *dai* fishery of the TSGL system. Thus, ENSO data was compared with the mean annual water level measured at Kampong Luong, to assess its influence on the historical flood level variations of the lake, and with the total catch, for the influence on the *dai* catch.

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[8] www.ncdc.noaa.gov

II) HISTORICAL ANALYSES OF THE TSGL SYSTEM'S FLOOD LEVEL

The historical variations in the lake's water level were estimated following the hydrologic year approach (Kummu and Sarkkula, 2008; Kummu *et al.,* 2014), where the daily water level (meters) at Kampong Luong were gathered at the 1st of May of a given year until the 30th April of the following year. The aim was to understand how much the lake's flood level has changed during a 19-year period, and by what magnitude and with what variability.

III) FLOW BALANCE: INFLOWS AND OUTFLOWS

The TSGL system's complex flow dynamics presents a challenge to correctly estimate the volume of water flowing in (F_{in}) and out (F_{out}) of the lake during the wet and dry seasons, associated to the two flow reversals (Kummu *et al.,* 2014). On the other hand, it is crucial to correctly estimate the amount of water flowing in and out of the lake, since the inflows/outflows not only contribute to the historical flood magnitudes (i.e. 54% according to Kummu *et al.,* 2014), but also to the sediment dynamics (Kummu *et al.,* 2008b). According to Kummu *et al.,* (2008b), the total suspended sediment flux (TSS) into the lake is highest during August, with nearly 2.5×10^9 kg of sediment from the Tonle Sap River, entering the lake, and an outflow to the Mekong river of 1.4×10^9 kg in late October. The flux of sediments carries nutrients that are key to boost the productivity of the system, particularly for enhancing food sources to fishes (Baran *et al.,* 2015).

The application of a standard rating curve for this particular system proves to be a challenge to correctly estimates the inflow/outflow of the lake and the sediment flux (Kummu *et al.,* 2008b;2014). Kummu *et al.,* (2014) employed a numerical model to provide a good fit of the Tonle Sap water balance. The study uses historical water level observations from Kampong Luong, Prek Kdam and Phnom Penh Port gauging stations, to infer a relationship between the flow values inside the lake (Kampong Luong gauging station) and the same day water level measured outside the lake (Phnom Penh Port gauging station). The study also considers the flow diversions from the Tonle Sap River, the principal tributary connecting the TSGL system to the Mekong river (Prek Kdam gauging station). Therefore, the following equation (Equation 4.1) from Kummu *et al.,* (2014) study reflected the flow pattern ($m^3 s^{-1}$) of the TSGL system and was used to estimate in this chapter the inflows (F_{in}; positive values) and outflows (F_{out}; negative values) of the lake's water balance for the period 1996-2014:

$$\boldsymbol{F_{in/out}} = (\boldsymbol{WL_{PK}})^{\mathbf{1.2}} * (|\boldsymbol{WL_{PP}} - \boldsymbol{WL_{KL}}|)^{\mathbf{0.5}} \qquad \textbf{(EQUATION 4.1)}$$

Where $F_{in/out}$ refers to the water flowing in and out of the lake daily. WL corresponds to the observed water levels (meters) for Kampong Luong (WL_{KL}), Prek Kdam (WL_{PK}) and Phnom Penh Port (WL_{PP}).

4.3.4 THE HISTORICAL IMPORTANCE OF THE FLOOD PULSE TO THE *DAI* FISHERY AND MIGRATORY GUILD SPECIES COMPOSITION

To understand how historical changes in the flood pulse (i.e. timing, duration, amplitude, smoothness and continuity) influenced the *dai* fishery and migratory guild species composition of the catch, different methodologies were applied in an analysis following Halls *et al.* (2013a) and DHI (2015). First, the total annual catch (tonnes) was related to the mean annual water level (meters) from Kampong Luong, to infer a relationship for the period (1996-2014). Then, to quantify the long-term influence of variations in floodplain flood duration and extent (i.e. flood index) on total catch and migratory guild species composition, the definition of a lake's flood event was first settled for the entire model period (1996-2014). This was done by, using the break slope analysis of the Landsat flood model equations for the dry and wet season periods (see sub-section 4.4.1), to estimate daily lake area. Because both dry and wet season's equations required water level inputs, daily water levels from Kampong Luong gauging station were used to calculate the correspondent lake's daily areas. Then, an average for each year lake area was calculated following the Julian calendar (1st January-31st December). After averaging the lake area per year, a total model period (1996-2014) average of the lake area was calculated (Equation 4.2). The calculated value was used as a threshold for defining a flood event, where areas above the value were considered as flood areas.

$$\textbf{FLOOD EVENT: } \frac{\sum_{\boldsymbol{Y=19}} \boldsymbol{La}}{\mathbf{19}} \qquad \textbf{(EQUATION 4.2)}$$

Where L_a represents the lake's averaged area measured each year during the 19-year period (Y=19). The sum of each year's lake averaged area was performed and divided by the number of year observations (19) to calculate the averaged model period (1996-2014) lake area (i.e. threshold area). After defining the threshold area, the sum of daily flood areas and duration count by the timing (start and end) were recorded (Table 4.5), to assess annual variations in the flood index. The Flood Index (FI) sums the flooded area on day d (F_a) above the mean flooded area of the model period. This index has been used to understand how combined changes in flood duration, timing and extent influence the productivity of a system (Halls *et al.,* 2013a). The flood index quantifies both extent and duration of the flood each year as (Equation 4.3):

$$\mathbf{FI_Y} = \sum_{d} Fay, d \qquad \textbf{(EQUATION 4.3)}$$

Where FI_y represents the flood index of a given year (km^2 days), measured by the sum of the flooded area per year (F_{ay}) and the duration (d), measured above the mean flooded area for the model period (1996-2014). The annual flood index was related with the annual *dai* catch to infer a long-term relationship. Also, a relationship was assessed between the annual flood index and each of the top five species composition of the annual *dai* catch, to assess the historical importance of variations in floodplain habitats flood dynamics to the fish production of each migratory fish guild species.

4.3.5 STATISTICAL ANALYSES

The statistical analyses in this chapter applied linear and non-linear regression fit analyses, to predict how one variable explained the other (Zar, 2010). One-way ANOVA analyses were employed to infer the significance of the regression fit. The statistical analyses focused on:

- Can the Landsat flood model predict a good fit relationship between the historical seasonal variations in the lake area and the observed water level from Kampong Luong gauging station?
- Has the flood level of the lake changed significantly during the study period?
- Is there a significant relationship between the annual variations in the lake's flood level and the observed *dai* fishery?
- Do historical variations in the annual flood index significantly influence the observed *dai* catches?

- Do historical variations in the annual flood index significantly influence the top five *dai* fishery species composition? What guilds are more affected?

All analyses were performed in SPSS 24.0 and interpreted with reference to the 95% confidence level (Zar, 2010).

4.4 RESULTS

4.4.1 THE LANDSAT FLOOD MODEL AND THE LAKE'S HISTORICAL FLOOD AREA DYNAMICS

The Landsat flood model for the entire 19-year period of Landsat-TM data collection is presented in Equation 4.4:

$$A_{(D)} = 1517.9 * W_{KL(D)} - 1178.9 \qquad \textbf{(EQUATION 4.4)}$$

Where $A_{(d)}$ is the area of the lake measured at a specific date and $W_{KL(d)}$ refers to the observed water level (meters) recorded from Kampong Luong gauging station at the specific date of the Landsat-TM image collection.

A comparison between the digitised lake area and corresponding water levels is presented in Figure 4.4. The linear regression fit analysis best describes the influence of the lake's water level (meters), observed at Kampong Luong gauging station, to the 19-year variations in the lake area from the Landsat-TM images collection. Moreover, the break slope analysis from Equation 4.4 (Figure 4.4) shows that during the dry season (Equation 4.5) the lake area remained relatively stable (black triangles), with an average area of 2,358 km^2 and a maximum water level of 4.5 meters. The transitional period from dry to wet season (orange circles; Equation 4.6) is marked by a rapid increase in the area, from an average of 2,358 km^2 to an average of 8,100 km^2 (early August), as water levels rise from 4.5 to nearly 6 meters. From 6 meters, the lake area shows a plateau of gradual increase, reaching a maximum flood area of 16,389 km^2 at a maximum water level of 10.5 meters. The strong association is described by the coefficient of determination (R^2 is a measure of the goodness of fit when plotting one variable against the other to infer how much one variable explains the variation observed in the other; Zar, 2010), showing that historical changes in the lake area are significantly explained, in 77% (R^2=0.77; ANOVA; $F_{(1,53)}$=73.46; p=0.00) during the dry season and 89% (R^2=0.89; ANOVA; $F_{(1,43)}$=345.97; p=0.00) during the wet season, by the variation in the water level.

DRY SEASON LANDSAT AREAS (KM^2): Y=62.365X+2275.3 (EQUATION 4.5)

WET SEASON LANDSAT AREAS (KM^2): Y=1913.1X-3775.5 (EQUATION 4.6)

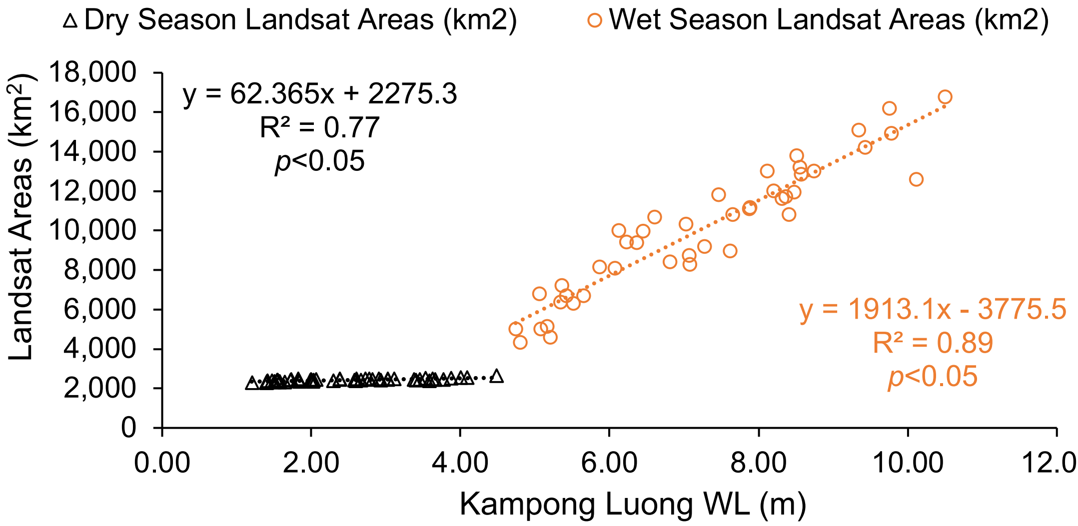


FIGURE 4.4 - BREAK SLOPE ANALYSIS SHOWING THE LINEAR FIT EQUATIONS, COEFFICIENT OF DETERMINATION (R^2) AND SIGNIFICANCE (*P*-VALUE) FOR THE DRY (BLACK) AND WET (ORANGE) SEASONS. BOTH SEASONS SHOW A SIGNIFICANCE ASSOCIATION BETWEEN THE LAKE'S WATER LEVEL (METERS) AND THE MEASURED LAKE AREA FROM THE LANDSAT-TM IMAGES.

Annual variations in the lake's mean area are presented in Figure 4.5. The annual mean areas were calculated from the daily areas, estimated using the break slope analysis' equations (Equation 4.5 and Equation 4.6) and the daily water level (meters) records at Kampong Luong gauging station, for the period 1996-2014. During the 19-year period, it is shown that the mean lake area does not vary significantly (ANOVA, $F_{(1,17)}$=1.19; p=0.29) at an annual scale. However, periods of low mean lake area (1998 and 2010) are visible (Figure 4.5).

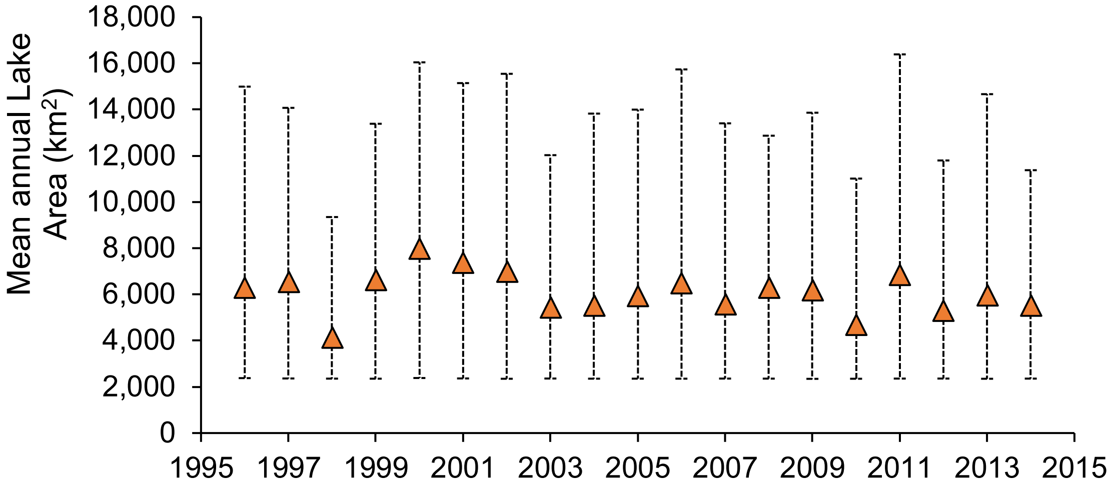
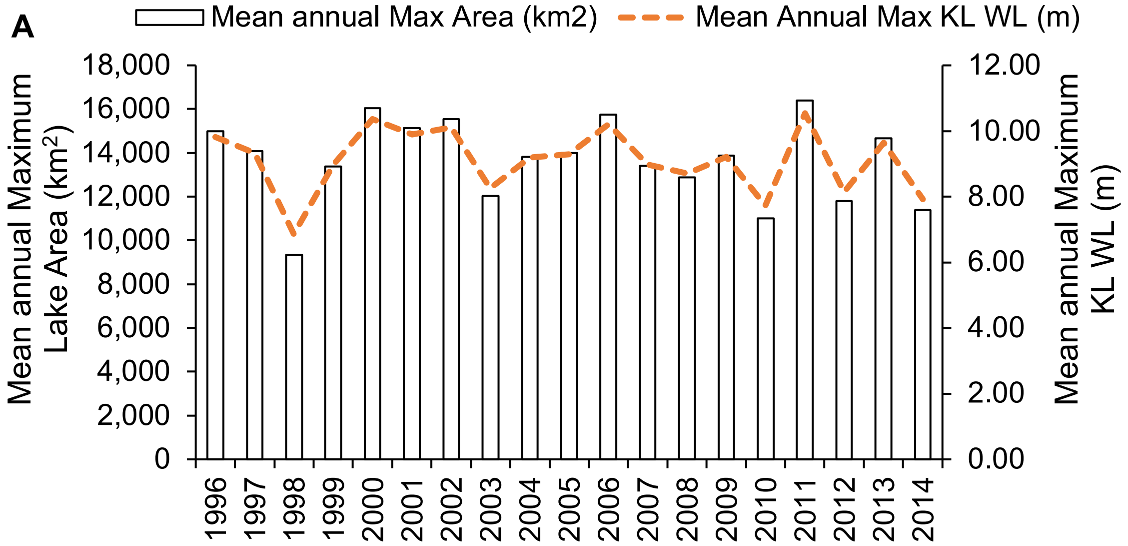


FIGURE 4.5 - HISTORICAL ANNUAL VARIATIONS IN THE LAKE AREA. THE MEASUREMENTS REFLECT THE USE OF THE LANDSAT FLOOD MODEL'S BREAK SLOPE EQUATIONS TO ESTIMATE DAILY AREAS. THE DASHED LINES REPRESENT THE MEAN MAXIMUM AND MINIMUM AREAS (KM^2), AND THE ORANGE TRIANGLE REPRESENTS THE MEAN AREAS (KM^2).

Additionally, inter-annual variations can be observed in the mean maximum lake area (Figure 4.6**; A**), reflecting annual variations in the mean maximum water level. Yet, the mean minimum lake area (Figure 4.6**; B**) remained relatively constant throughout the 19-year period, as previously identified in the break slope analysis (Figure 4.4), reflecting the historical low level of variations in the mean minimum water level of the lake (Figure 4.6). These results help to understand the importance of the historical predictable seasonal flood magnitude variations (i.e. for the wet and dry season variations in water level) of the lake in influencing the variations in the lake area.



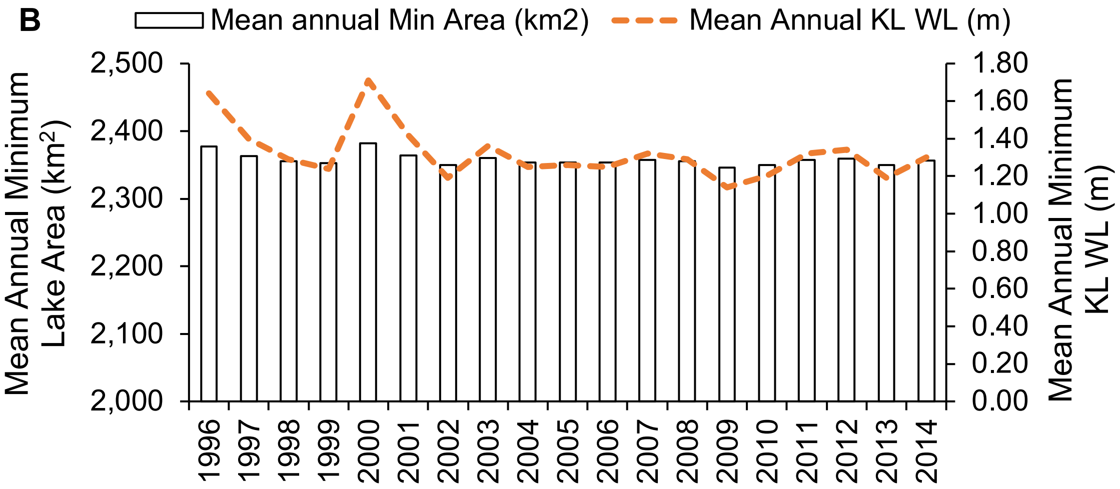


FIGURE 4.6 - HISTORICAL ANNUAL VARIATIONS IN THE LAKE'S MEAN MAXIMUM **(A)** AND MEAN MINIMUM **(B)** AREAS, REFLECTING THE VARIATIONS IN THE MEAN MAXIMUM AND MINIMUM WATER LEVELS (METERS) OBSERVED AT KAMPONG LUONG GAUGING STATION. COLUMNS REPRESENT THE AREAS (KM^2) AND THE ORANGE DASHLINE THE WATER LEVEL (METERS).

The historical monthly variability of the lake's water level (meters) measured at Kampong Luong gauging station is present in Figure 4.7. The blue and yellow lines represent the mean maximum (blue) and mean minimum (yellow) flood levels of the entire period, observed in 2011-2012 and 1998-1999, respectively. Furthermore, a detailed annual analysis (following the hydrologic year approach) for the 19-year period, showed that from 1996-2014 the mean monthly water level did not significantly change (ANOVA; F= 2.52; p=0.57) between years. Therefore, the dashed red line represents the average of the monthly water level observations for the period 1996-2014. Of note, it is possible to see a peak flood occurring in October, followed by a steady decrease from October until April, marking the end of the wet season and beginning of the dry season. The results show the historical predictability and seasonality of the flood pulse dynamics of the lake, with the peak flood for all years and duration of both seasons, being relatively stable during the model period. The effects of the relatively stable flood pulse dynamics on floodplain habitats flood dynamics and fishery resources will be next assessed.

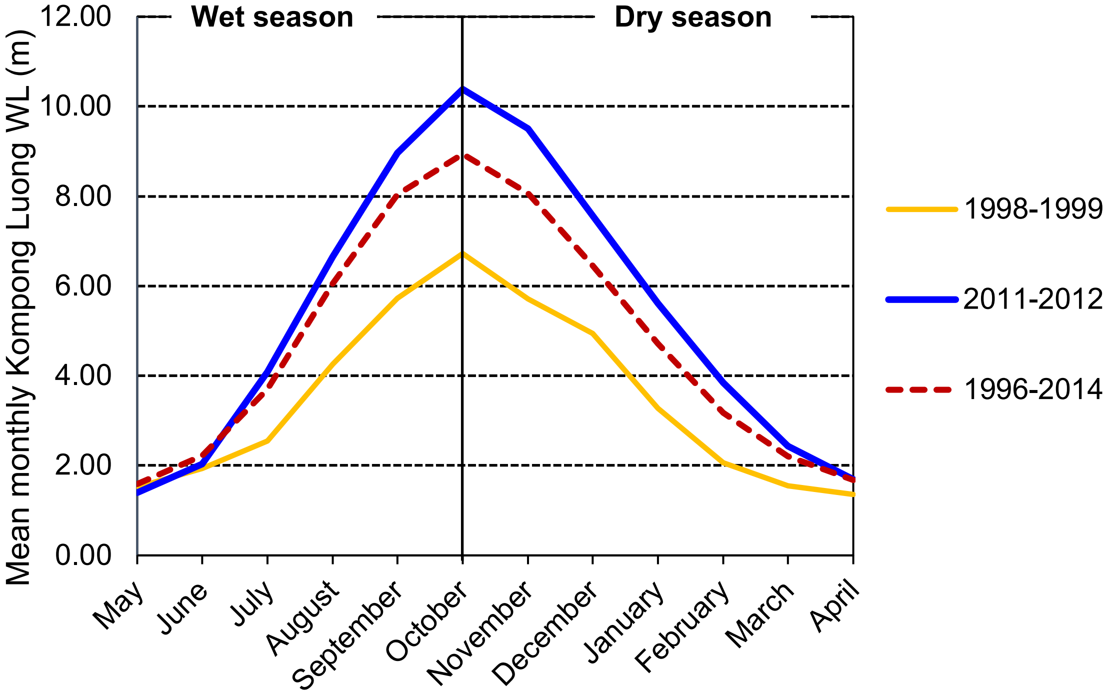


FIGURE 4.7 - INTRA-ANNUAL (MONTHLY) VARIATIONS IN THE LAKE'S FLOOD LEVELS OBSERVED AT KAMPONG LUONG, FOLLOWING THE HYDROLOGIC YEAR APPROACH. THE BLUE LINE REPRESENTS MEAN MAXIMUM OBSERVED WATER LEVEL (METERS) FOR THE ENTIRE PERIOD, THE YELLOW LINE REPRESENTS MEAN MINIMUM OBSERVED WATER LEVEL (METERS) FOR THE ENTIRE PERIOD, AND THE RED DASHLINE REPRESENTS THE MEAN FOR THE 1996-2014 PERIOD.

4.4.2 THE STATE OF THE *DAI* FISHERY: TOTAL ANNUAL CATCH, EFFORT AND SPECIES COMPOSITION

Figure 4.8 presents the annual *dai* fishery catch records of the TSGL system for the 1994-2014 period. The inter-annual percentage (%) variation in the total catch is also shown. The sum of catch for the 20 years period (1994-2014) was 384,385 tonnes, with an average of 19,219 tonnes. Regarding observed inter-annual variations in the total catch, although an overall stability is seen for the 20-year period, marked variations are present for few individual fishing seasons. Thus, a 137% increase in the total catch of the 2000-2001 fishing season (29,140 tonnes) is observed, compared with the previous fishing season (12,281 tonnes). The same was detected for the 2004-2005 fishing season with an increase of the catch for that year of 144% (25,895 tonnes), compared to the previous year catch of 10,596 tonnes. The 2011-2012 fishing season exhibited the highest catch for the entire period, with 46,000 tonnes and an increase of 437% compared with the previous year that registered the lowest catch (8,560 tonnes) and a 35% decrease in catch, compared with the previous year (13,216 tonnes). The 2012-

2013 period showed a 65% decrease in catch (15,989 tonnes), followed by a 24% increase in the following year.

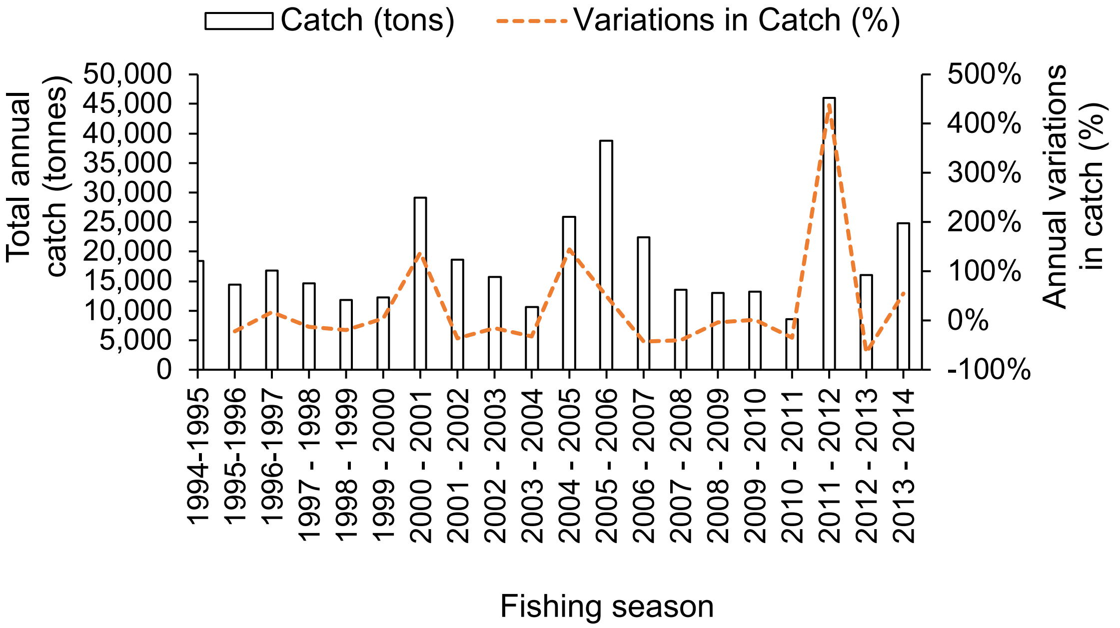


FIGURE 4.8 - COMPARISON BETWEEN THE TOTAL ANNUAL *DAI* CATCH (TONNES) AND INTER-ANNUAL VARIATIONS IN CATCH (%). COLUMNS REPRESENT THE CATCH (TONNES) AND THE ORANGE DASHLINE REPRESENTS THE VARIATIONS IN CATCH (%).

Halls *et al.,* (2013a) analysed in Figure 4.9 the monthly (October-March) variations in the catch effort ($\log_e$-transformed CPUE, kg *dai*$^{-1}$ day^{-1}) from each year's fishing season during 1997-2009. Significant (ANOVA; $F_{(1,72)}$=170.97; *p*=0.001) variations were detected between and within the six months comprising each year's fishing season, with the catch effort peaking in January (mean= 10,818 ± 18.709 kg *dai*$^{-1}$ day^{-1}) and being the lowest in October (mean= 86.9 ± 438.7 kg *dai*$^{-1}$ day^{-1}). Moreover, between fishing seasons, it is possible to see an overall (i.e. mean catch effort for all months) increasing pattern in the fishing effort, although, for individual months of some fishing seasons (e.g. the November catch effort of 2006-2007, compared to the November catch effort of 2007-2008) decreases in the fishing effort are also observed.

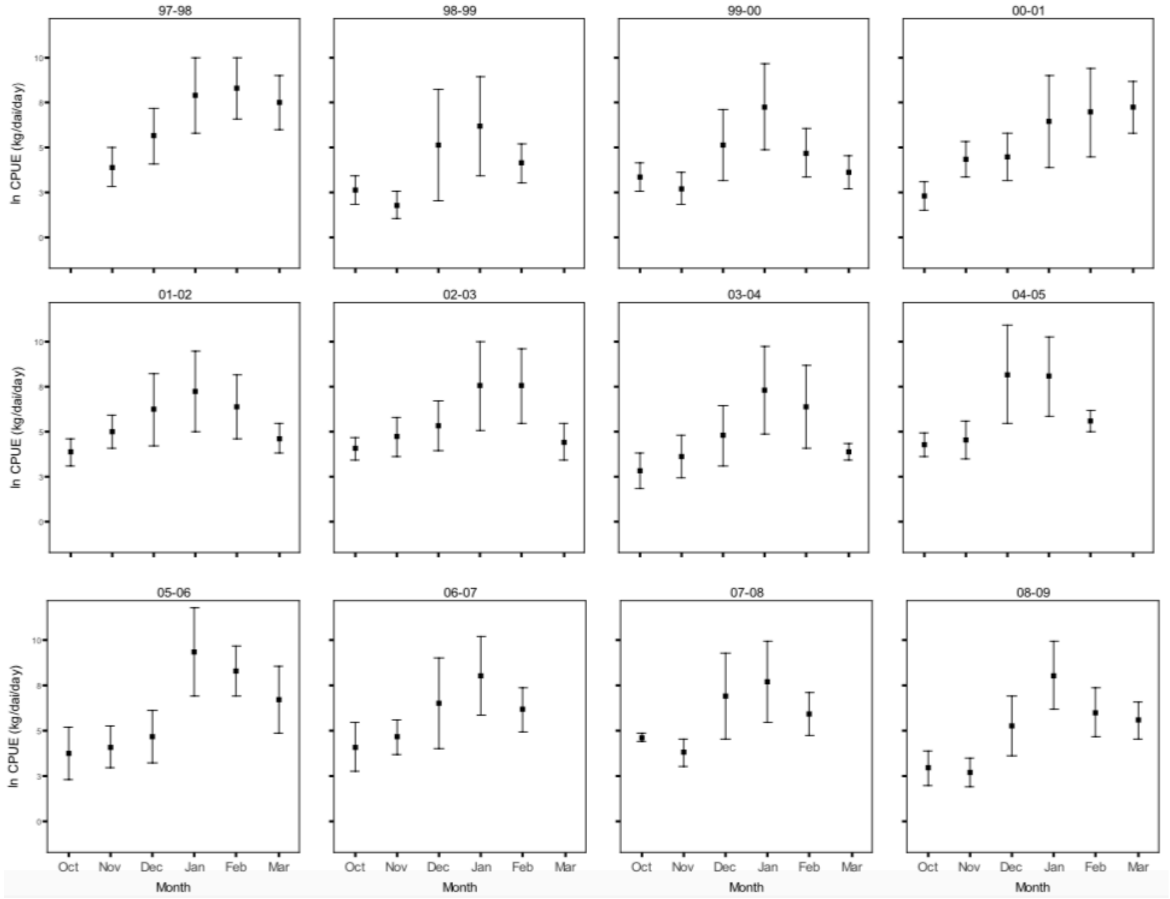


FIGURE 4.9 - MONTHLY VARIATIONS IN THE *DAI* FISHERY CATCH EFFORT (CPUE; LOG$_E$-TRANSFORMED KG *DAI*$^{-1}$ DAY^{-1}) FROM THE OPEN FISHING SEASON (OCTOBER-MARCH), COVERING THE PERIOD 1997-2009. THE MEAN CATCH EFFORT IS SHOWN IN BLACK CIRCLES, WHILE LINES REPRESENT THE STANDARD DEVIATION (SOURCE: HALLS *ET AL.*, 2013A).

The *dai* fishery primarily targets small cyprinids of the *Cirrhinus*, *Labiobarbus* and *Henicorhynchus* genus, traditionally known in Cambodia as *trey riel* (in Khmer) and classified as whitefish (Rainboth, 1996; Poulsen *et al.,* 2002; Halls *et al.,* 2013a). Other species contributing to the overall catch are pelagic river carp and loaches. Thus, this fishery aims to target the small body-size migratory fish species leaving the lake during the onset of the dry season (Baran *et al.,* 2001; van Zalinge *et al.,* 2003; Halls *et al.,* 2013a; Termvidchakorn and Halls, 2013; DHI, 2015). Medium to large body-size fish species have also been caught in this fishery (e.g. Pangasiidae family; whitefish; Baran *et al.,* 2001; Halls *et al.,* 2013a). Table 4.3 shows the annual reported catch of the top five species during the 1997-2013 period. The total catch of the top five species, for each year and the annual percentage contribution to the total *dai* fishery of that year is presented. Furthermore, each species overall catches and the percentage contribution for the 1997-2013 period is shown. The overall total catch and the percentage contribution for the 1997-2013 catch are present as well.

The *Henicorhynchus sp*. (Cyprinidae) group small body-size whitefishes (information on all species maximum body-size is presented in Table 4.6 in the Discussion section) like: *Henicorhynchus lobatus, Henicorhynchus siamensis* and *Henicorhynchus cryptopogon* (Termvidchakorn and Halls, 2013). This genus makes the highest overall contribution in the observed catch, with estimations of 103,241 tonnes and 33.3% for the 1997-2013 period. The Other sp. refers to the group of individual species that contributed individually less in the fishery. These are: *Puntioplites proctozysron* (Cyprinidae), *Pangasius pleurotaenia* (Pangasiidae), *Botia modesta* (Cobitidae), *Clupeichthys aesarnensis* (Clupeidae), *Pangasius larnaudii* (Pangasiidae), *Labiobarbus siamensis* (Cyprinidae), *Cirrhinus microlepis* (Cyprinidae) and *Syncrossus helodes* (Cobitidae; Halls *et al.,* 2013a). This group presents the highest annual catch from 1997-2003, after that, a decline can be observed in the annual catch of species belonging to this group. The group also possesses the second highest observation in the overall catch, with 79,099 tonnes and 25.51% contribution to the 1997-2013 fishing period. *Paralaubuca barroni* (Cyprinidae), *Labiobarbus lineatus* (Cyprinidae), *Labeo chrysophekadion* (Cyprinidae) and *Thynnichthys thynnoides* (Cyprinidae) make the third, fourth and fifth contribution to the overall catch, with 10.61%, 7.59%, 2.32% and 1.98% contribution for the 1997-2013 period, respectively. High inter-annual variations in the reported catch for the top five species can be observed. Overall, the Cyprinidae family dominates the landings with the majority belonging to the white and greyfish guilds, and the top five species in the *dai* fishery contributed annually on average over 60% to the catch and over 80% to the catch of the entire period (Table 4.3)

TABLE 4.3 - TOP FIVE SPECIES PRESENT IN THE ANNUAL *DAI* FISHERY (TONNES) FROM 1997-2013. THE ANNUAL TOTAL CATCH AND PERCENTAGE (%) CONTRIBUTION TO THE *DAI* FISHERY OF THAT YEAR OF THE FIVE SPECIES IS HERE PRESENT. ALSO, INDIVIDUAL SPECIES TOTAL CATCH AND CONTRIBUTION (%) TO THE OVERALL PERIOD (1997-2013) IS SHOWN, ALONG WITH THE TOTAL CATCH AND PERCENTAGE CONTRIBUTION (%) TO THE ENTIRE FISHING SEASON (1997-2013). THE PERCENTAGE (%) CONTRIBUTIONS WERE CALCULATED BASED ON THE TOTAL REPORTED CATCHES OF EACH FISHING SEASON PRESENT IN FIGURE 4.8.

Species composition	97-98	98-99	99-00	00-01	01-02	02-03	03-04	04-05	05-06	06-07	07-08	08-09	09-10	10-11	11-12	12-13	Total	97-13 (%)
Henicorhynchus sp.	4,270	3,333	2,759	6,184	3,642	2,863	2,715	9,386	12,712	9,321	4,844	5,126	4,519	3,878	21,890	5,799	103,241	33.30
Labeo chrysophekadion	400	140	535	440	597	417	181	400	769	774	293	235	180	157	1,425	236	7,179	2.32
Labiobarbus lineatus	671	207	222	807	1,486	1,090	594	859	1,814	2,336	1,279	1,327	898	1,589	5,767	2,573	23,519	7.59
Paraubuca barroni	1,345	1,179	775	2,741	605	814	462	2,508	6,754	3,386	1,426	1,081	890	951	6,306	1,655	32,878	10.61
Thynnichthys thynnoides	592	336	369	937	290	475	177	224	455	670	271	246	122	77	719	180	6,140	1.98
Other sp.	4,874	3,824	7,155	9,423	11,886	9,230	1,695	5,397	10,663	5,481	2,707	2,174	420	1,120	2,307	743	79,099	25.51
Total	**12,152**	**9,019**	**11,815**	**20,532**	**18,506**	**14,889**	**5,824**	**18,774**	**33,167**	**21,968**	**10,82**	**10,189**	**7,029**	**7,772**	**38,414**	**11,186**	**252,056**	**81.31**
% Contribution to Total Catch	**83.23**	**76.52**	**96.21**	**70.46**	**99.62**	**94.73**	**54.96**	**72.50**	**85.65**	**98.11**	**80.01**	**78.32**	**53.19**	**90.79**	**83.51**	**69.96**	**81.21**	

Table 4.4 shows the guild analysis for each of the top five species and the proportion in the total catch for the fishing period (1997-2013). The categories “very high”, “medium” and “little or no impact” are for the likely effects of hydropower development on the migration pattern of these species, following the methodology applied in Halls and Kshatriya (2009). The guilds 3 and 4 together represent 53.82% contribution to the overall catch for the period 1997-2013. This means that more than 50% of the *dai* fishery is composed by white and grey fish that seasonally perform long and medium distance migrations from and to the lake, and will likely be affected by hydropower development expected to be built in the Mekong basin (see the Discussion section; Halls and Kshatriya, 2009).

TABLE 4.4 - GUILD ANALYSIS FOR THE TOP FIVE SPECIES PRESENTED IN THE *DAI* FISHERY FOR THE PERIOD 1997-2013. THE PROPORTION OF CATCH (%) AND THE LIKELY EFFECTS OF HYDROPOWER DAM DEVELOPMENT FOLLOWED THE METHODOLOGY APPLIED IN HALLS AND KSHATRIYA (2009) STUDY.

Species name	Guild	Proportion of Catch (%)	Likely impacts of mainstream dams on migration
Henicorhynchus sp.	3	35.62	Very high
Labeo chrysophekadion			
Labiobarbus lineatus	4	18.20	Medium
Paralaubuca barroni			
Thynnichthys thynnoides	5	1.98	Little or no impact
Other sp.		25.51	

The Other sp. group presents 25.51% contribution to the overall catch. This group is composed by *Puntioplites proctozysron* and *Pangasius pleurotaenia* belonging to **guild 3**; *Botia modesta*, *Pangasius larnaudii*, *Labiobarbus siamensis* and *Syncrossus helodes* belonging to **guild 4**; *Clupeichthys aesarnensis* belonging to **guild 1** and *Cirrhinus microlepis* belonging to **guild 5** (Halls and Kshatriya, 2009; Halls, 2010). Adding to the results of Table 4.3, it is likely that nearly 80% of the species caught in the *dai* fishery are composed by medium to long distance migratory species (guilds 4 and 3, respectively).

4.4.3 HISTORICAL EFFECT OF THE TSGL SYSTEM FLOOD PULSE ON THE *DAI* CATCH

Since the lake's flood level showed relatively stable inter and intra-annual variations during the 19-year period, the next analysis shows how historical annual variations in the flood level influenced the *dai* fishery (Figure 4.10). The water level (meters) presented are mean annual values for the fishing season.

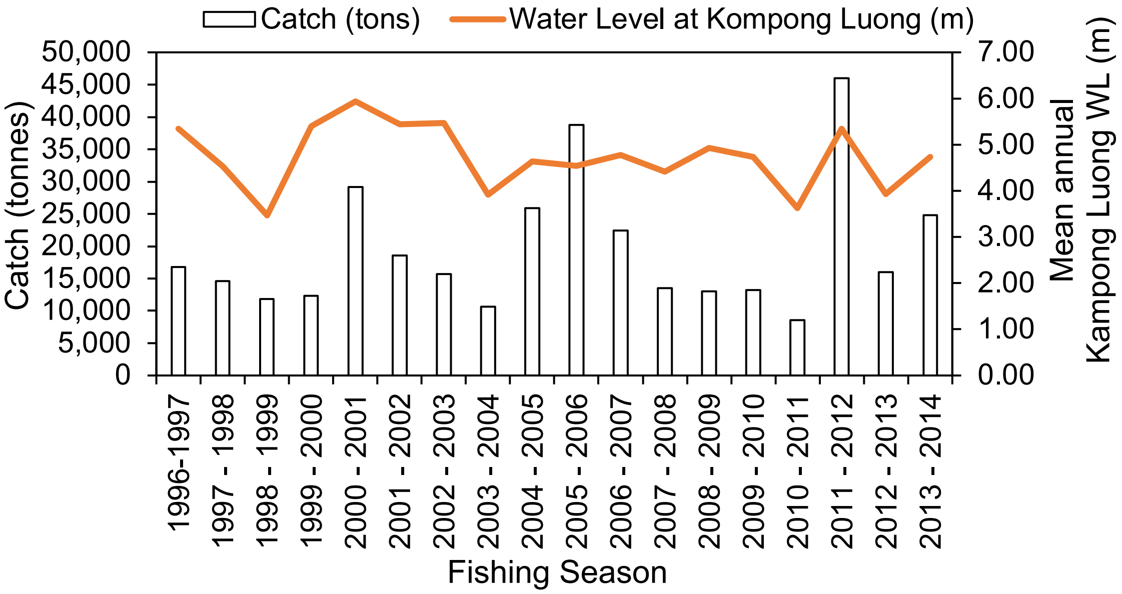


FIGURE 4.10 - HISTORICAL VARIATIONS IN THE ANNUAL *DAI* CATCH AND ITS RELATIONSHIP WITH THE MEAN ANNUAL WATER LEVEL (M) RECORDS FROM THE KAMPONG LUONG GAUGING STATION. COLUMNS REPRESENT CATCH AND THE ORANGE LINE REPRESENTS WATER LEVEL.

The results from Figure 4.10 show that increases and declines in the water level of the lake follow the observed increases and declines in the reported total annual catch. Exceptions to this pattern are observed for the 2002-2003 period, where an increase in the mean water level, does not show the same trend in the reported total catch for that period. Also, the fishing seasons corresponding to 2005-2006 and 2011-2012 show higher total catches compared to the mean water levels.

The influence of the lake's mean annual water level on the reported total catches from 1996-2014 is presented using a logarithmic regression fit analysis (Figure 4.11; Equation 4.7). The logarithmic regression fit was applied instead of a linear regression fit in accordance with previous work from Baran *et al.,* (2001), who showed that biological responses to environmental variations are not linear, but asymptotic with the logarithmic fit best describing the trend. Although a non-statistically significant relationship was found, the lake's mean annual water level variations explained in 18% (R^2=0.18; ANOVA; $F_{(1,17)}$=3.46; *p*=0.08) the variation in catch.

CATCH (TONNES) RELATION WITH KL WL (M): Y=28275LN(X)-24105 (EQUATION 4.7)

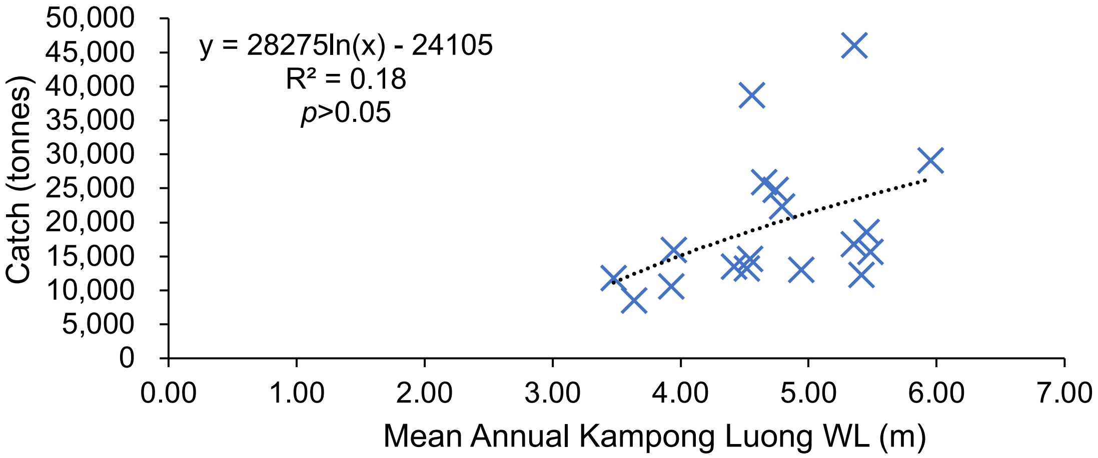


FIGURE 4.11 - INFLUENCE OF THE MEAN ANNUAL WATER LEVEL (M) OF THE LAKE TO THE TOTAL ANNUAL *DAI* CATCHES (TONNES) FOR THE 1996-2014 FISHING PERIOD. THE LOGARITMIC REGRESSION FIT ANALYSIS SHOWED A NON-STATISTICALLY SIGNIFICANT EFFECT (P>0.05).

To understand how historical variations in the flood pulse-driven floodplain flood dynamics influenced the reported *dai* catches, first a flood event was established following the methodology described in sub-section 4.3.4. The threshold was settled at **6,093 km^2** for the entire period (1996-2014). That is to say that areas in excess of this value were deemed as flood areas. Subsequently, using the threshold value, the sum of the areas above it and the duration for each year were calculated, to infer the flood index (km^2 days; Equation 4.3) of each year. The flood index describes variations in floodplain flood duration and extent, which is argued to influence the *dai* fisheries (Baran *et al.,* 2001; Halls *et al.,* 2013a). Thus, Table 4.5 presents the floodplain flood start and end dates, based on the duration of a flood event. The long-term variations in floodplain flood timing and duration show the predictable and seasonal influence of the relatively stable dry (November-April) and wet seasons flood levels (May-October; Figure 4.7), with floodplain flooding starting in July-August and December-January marking the decrease in flooding and consequent second flow reversal, with the waters flowing out of the lake. Likewise, a relatively stable pattern of flood index is observed for the entire period (averaged for the entire period of 721,626 km^2), although marked amplitude variations are observed for the maximum flood index (1,225,074 km^2 days; 2000-2001) and minimum flood index (157,057 km^2 days; 1998-1999; Table 4.5), which will be further explored in the Discussion for the linkage with the same flood season observed maximum and minimum flood level variations (Figure 4.7) and role of ENSO events (see sub-section 4.4.5).

A statistically significant exponential regression fit analysis was inferred between the historical flood index and the total annual catches (Figure 4.12; Equation 4.8). The application of an exponential fit is in accordance with the methodology applied by Halls *et al.,* (2013a), describing an exponential effect of the variations in the annual flood index on the *dai* fishery. The coefficient of determination shows that 31% (R^2=0.31; ANOVA; $F_{(1,17)}$=4.78; p=0.03) of the annual variations in the *dai* catch were explained by the variations in the annual flood duration and extent (flood index), for the 19-year period.

The importance of the historical variations in the floodplain habitats flood pulse dynamics (particularly for the flood index) on the top five species composition of the *dai* fishery was quantified (Figure 4.17) and will be presented and discussed in the Discussion section (see sub-section 4.5.3).

CATCH (TONNES) RELATION WITH FLOOD INDEX (KM^2 DAYS): Y=9193.3E^9E-07X (EQUATION 4.8)

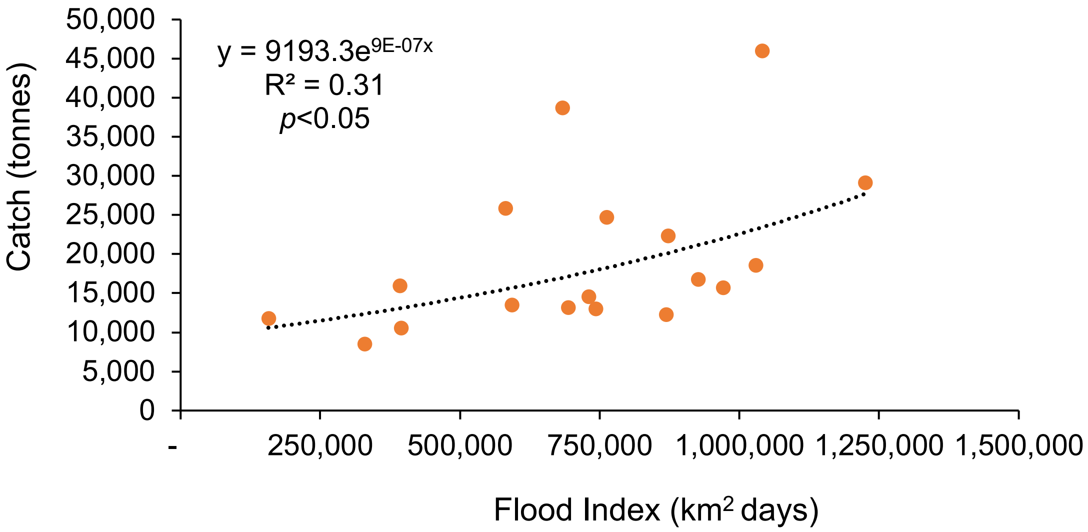


FIGURE 4.12 - EXPONENTIAL REGRESSION FIT ANALYSIS TO INFER A RELATIONSHIP BETWEEN ANNUAL VARIATIONS IN THE FLOOD INDEX AND THE TOTAL *DAI* CATCH FOR THE 19-YEAR PERIOD (R^2). STATISTICALLY SIGNIFICANT DIFFERENCES (P<0.05) WERE DETECTED.

TABLE 4.5 - HISTORICAL ANALYSES (1996-2014) OF THE FLOOD START, END, DURATION (DAYS), FLOOD INDEX (KM^2 DAYS) AND CATCH (TONNES). THE POSITIVE AND NEGATIVE VALUES REPRESENT THE INTER-ANNUAL VARIATIONS IN THE DURATION, FLOOD INDEX AND CATCHES.

Flood Season	Flood start	Flood end	Duration (days)	Variation in days	Flood Index (km^2 days)	Variation in Flood Index	Catch (tonnes)	Variations in Catch (tonnes)
96-97	09/08/1996	01/02/1997	177		926,316		16,800	
97-98	04/08/1997	27/12/1997	146	-31	730,242	-196,074	14,600	-2,200
98-99	03/09/1998	01/12/1998	90	-56	157,057	-573,185	11,787	-2,813
99-00	08/07/1999	24/01/2000	201	111	868,768	711,711	12,281	494
00-01	03/07/2000	24/01/2001	206	5	1,225,074	356,306	29,140	16,859
01-02	15/07/2001	20/01/2002	190	-16	1,028,765	-196,308	18,577	-10,563
02-03	21/07/2002	30/01/2003	194	4	970,141	-58,624	15,718	-2,859
03-04	21/08/2003	16/12/2003	118	-76	394,789	-575,352	10,596	-5,122
04-05	08/08/2004	04/01/2005	150	32	581,320	186,531	25,895	15,299
05-06	11/08/2005	03/01/2006	146	-4	683,123	101,803	38,726	12,831
06-07	05/08/2006	03/01/2007	152	6	871,773	188,651	22,392	-16,334
07-08	18/08/2007	07/01/2008	143	-9	592,260	-279,513	13,524	-8,868
08-09	27/07/2008	14/01/2009	172	29	742,617	150,357	13,010	-514
09-10	29/07/2009	02/01/2010	158	-14	692,898	-49,719	13,216	206
10-11	03/09/2010	24/12/2010	113	-45	328,944	-363,954	8,560	-4,656
11-12	31/07/2011	23/01/2012	177	64	1,040,777	711,833	46,000	37,440
12-13	17/08/2012	14/12/2012	120	-57	392,270	-648,507	15,989	-30,011
13-14	10/08/2013	16/01/2014	160	40	762,139	369,868	24,764	8,775

4.4.4 THE LAKE'S HISTORICAL COMPLEX FLOW DYNAMICS

The flow balance (inflows and outflows) of the TSGL system is presented in Figure 4.13. It is possible to see that the peak inflow (positive orange curve) was on average nearly 10,000 m^3 s^{-1} and occurred in July/August, while the peak outflow (negative orange curve) had a value of approximately 9,000 m^3 s^{-1} and peaked in late October – early November. These results help to understand the verified fluctuations in the flood start and end presented in Table 4.5. Furthermore, little inter-annual variations were found for the 1996-2014 period. Of note, although Kummu *et al.,* (2014) only covered the period 1997-2004, the rating curve extended here shows a good fit in predicting the 19-year period of water balance. The observed variations in the inflows/outflows will be covered in the Discussion section, for the Mekong basin's historical flow conditions, importance for the bi-directional flow reverse and contribution to the flood dynamics in the TSGL system for the aquatic biota supported, and the likely impacts from water infrastructure development (Grumbine and Xu, 2011; Cochrane *et al.,* 2014).

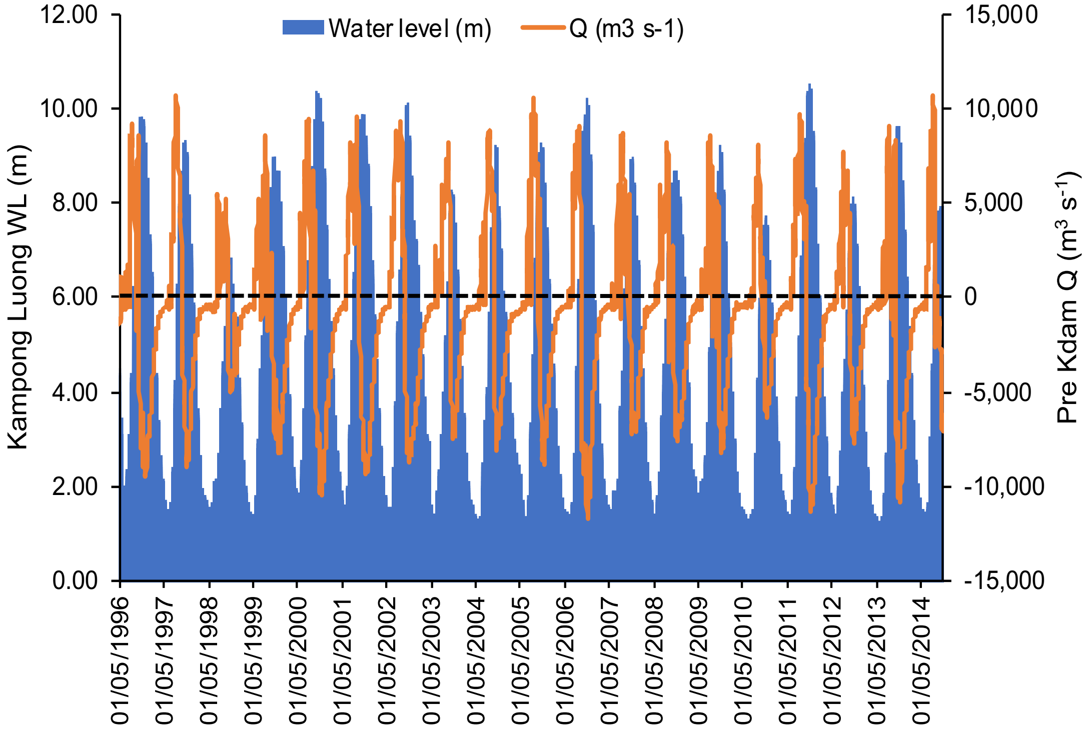


FIGURE 4.13 - ANNUAL INFLOWS (POSITIVE ORANGE CURVE) AND OUTFLOWS (NEGATIVE ORANGE CURVE) OF THE TSGL SYSTEM FOR THE PERIOD 1996-2014. THE ESTIMATIONS WERE BASED ON THE METHODOLOGY APPLIED BY KUMMU *ET AL.,* (2014), USING THE DAILY WATER LEVELS (BLUE CURVES) FROM KAMPONG LUONG AND PHNOM PENH PORT, TO INFER THE AMOUNT OF WATER FLOWING IN AND OUT OF THE LAKE IN THE TONLE SAP RIVER (PREK KDAM GAUGING STATION). A DARK DASHLINE IS PRESENT TO MARK THE NEGATIVE (OUTFLOW) AND POSITIVE (INFLOW) VALUES.

4.4.5 HISTORICAL INFLUENCE OF ENSO EVENTS ON THE OBSERVED FLOOD LEVEL AND FISHERY CATCH

The effect of ENSO events on the observed mean annual variations in Kampong Luong water level (meters) were assessed in Figure 4.14 for the period 1996-2014. From the data available it was not possible to quantify the percentage contribution of ENSO events on the annual variations in water level. Nevertheless, the results show an influence of El Niño (positive values) and La Niña (negative values) years on the observed decrease and increase, respectively, in the flood level and variations in the duration of the floods, with the effects being highlighted in the following year of each El Niño/La Niña event. Thus, 1996 was preceded by a strong La Niña (1995), which lasted until April/96. Consequently, the higher observed flood level (nearly 10 meters at the Kampong Luong gauge in September 1996) might have been caused by La Niña events. The 1997-1998 period shows a strong El Niño, which might have contributed to the observed decrease in water level in the wet season of 1997, with the effect being more pronounced at the end of 1998 - wet season (October), marking the lowest wet season water amplitude (from 9.18 meters in 1997 to 6.72 meters in 1998) of the entire period (1996-2014). The period from 1999-2001 is marked by a strong 2-year La Niña, with continuous (i.e. duration) observed increases in water levels (from 8.5 meters in 1999-wet season to 10 meters in 2001-early January). 2002-2003 displays a strong El Niño, with the reduced flood level being felt only during the wet season of 2003 (8.10 meters in July), possibly because of the previous 2-year La Niña, which might have provided higher floods, and consequent enough water to cope with a decrease in the precipitation pattern from an El Niño event. 2004-2006 exhibits the same trend, with a steady increase in the water level (from 8.44 in the wet season of 2004 to 9.33 in the wet season of 2006). 2006-2009 is again marked by a strong La Niña, although a short El Niño during the dry season of 2006/2007, might have contributed to the decrease in the flood level from nearly 10 meters in October 2006 to nearly 8 meters in September 2007. The 2009-2010 period displays a strong El Niño, and 2010-2012 was marked by a strong 2 year La Niña, with the highest water level (nearly 11 meters) observed in October 2011, followed by a decrease in water level in 2012 to nearly 8 meters in October and increase in the following year (9.44 in October 2013).

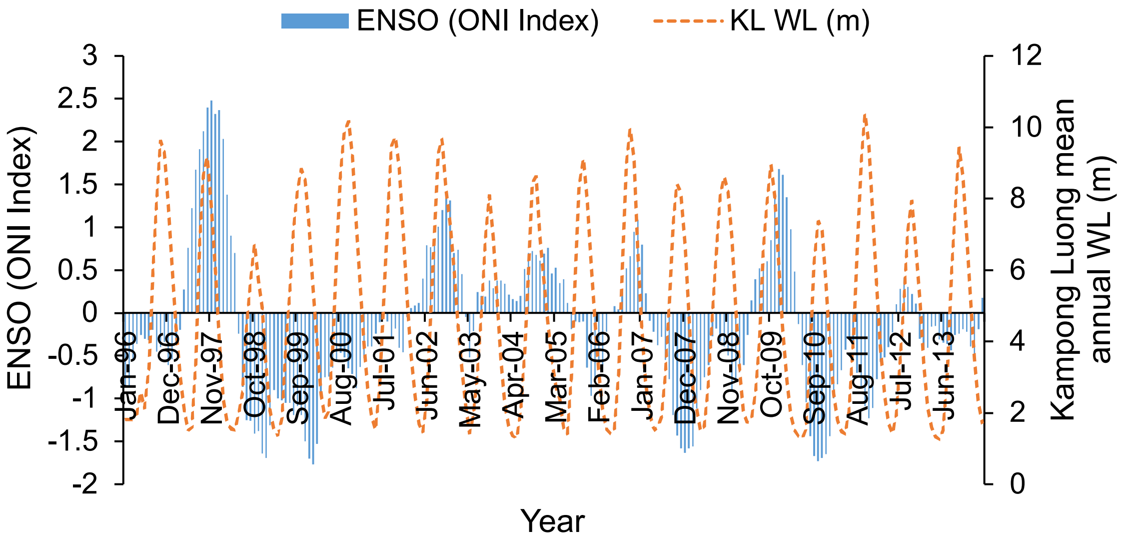


FIGURE 4.14 - HISTORICAL ASSESSMENT OF THE EFFECT OF ENSO EVENTS (BLUE COLUMN) ON THE OBSERVED MEAN ANNUAL WATER LEVEL (ORANGE DASH LINE; METERS) MEASURED AT KAMPONG LUONG. LA NIÑA EVENTS ARE MARKED BY HIGH FLOODS AND NEGATIVE VALUES IN THE ONI INDEX. EL NIÑO EVENTS ARE MARKED BY REDUCED FLOODS AND POSITIVE VALUES.

The (1996-2014) influence of ENSO events on the total annual catch (tonnes) is assessed in Figure 4.15. From the available data it was not possible to quantify the percentage contribution of ENSO events on the total annual catch. However, the 1997-1998 fishing period was under a strong El Niño, which might have influenced the decrease in fish catch (of more than 4,000 tonnes) until 2000, linked to a decrease in the flood level (Figure 4.14). The recovery in catch (increase of nearly 17,000 tonnes) occurred from 1999-2001, which was also followed by a 2 year La Niña period. This was followed by a decrease in catches from 2001-2004 (nearly 19,000 tonnes), when El Niño years occurred. From 2004-2006 the catches increased greatly (nearly 28,000 tonnes), although El Niño (2004-2005) and La Niña (2005-2006) events occurred. The 2006-2007 fishing period was under a strong El Niño, with a consequent decrease in total catch (nearly 16,000 tonnes), although the following two years (2007-2009) exhibited La Niña events, with the catches seemed to stabilise (average of 13,200 tonnes), instead of increasing, as it was expected, since La Niña events brought higher flood levels (Figure 4.14). 2009-2010 had a strong El Niño, resulting in a decrease in total catch to the lowest observed for the entire period (i.e. 8,560 tonnes) seen in next year fishing season (2010-2011). Also, a strong La Niña occurred in 2010-2011, with the effect being highlighted next year (2011-2012), by the highest observed flood levels (Figure 4.14), which might have contributed to the highest total catch reported for the entire period (46,000 tonnes). The following years (2012-2014) show a decrease (nearly 30,000 tonnes; 2012-2013), followed by an increase (nearly 9,000 tonnes) in fish catch, which connected to Figure 4.14, might have been associated to the observed floods.

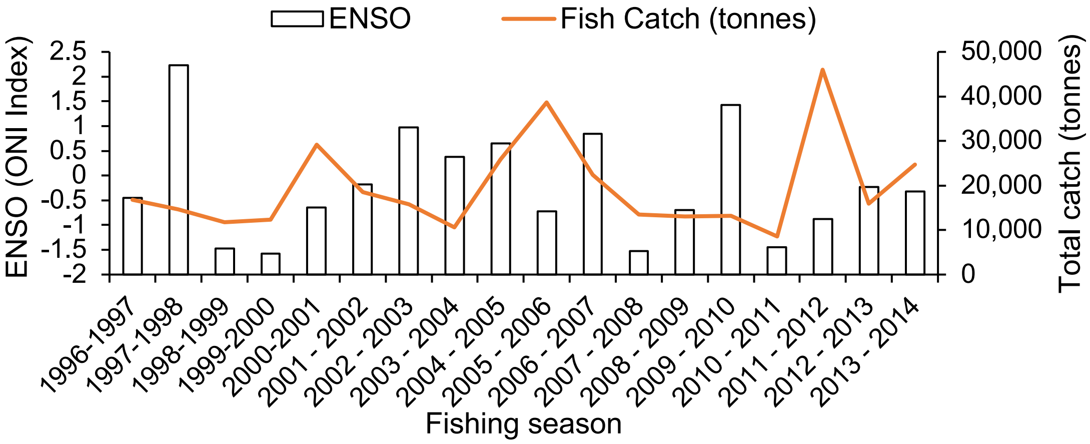


FIGURE 4.15 - LONG-TERM (1996-2014) ASSESSMENT OF THE EFFECT OF ENSO EVENTS (BLACK COLUMN) ON THE OBSERVED TOTAL CATCH (TONNES; ORANGE LINE). LA NIÑA EVENTS ARE MARKED BY HIGH FLOODS AND NEGATIVE VALUES IN THE ONI INDEX. EL NIÑO EVENTS ARE MARKED BY REDUCED FLOODS AND POSITIVE VALUES IN THE ONI INDEX.

4.5 DISCUSSION

4.5.1 AN ASSESSMENT OF THE USE OF EARTH OBSERVATION TO DESCRIBE THE HISTORICAL FLOOD PULSE DYNAMICS OF THE TSGL SYSTEM

The TSGL system exhibits a climate-driven long-term predictable seasonal flood pulse, characterised by a period of increased flood level (during the wet season) and a period of reduced flood level (during the dry season). These variations in the flood pulse dynamics influence the lake's catchment area, playing a critical role for different fish species' life cycles, fisheries and consequent food security, for the people inhabiting the lake and relying on fish as their first source of protein intake (Lamberts, 2006;2008; Campbell *et al.,* 2009; Hortle, 2007). In light of the previously said, applying effective methodologies capable of detecting, with high degree of accuracy, historical temporal and spatial variations in the lake's catchment area, related to the seasonality of the flood pulse dynamics, assumes a vital role for understanding the importance to the fisheries and fish production, and how shifts in the predictability and seasonality of the flood pulse-driven eco-hydrologic interactions might affect the profitable ecosystem services provided (Junk and Wantzen, 2004; Lamberts, 2006;2008; Campbell *et al.,* 2009).

Figure 4.16 provides a comparison analysis of the seasonal variations (1996-2014) in the lake area, estimated using the Landsat flood model (Figure 4.4) and an extended version[9] of the Digital Bathymetric Model (DBM) from Kummu *et al.,* (2014). Both models rely on the observations of the Kampong Luong water level (meters) to predict the seasonal variations in the lake area. Thus, by observing Figure 4.16 it is possible to see that both models are able to strongly predict the seasonal variations in the lake area by the observed water level (meters; 99% explained variance for both dry and wet seasons for DBM, and 77% and 89% explained variance for dry and wet seasons, respectively, for the Landsat model). However, looking at the red dash line corresponding to the dry season period for both models, it seems that the DBM model over-estimates the dry season area, while the Landsat model presents a relatively stable average area of 2,358 km^2 during the 19-year period, as previously described (Figure 4.4).

The Landsat lake area prediction for the dry season is in accordance with studies reporting the lake's average area of 2,500 km^2 during the dry season (Lamberts, 2008; Kummu and Sarkkula, 2008; Campbell *et al.,* 2009). It is important to note that the Landsat flood model itself possess measurement errors, which varied from (70-492 km^2)[10]. On the other hand, and paying attention to the dry season, the DBM shows over-estimations of the lake area of nearly 3,000 km^2 at for example 4 meters (yellow arrow), which is a higher estimation, compared with the reported literature for the dry season area, even considering the errors possibly made during the Landsat-TM images' analyses. Also, during the wet season (green dashline for both models), both models seem to follow the same pattern of the lake area, with slight over-estimations from the Landsat flood model, possibly due to the errors made in the measurements (Figure 4.16).

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[9] The Digital Bathymetric Model (DBM) applied by Kummu *et al.,* (2014), covers daily lake area measurements with the inputs of daily water levels (meters) from Kampong Luong gauging station, for the 1997-2005 period. Thus, using the DBM model's equation, the model was extended until 2014, to be possible to compare it with the Landsat flood model (Figure 4.16). Noteworthy, the DBM areas used for the present analysis were the ones corresponding to the same day of the Landsat areas, to keep the consistency.

[10] These variations were calculated based on the assumption of the pixel error stated in the methodology section of this chapter. Thus, for the minimum observed area of 2,346 km^2, this resulted in an error or 70.4 km^2 (2,346 x 0.03 km^2). For the maximum observed lake's area of 16,389 km^2, the estimated error was 491.7 km^2.

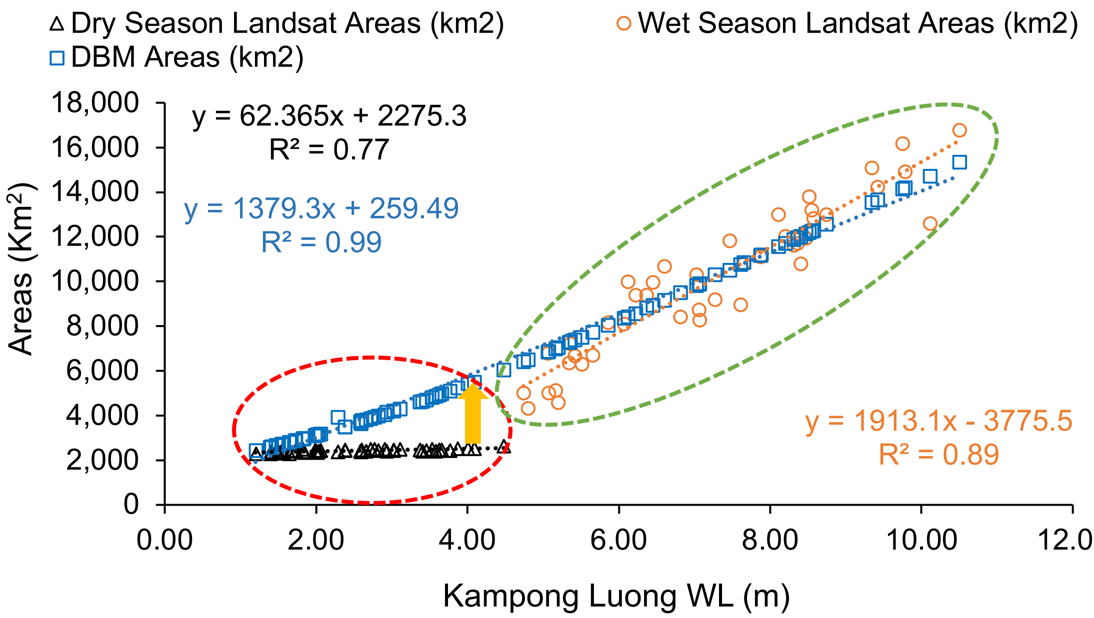

FIGURE 4.16 - COMPARISON ANALYSIS ON THE HISTORICAL (1996-2014) VARIATIONS IN THE LAKE AREA, USING THE LANDSAT FLOOD MODEL BREAK SLOPE ANALYSIS (FIGURE 4.4) AND THE EXTENDED DIGITAL BATHYMETRIC MODEL (DBM) FROM KUMMU *ET AL.,* (2014). LINEAR FIT ANALYSIS AND THE COEFFICIENT OF DETERMINATION ARE PRESENTED FOR BOTH MODELS. THE YELLOW ARROW SHOWS THE AMPLITUDE VARIATION BETWEEN THE DRY SEASON AREA PREDICTIONS FROM THE TWO MODELS AT 4 METERS. THE RED AND GREEN DASHLINES REPRESENTS THE DRY AND WET SEASONS COMPARISONS, RESPECTIVELY, FOR BOTH MODELS.

The fact that the DBM Model seems to over-estimate the dry season lake area, might be associated to the characteristics of the model itself and limitations in the parameters used, although the model measurement errors were not mentioned in the study. The Digital Bathymetric Model applied by Kummu *et al.,* (2014) was derived from three spatial datasets: a hydrographic survey carried by the MRC in 1999 to compute the contours for the dry season lake area and Tonle Sap River; the Certeza survey map made in 1964, for mapping the floodplains, and SRTM data (shuttle radar topography mission) for mapping the surrounding areas of the lake. Moreover, the DBM used GIS analyses for setting-up the model from the acquired datasets in conjunction with historical water level records from the hydrologic gauging stations in the TSGL system (Kampong Luong, Prek Kdam and Phnom Penh Port; Kummu *et al.,* 2014). Looking at the spatial datasets used to set-up the DBM Model, this is calibrated using survey data from two different sources, which, given the 35 years difference (i.e. 1964 and 1999), are likely to have applied different methodologies. Moreover, the survey campaigns were based on field measurements, with inherent estimation errors, regarding the TSGL system’s topography, extent of vegetated area, catchment area, among other factors. Therefore, it is likely that the field measurements made could not cover in detail the spatio-temporal variations in the lake area. Moreover, the periodicity in which those surveys were made, could have contributed to the resulting over-estimates. Although, from the Landsat flood model and available literature, the lake area does not change substantially during the dry season (Campbell *et al.,* 2006;2009; Lamberts, 2006;2008; Kummu and Sarkkula, 2008). Consequently, even if the surveys were performed every two months, this would still be able to capture the stable variations in the lake area during the dry season. Hence, the most plausible explanation is likely to rely on potential inconsistencies on the spatial datasets used and inherent methodological approach during the model calibrations and simulations made.

The implications of the dry season lake area over-estimations from the DBM Model are the reduced accuracy in estimating and understanding the historical importance of the predictability and seasonality of the flood pulse dynamics in driving the seasonal variations in the lake area, and consequent influence on the fish dynamics, abundance and *dai* fishery supported, which are key to understand the sustainability of the system and for the correct implementation of management plans in Strategical Development Plans (e.g. MRC Technical Reports; MRC, 2009b). This is remarkably important in light of future water infrastructures development (e.g. hydropower and irrigation expansion) in the basin, predicting to increase dry season's flood area of the lake in nearly 21% and reduce it in nearly 22% in the wet season (Arias *et al.,* 2012). Therefore, the over-estimation of the lake area would have implications on the correct understanding of the migratory behaviour of more than 50% fish species that leave the lake in the dry season to find shelter, spawn and feed in upstream areas of the mainstream river (Poulsen *et al.,* 2002;2004; Campbell *et al.,* 2009; Halls *et al.,* 2013a; DHI, 2015). This is because an increase in the flood area, would likely disrupt the migratory trigger (i.e. reduced flood level and consequent shrinkage on floodplain habitats availability; Baran, 2006) for fishes to leave the lake during the dry season, which will then translate in a decrease in the *dai* fishery yields (Baran, 2006; Campbell *et al.,* 2009; Arias *et al.,* 2012;2013;2014), since fishes would spend longer times in flooded floodplains, with consequent alterations in the spawning cycle likely to compromise the population dynamics and next year fishery recruitment (Baran and Mychowoda, 2008;2009). Additionally, the higher lake area does not necessary translate to an increase in food sources, since the vegetation surrounding the lake exhibits strict tolerance to the flood dynamics, particularly for permanent flooding, with the death of vegetation species likely to increase the anoxic conditions in floodplains, posing challenging adaptations to fishes (Sarkkula *et al.,* 2004; Lamberts, 2006;2008). Ultimately, all these factors would substantially modify the annual provision of fish protein, which more than 1.2 million people are highly dependent, increasing food security issues in one of the poorest countries in Southeast Asia (for more information see Chapter 5; UNEP, 2006; Hortle, 2007; So,2010). Therefore, the DBM Model's over-estimation of the dry season lake area provide a baseline knowledge of a higher flooded area of the lake, which applied to compare with the future projections of the lake flooded area, under water infrastructure development and climate change scenarios, would influence the accurate predictions of the future of the fishery resources and migratory fish supported.

The Landsat flood model provides a higher level of accuracy in assessing the spatio-temporal variations in the lake area, particularly during the dry season, following available literature (Kummu and Sarkkula, 2008; Campbell *et al.,* 2009). Thus, with the earth observations and time-series hydrologic analyses of the historical variations in the lake area, driven by the predictability and seasonality of the climate-driven flood pulse regime, it is possible to accurately establish key relationships on the importance of both seasons' flood dynamics for the eco-hydrologic interactions established in floodplains, and the role on the long-term fisheries and fish species composition supported (Figure 4.8 and Table 4.3). Importantly, it allows a reliable quantification and insight understanding on the historical adaptive strategies of different fishes to the timing, duration, amplitude, smoothness and continuity of the flood to occupy (wet season) or leave (dry season) the TSGL system (see later in the Discussion section; Baran, 2006; Campbell *et al.,* 2006;2009; Chea *et al.,* 2016). Also, the Landsat flood model applies satellite images, which are free to access, cover a long daily period of image collection (40 years), and importantly, cover the entire lake's catchment area. This enables the assessment of the historical fluctuations in the lake's total catchment area, without the need to rely on field surveys or match different mapping sources, which would potentially increase the errors in the area estimations. As so, the use of remote earth observation tools for assessing historical spatio-temporal variations in the flood pulse dynamics and catchment area of freshwater systems, and the relation with the fish abundance and dynamics supported, should be encouraged for the high level of detail provided, particularly in tropical system, where the ecosystem services (i.e. fisheries) provided are vital to the people for food security (Lamberts, 2006;2008; Campbell *et al.,* 2009; MacAlister and Mahaxay, 2009; Fujii *et al.,* 2010; Craig, 2016; Fragal *et al.,* 2016). Of note, this would allow a higher accuracy on the future projections of the variations in the flood pulse dynamics and flooded area, and the quantifications of the impacts upon the fishery resources and migratory species, driven by future environmental change scenarios, which would provide the reliable scientific knowledge to be integrated in Strategic Development Plans, for applying mitigation measures (see further in Chapter 5; Baran, 2010; DHI, 2015). As presented in the results section of this chapter, over a 19-year period (1996-2014), the lake's mean annual area and variations in the mean annual maximum and minimum lake area showed low fluctuations, reflecting the predictability and stable seasonal variations in the lake's flood level (Figure 4.5; Figure 4.6 and Figure 4.7, respectively). Notably, this indicates that although the Mekong basin has been under increasing hydropower development (mainly in tributaries and upstream sections; see Chapter 3), the historical flood regime in the mainstream river has not changed substantially to affect the unique bi-directional flow reversals for the timing, duration and magnitude of water in the lake (Kummu *et al.,* 2005a; Lu and Siew, 2006; Lauri *et al.,* 2012; Cochrane *et al.,* 2014).

The mainstream dams in operation are all concentrated in the upstream section of the river in China[11] (Lu and Siew, 2006; Grumbine and Xu, 2011). Since the TSGL system has a distance from the upstream Chinese dams of over 1,000 km and the extensive lateral tributaries surrounding the Mekong river play a role in providing downstream flows (see Chapter 3; Adamson *et al.,* 2009), it is unlikely that significant flow modifications would be felt as downstream as the lake, as already reported by other studies (Adamson *et al.,* 2009; Campbell *et al.,* 2009; Cochrane *et al.,* 2014). Yet, plans for the construction of the Don Sahong (installed capacity: 256 MW) and Xayaboury (installed capacity: 1,285 MW) first mainstream dams in Lao PDR will likely pose significant modifications in the flow conditions, fish migratory behaviour and sediment flux downstream of Lao PDR

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[11] The Chinese plan for building eight cascade dams in the Lancang River (Chinese portion of the Mekong river) is soon to be completed. The Manwan dam was the first to be completed in 1993 with an installed capacity of 1,670 Megawatts (MW). Then, the Dachaoshan was completed in 2002 with 1,350 MW followed by the Jinghong in 2009 (1,750 MW), Xiaowan in 2010 (4,200 MW), Gongguoqiao in 2012 (900 MW), Nuozadu in 2014 (5,850 MW), Miaowei in 2016 (1,400 MW), and Dahuaqiao are due to be finish in 2018 (920 MW; Lu and Siew, 2006; MRC, 2009a; Grumbine and Xu, 2011).

(Baird, 2011; Baran *et al.,* 2011). Furthermore, the Sambor dam (installed capacity: 2,600 MW; Sithirith, 2016), will be the first mainstream dam to be built in Cambodia and is a major threat to the relatively pristine flow regime of the Mekong river, which will likely disrupt the timing and volume of the bi-directional flow reversals, impacting upon the flow regime and carrying of sediments into and out of the TSGL system downstream, while disrupting the lake's historical spatio-temporal catchment area, floodplain habitats flood pulse dynamics, fish migratory pathway and cues (the influence of hydropower development on the TSGL system flood pulse, *dai* fishery and species composition, will be assessed in Chapter 5; Baran, 2006; Kummu *et al.,* 2008b; Baran and Mychowoda, 2009; Arias *et al.,* 2012;2013;2014; Kummu *et al.,* 2014; Hecht *et al.,* 2019).

The historical balanced flow pattern (Figure 4.13) support the argument that the seasonal flood pulse of the lake has not substantialy changed over the 19-year period. Thus, the observed historical variations in the area of the lake, connected with the predictability and seasonality of variations in the flood level, reflect the influence of the balanced inflows and outflows on the seasonal flood magnitude, as described by Kummu *et al.,* (2014). Moreover, the stability in the flow pattern, resulting in relative unchanged conditions in the sediment flux (Kummu *et al.,* 2008b), help to understand the historical productivity of the system for its fisheries (Figure 4.8) and species composition (Table 4.3), which rely on the sediment dynamics and floodplain habitats inundation pattern for the nutrient recycling that provided food source, spawning and shelter grounds (Lamberts, 2008).

Although the historical (1996-2014) analysis of the monthly water level of the lake lead to the inference that the flood level has not profoundly changed, as seen in Figure 4.7, low and high flood levels were observed for the 1998-1999 and 2011-2012 hydrologic years, respectively. Thus, it is likely that these marked variations, compared to the 1996-2014 period, were related to climatic events in the basin (e.g. ENSO events), since the monsoon climate strongly influence the flow pattern in the Mekong basin (for revising information see Chapter 3; Holmes *et al*., 2009 Delgado *et al.,* 2012; Darby *et al.,* 2013; Räsänen and Kummu, 2013). From Figure 4.14 it appears that for the observed low flood level of 1998-1999, although the year is marked by a La Niña event, it seems that the flood level was significantly affected by the strong El Niño event in 1997-1998, which resulted in the next hydrologic year (1998-1999) low water level. The influence of ENSO events one or two years after an El Niño/La Niña events on the flood level was mentioned in the study of Räsänen and Kummu, (2013) for the Mekong river, where the authors identified the same trend for the 1998-1999 and 2011-2012 hydrologic years. Therefore, for the maximum observed flood level of 2011-2012, the same argument can be applied when paying attention again to Figure 4.14. In fact, this hydrologic year is preceded by a La Niña event starting in the wet season of 2010, continuing for the entire 2011 and ending in 2012. As so, the annual variations seen in the lake area in Figure 4.5 and Figure 4.6 are likely to have been affected by ENSO events contribution to the observed variations in the flood level (Figure 4.14), which also helps to understand the increased discharge pattern observed in Figure 4.13. Regarding the *dai* fishery (Figure 4.15) the annual variations in the flood level of the lake, influenced by ENSO events, might help to understand the lowest catch in 2010-2011 (8,560 tonnes), which, although corresponding to a La Niña year, was preceded by a strong El Niño year. Moreover, the highest observed catches (46,000 tonnes) in 2011-2012, were followed by a La Niña year. The same pattern was observed for the Amazon basin fisheries by Pinaya *et al.,* (2016), with years of El Niño (La Niña) events, resulting in next year's decrease (increase) in the CPUE of the fishery and modifications in the species composition, with species that require longer flood durations, being affected by decreases in flood level and inundation pattern. Unfortunately, the study does not quantify the percentage contribution of ENSO events in the fisheries and percentage change in the species composition of the fishery, due to constrains in the available ENSO data (Pinaya *et al.,* 2016). The same was observed in the current analysis.

Consequently, from the long-term (1996-2014) analyses of the monthly flood level (Figure 4.7) and catches (Figure 4.8), it is possible to see that ENSO events have not substantially modified the predictability and seasonality of the flood pulse dynamics of the lake and resulting fishery's yields, with contributions to the observed variations seen in Figure 4.14 and Figure 4.15. Yet, this apparent stable pattern might be severely disrupted in the near future by climate change projections and water infrastructure development in the basin, which are expected to severely affect the predictability and seasonality of the flood pulse dynamics, disrupting the eco-hydrologic floodplain interactions and fish production. Importantly, the consequences of these alterations will likely reflect on a modification in *dai* fishery's yields, posing high food security challenges in the region (Kummu *et al*., 2005a; Lamberts, 2006;2008; Arias *et al.,* 2012;2013;2014; Cochrane *et al.,* 2014; DHI, 2015). Notably, the gaps in studies quantifying the expected impacts of the individual and combined actions of the multiple drivers of environmental change, regarding the fishery yield and species composition, make it difficult to effectively understand how the system will be affected, and the challenges to people for food security. Yet, these studies should be tackled for the strict influence here shown of the flood pattern on variations in the lake area (Figure 4.5; Figure 4.6), which are also likely to be driven by ENSO events (Figure 4.14), ultimately influencing the fishery yields (Figure 4.8 and Figure 4.15) and food protein to more than 1.2 million people. Therefore, Chapter 5 will focus on these remarkably important issues.

4.5.2 THE LONG-TERM STABILITY OF THE *DAI* CATCH AND IMPORTANCE OF THE TSGL SYSTEM FOR FISH MIGRATIONS

The 20-year period of *dai* fishery analysis (Figure 4.8) shows that the annual catches have not been declining as suggested by Watershed (2002), MRC (2003), Pearce (2004) and Wain (2004). In fact, it is remarkable the ability of the fishery to recover from year to year, as exemplified by the lowest observation in the fishing season of 2010-2011 (8,560 tonnes; Figure 4.8) and the next year maximum reported catch of 46,000 tonnes. These findings are in accordance with Baran and Myschowoda (2008), who stated that the *dai* fishery approximately doubled from 1940-1995. Halls *et al.,* (2013a) and DHI (2015) also support the argument of a long-term (1994-2014) stability in the annual catch yields, stating the influence of the flood pulse of the system in driving the high yields. The hypothesis of the observed stability (and in some years increases in the fishery catches; Figure 4.8) being associated with the flood pulse of the lake will be further covered here. Moreover, from Figure 4.14, it is likely that climatic events impacted the flood level, playing an important role in the eco-hydrological interactions established in floodplains, with increased periods in the flood inundation (La Niña events), promoting extensive habitat for fishes to grow and feed by the recycle of nutrients. Also, during the El Niño phase the reduced flood level resulted in the shrinkage of habitats and consequent migration of fish out of the lake. Thus, the *dai* fishery annual yields reflect the fish leaving the lake, with the flood pulse dynamics influencing the fish that were growing in the floodplains, and after caught in the fishery (Baran *et al.,* 2001; Halls *et al.,* 2013a).

On the other hand, what has changed (1997-2009) is the effort applied in the catch (Figure 4.9). Ahmed *et al.,* (1998), Baran and Myschowoda (2008) and Halls *et al.,* (2013a) mentioned intra and inter-annual variations in the catch effort from the six months period that the *dai* fishery is open (October-March). The authors agreed that the catch per fisher had been reduced by almost half of what it was six decades ago, although exact numbers are not provided (Baran and Myschwoda, 2008). This has resulted in the observed overall increase in the fishing effort (Figure 4.9), associated with more operators, more boats, the increased dimension of gears and reduction of the mesh size (Ahmed *et al.,* 1998; Baran and Myschowoda, 2008; Halls *et al.,* 2013a). Also, the decline in medium and large body-size fish species for small body-size fish, shows that the *dai* fishery is following the downside fishery concept (van Zalinge *et al.,* 2000; Sverdrup-Jensen, 2002). This concept states that large body-size fish species, usually with long-life cycles are replaced by small size short life-span species, which has already been observed in other tropical fisheries (e.g. Amazon, Lake Victoria and Okavango delta; Welcomme, 1995; Mosepele, 2014; Castello *et al.,* 2015). Consequently, cautions need to be taken when stating that the *dai* fishery has been declining, when the reality is that the fishery still keeps its historical annual catch (Figure 4.8). Also, the increase in catch effort should not imply that fishes are becoming scarce in the system, but that the increase is associated with higher fishing pressure (i.e. more boats and hours fishing), to then capture small body-size species that are more abundant than large body-size species (Lim *et al.,* 1999; van Zalinge *et al.,* 2000; Baran *et al.*, 2001; Sverdrup-Jensen, 2002). Importantly, the reasons behind the decline in medium and large body-size fish species need to be carefully examined for the consequences that might arise regarding the trophic interactions established in the system (Barlow *et al.,* 2008). The long-term (1997-2013) *dai* fishery is composed by nearly 80% of medium (guild 4) to long (guild 3) distance migratory white and greyfish species (Table 4.3 and Table 4.4). This high percentage shows the vital importance of the lake for fishes that seasonally utilise it for completing their life cycle (see Figure 4.1; Baran, 2006), which will then contribute to the fishery yields and fish protein consumed by the local population in the system, regarded as the highest in Southeast Asia (70 kg person^{-1} year^{-1}; So, 2010). Also, decreases in dissolved oxygen levels and turbidity affect fishes, resulting in medium to large body-size fishes to migrate when the lake has dissolved oxygen levels lower than 4 mg l^{-1}. Thus, during the dry season the lake has low dissolved oxygen levels (< 4 mg l^{-1}) and high turbidity, while during the wet season the lake possesses dissolved oxygen levels higher than 4 mg l^{-1} and high turbidity from the inflows of the Mekong river (see the analysis in section 3.3 of Chapter 3; Sarkkula *et al.,* 2003; Campbell *et al.,* 2009). Therefore, these water quality variations, particularly in the dry season, might help to understand the migratory behaviour exhibited by some white and greyfish species that are caught in the fishery. Also, the migration pattern of white and greyfish species are highly triggered by variations in the flood level (Poulsen *et al.,* 2002;2004; Baran, 2006; Campbell *et al.,* 2006; Termvidchakorn and Halls, 2013; Chea *et al.,* 2016), which will be further assessed in the section below. Table 4.6 provides the maximum body length (cm) of the species contributing to the *dai* fishery. The guilds of each species are also present. The estimated maximum body length is based on Rainboth (1996), Termvidchakorn and Halls (2013) and supplemented by the FishBase website (www.fishbase.org).

TABLE 4.6 - THE ESTIMATED MAXIMUM BODY LENGTH (CM) OF EACH SPECIES COMPOSING THE *DAI* CATCH, THEIR FAMILY AND GUILDS.

Species	Family	Max body length (cm)	Guild
Henicorhynchus sp.	Cyprinidae	15	3
Labeo chrysophekadion	Cyprinidae	90	
Puntioplites proctozysron*	Cyprinidae	30	
Pangasius pleurotaenia*	Pangasiidae	35	
Labiobarbus lineatus	Cyprinidae	15.5	4
Paralaubuca barroni	Cyprinidae	15	
Botia modesta*	Cobitidae	25	
Pangasius larnaudii*	Pangasiidae	130	
Labiobarbus siamensis*	Cyprinidae	22	
Syncrossus helodes*	Cobitidae	30	
Thynnichthys thynnoides	Cyprinidae	25	5
Cirrhinus microlepis*	Cyprinidae	65	
Clupeichthys aesarnensis	Clupeidae	7	1

*Refers to the species belonging to the category Other sp. from the results section.

The *Henicorhynchus* sp., *Labiobarbus lineatus* and *Paralaubuca barroni* together contributed to nearly 54% of the 80% top five species from 1997-2013 *dai* fishery (Table 4.3). These species are small body-size species (average body-size: 15

cm), with short life cycles, and triggered by hydrologic modifications, starting the migration at the start of the flood season, entering the lake for spawning and feeding in the productive inundated floodplains (Rainboth, 1996; Poulsen *et al.,* 2002; Baran, 2006). Thus, the fact that these small body-size species play a high contribution to the overall catch can help to explain the downside fishery concept observed in the *dai* fishery (van Zalinge *et al.,* 2000; Sverdrup-Jensen, 2002). Moreover, their quick life-span (i.e. quick growth rates, reaching the reproductive maturation at earlier stages and spawning more than once a year) provide useful information on the continuous annual recruitment, which might reveal that the ratio juvenile/adult in the *dai* fishery does not follow the same trend as observed in other tropical fisheries (Mosepele, 2014; Castello *et al.,* 2015; Pinaya *et al.,* 2016). Supporting evidence for this assumption can be explained not only by the species quick life-span, but also by the fact that the *dai* nets in operation during the fishing season possess differences in mesh sizes, according to the target species, with reported high intra-variability in the minimum and maximum mesh sizes in use (often exceeding the legal maximum and minimum sizes stated here; van Zalinge *et al.,* 2003; Halls *et al.,* 2013a). During the first two months (October-November) the *dai chieu* (mean minimum mesh size: 24 mm and mean maximum mesh size: 182 mm) net is used to target medium to large body-size fish species, which are believed to migrate earlier than small fish (Hall *et al.,* 2013a).

Termvidchakorn and Halls (2013) provide useful information on the average size of larvae and juvenile stages of common species in the Mekong. *Henicorhynchus sp., Paralaubuca sp*. and *Labiobarbus lineatus* have an estimated body-size of larvae to juvenile stage of nearly: 23, 26 and 22 mm, respectively. Thus, even larvae or juveniles of these species, if starting the migration earlier with the medium to large body-size fishes, are likely to pass by the mesh and, therefore, contribute to the next year recruitment, when they return to the lake (Halls *et al.,* 2013a). Moreover, strong water currents during the *dai* fishing season limit the *dai* nets that can be in operation, which might imply that fish are migrating with a considerable proportion not being caught (Halls *et al*., 2013a). From December to February, the *dai nheuk* (mean minimum mesh size: 16 mm and mean maximum mesh size: 118 mm) is in operation to target small body-size species: *Henicorhynchus sp., Paralaubuca sp*. and *Labiobarbus lineatus* (Halls *et al.,* 2013a). As so, depending on the minimum and maximum mesh size used, type of *dai*, the start of the migration pattern, and the fact that the majority of these species come to the floodplain areas to grow and spawn; the fish that are caught in the *dai* nets are more likely to be adults than juveniles. The ones able to escape from the different mesh-sizes in use, will likely return to the lake next year and contribute to the continuous yearly fish stock recruitment and low inter-annual variability in the catch observed in Figure 4.8 (Halls *et al.,* 2013a).

Although exhibiting a proportional smaller contribution to the overall catch (i.e. ~30% of the catch is composed by large body-size species - average body-size: 50 cm, while 53% is composed by small body-size species - average body-size: 15 cm) medium to large body-size species (e.g. *Labeo chrysophekadion, Thynnichthys thynnoides, Puntioplites proctozysron, Pangasius pleurotaenia, Botia modesta, Pangasius larnaudii Labiobarbus siamensis, Syncrossus helodes* and *Cirrhinus microlepis)* should be considered for their ecological role in the food chain (Baran, 2006). These species possess higher maximum body length when compared to the previous group, which means they require longer maturation times and are more susceptible to variations in the flood pulse (Poulsen *et al.,* 2002;2004; Baran, 2006; Lamberts, 2008, Campbell *et al.,* 2009). Consequently, the fact that these species require longer maturation times to grow and spawn might explain the relatively smaller contribution in the overall *dai* catch and the higher inter-annual variations in the total catches (Table 4.3). The increasing effort applied to this fishery might mean that most of medium to large body-size fish species will not be able to reach the adult stage, since fishes will be caught even at juvenile stages, and therefore contributing to slight changes in the adults/juveniles ratio (although this ratio is likely to still keep the adult stage as the majority of proportion, since large body-size species contribute less to the overall catch). This is often the case reported in other tropical fisheries (Mosepele, 2014). However, care should be taken with this assumption, as no information was available concerning daily species length (cm) at the time of the fishery.

According to Baran (2010), Halls and Kshatriya, (2009) and DHI (2015) the medium to large body-size white and greyfishes are likely to be impacted by hydropower development in the basin, due to their inability to successfully use the fish passage in dams. Furthermore, the ones that are able to pass require flowing water conditions (lotic environments) and water quality parameters (high dissolved oxygen and nutrients), which are often absent in the catchments before and after the dams (usually changing to a lentic system and low dissolved oxygen content; Ongley, 2009; Baran *et al.,* 2015). Yet, even with dams blocking the migration routes, small body-size fish species that perform medium to long distance migrations are predicted to be less affected by dams and are more able to successfully utilise their passages (survival rates higher than 75%), according to a model study from Halls and Kshatriya (2009). Despite of that, when compared to large body-size species (*Pangasius sp. Probarbus sp.* among another medium to large body-size species used in the model study), the mortality rates were higher than 80% (Halls and Kshatriya, 2009).

As such, it is safe to assume that if continuous hydropower development persists in the Mekong basin (both tributary and mainstream dams; Grumbine and Xu, 2011; Lauri *et al.,* 2012; Cochrane *et al.,* 2014) and the fish effort continues to increase, with more boats in operation along with more fishing hours (Halls *et al.,* 2013a), the *dai* fishery will continuously be regarded as a small body-size species' fishery, and the medium to large body-size species will become increasingly rare, as already observed in other tropical fisheries (Mosepele, 2014; Castello *et al.,* 2015; Pinaya *et al.,* 2016). This will exacerbate biological dramatic changes in the food chain, since the medium to large body-size fishes are regarded as top predators, feeding from the small fishes as well as macroinvertebrates and plants in the lake, and keeping the equilibrium in the number of small body-size fish and energy flow in the chain (see Chapter 2; Campbell *et al.,* 2006; Chea *et al*., 2016).

It is also important to clarify (although exact revenue values could not be found in the literature, apart from Barlow *et al.,* (2008) who stated the first-sale value of migratory fishes to be estimated at US$ 1.89 per kg) that these top size fishes are highly pursued by fishermen as fishing trophies due to their price per kg and iconic symbol for the countries where these fishes are caught (Barlow *et al.,* 2008).

Hence, although the majority of the fishery (more than 50%) is composed by small body-size medium to long distant migratory fish species, less affected by dam developments in the basin, these species are influenced by the historical predictability and seasonality of the flood pulse to perform migrations.

4.5.3 INFLUENCE OF THE LAKE'S HISTORICAL FLOOD PULSE ON THE FISHERY YIELDS AND SPECIES COMPOSITION OF THE CATCH

Previous studies have reported a close relationship found between historical variations in the flood pulse of a river or lake and the observed fisheries yield. In the Amazon basin, which follows the flood pulse concept (Junk *et al.,* 1989;2010), 62% of the increases in the reported fish catch (1993-2004) were associated with increases in the flood level and the inundation extent of the floodplain areas. Also, the timing of the flood influenced the early or delay in the migratory pattern of more than 60% of the species composing the fishery (Castello *et al.,* 2015). Importantly, when higher flood duration and extent were observed in floodplain areas, these strongly explained (R^2=0.85) the increases in the top ten species caught in the fishery (Castello *et al.,* 2017).

From Figure 4.11 it is possible to see a non-statistically significant relationship between the historical annual variations in the lake's flood level (measured at Kampong Luong) and the variations in catch (R^2=0.18; ANOVA; $F_{(1,17)}$=3.46; p=0.08). This finding differs from that of Baran *et al.,* (2001) who reported an association of 85% between the *dai* catch and variations in the flood level measured at Kampong Chnnang (1995-2000). The Kampong Chnnang gauging station is situated in the Tonle Sap River, where the fishery occurs, which might explain a better fit than the findings in this chapter. Yet, this gauging station is not present in the MRC Data Portal and so it was not possible to obtain daily water level records. Noteworthy, using the Kampong Chnnang gauging station, Baran *et al.,* (2001) were only quantifying the direct effect of the system's water level at the time of the fishing period. Therefore, the authors were not considering the importance of the lake itself to support the fish, as a result of the inundation of the productive floodplains, which provide essential nutrients that will enhance the growth rates of the species that seek these habitats to grow, feed and spawn (Lamberts, 2006;2008; Campbell *et al.,* 2006;2009). Moreover, the study only assessed 5 years of data, while the present study covered a significantly longer time-frame, capturing longer historical trends. Importantly, the variability in the flood pulse, with periods of higher flood magnitude and reduced duration and reduced flood extent but increase in inundation duration (Table 4.5), can help to understand the disparity of the water level influence on catch, for the role in driving the maturation and spawning behaviour of the species composing the catch as previously described by Baran *et al.,* (2001) and Baran (2010). Consequently, although the findings in this study present a non-statistical relationship and the historical variations in the flood level could only explain in 18% the variations observed in the *dai* catch, the importance of the lake for providing extensive inundated floodplains, where high recycling of nutrients occurs, should be acknowledged for its beneficial effect on fish growth. Following this argument,

Figure 4.12 corroborates this hypothesis. The significant role (ANOVA; $F_{(1,17)}$=4.78; p=0.03) of the historical seasonality and predictability of the flood duration and extent (flood index; Table 4.5), help to understand the 31% contribution to the observed variations in the *dai* catch, over a 19-year period. This result is similar to the findings from Halls *et al.,* (2013a) who reported a 59% influence of the flood index in the weight (kg) variations of the *dai* catch. Moreover, although lower than the observed for the Amazon basin from Castello *et al.,* (2015;2017), the same trend is seen for the two tropical basins, seasonally influenced by the flood pulse, demonstrating the key role of the historical predictability and seasonality of the flood pulse to the seasonal flooded floodplain habitats and the eco-hydrologic interactions established (Junk *et al.,* 1989).

Since historical variations in the flood duration and extent significantly influenced the observed catch and knowing that nearly 80% of the *dai* catch is composed by species that perform medium to long distance migrations (guilds 3 and 4), Figure 4.17 assesses the historical role of the flood index on the top five species composing of the catch. This figure reveals that small body-size fish species (**A** - *Henicorhynchus sp., Labiobarbus lineatus* and *Paralaubuca barroni*), characterised by short life-span, are not significantly (ANOVA; $F_{(1,15)\textit{Henycorhynchus sp.}}$=1.19; p=0.29; $F_{(1,15)\textit{Labiobarbus lineatus}}$=1.24; p=0.28; $F_{(1,15)\textit{Paralaubuca barroni}}$=1.04; p=0.33) influenced by variations in the flood index. Thus, for these species longer flood durations and an increase in the flood extent, does not seem to play a role in the catch composition of these species. This finding is in accordance to the studies from Poulsen *et al.,* (2002;2004), Baran (2006) and Termvidchakorn and Halls (2013), who have all reported that small body-size fish are less influenced by variations in the flood duration and extent for their growth. However, the authors also stated that these species have their migratory pattern linked with the timing of the flood. Thus, from Table 4.5, it is possible to see variations in the flood start and end, which might help to explain the inter-annual variations in the total catch of these species, present in Table 4.3. Also, these species possess short life-span, reproducing more than once per year (Poulsen *et al.,* 2002;2004). Therefore, the continuous presence of these species in the catch and the high percentage (53%) contribution to the long-term catch, leads to the conclusion that the variations in flood start/end were not significant to disrupt the migratory pattern and consequent spawning behaviour for these species. Nevertheless, and as mentioned before, this pattern might soon change, due to the increase of the multiple drivers of environmental change in the basin, likely to severely disrupt the catch yields and food security (Hortle, 2007;2009; Baran, 2010; DHI, 2015).

On the other hand, for the medium and large body-size fish species (**B -** *Labeo chrysophekadion, Thynnichthys thynnoides* and Other sp., which all apart from *Clupeichthys aesarnensis*, possess large body-sizes than the previous group, for revising information, see Table 4.6), the variations in flood duration and extent, significantly (ANOVA; $F_{(1,15)\textit{Labeo}\ \text{chrysophekadion}}=16.71$; $p=0.01$; $F_{(1,15)\textit{Thynnichthys}\ \text{thynnoides}}=11.48$; $p=0.04$; $F_{(1,15)\text{Other sp.}}=5.25$; $p=0.03$) influenced the observed catch composition (Figure 4.17). This reveal that combinations of increased flood durations and magnitude promote the growth of these species, and thus contribute to the proportion in catch seen in Table 4.3. This has already been mentioned in other studies (Baran *et al.,* 2001; van Zalinge *et al.,* 2000;2003; Campbell *et al.,* 2009; DHI, 2015), where increases in flood magnitude and duration allowed an increase in the recycling of nutrients and, therefore, more availability of food. Also, the magnitude of inundated habitats allowed a higher refuge area for juveniles to protect from predators, while feeding and growing (see Chapter 2; Lamberts, 2006;2008). Yet, the proportion in catch of these large body-size species were comparable lower than for small body-size species, which reveal that although years with prolonged flood duration and extent, promoted the growth of these species, the increase in the fishing effort from the *dai* fishery, and potentially from the other fishery activities in the system (Table 4.2) that also develop in the floodplain areas of the lake, might have been responsible to caught the majority of the large body-size fish species, significantly depleting the populations. Although, being a hypothesis, van Zalinge *et al.,* (2000) and Baran *et al.,* (2007) mentioned the same theory for the role of the other fisheries in reducing the stock populations and next year recruitment. As so, it is important to improve the fisheries record of the lake, to effectively assess the population dynamics, particularly for large body-size fish species, which, due to their maturation time, would be severely impacted by the increase in fishing effort (van Zalinge *et al.,* 2000; Halls *et al.,* 2013a).

The diversity of species composing the *dai* fishery is noteworthy and means that small body-size fish species will not be significantly impacted by historical variations in the flood duration and extent. However, the contrary is observed for the large body-size species that require longer maturation times (Lamberts, 2006;2008). However, for both small, medium and large body-size medium to long distance migratory fish species (guilds 3 and 4), variations in the timing of the flood are likely to impact the migratory cues, which will create variations in the reported catches. These variations in the flood timing have been related to future projections of water resources development and climate change, which studies refer the likely disruptions on the historical flood pulse dynamics and consequent predictability and seasonality of floodplain habitats flood dynamics and key eco-hydrologic interactions, also affecting the connectivity of migratory pathways that together are expected to severely affect the migratory dynamics of the top-five fish species composition of the *dai* catch into and out of the lake (Arias *et al.,* 2012;2013;2014; Hoang *et al.,* 2016;2019). Ultimately, these disruptions will result in alarming negative consequences to the *dai* fishery, the medium to long distance migratory fish species composition and contribution to the catch and to fish protein availability to future populations (Hortle, 2009; So, 2010). Of note, effective quantifications of the future of the *dai* fishery resources and food security threat from the multiple drivers of environmental change in the basin remain largely unassessed, with Chapter 5 filling this gap and providing, for the first time, crucial projections on the future of the fishery resources and fish protein to future populations, driven by individual and combined impacts of hydropower, irrigation and climate change scenarios on the flood pulse and floodplain flood dynamics.

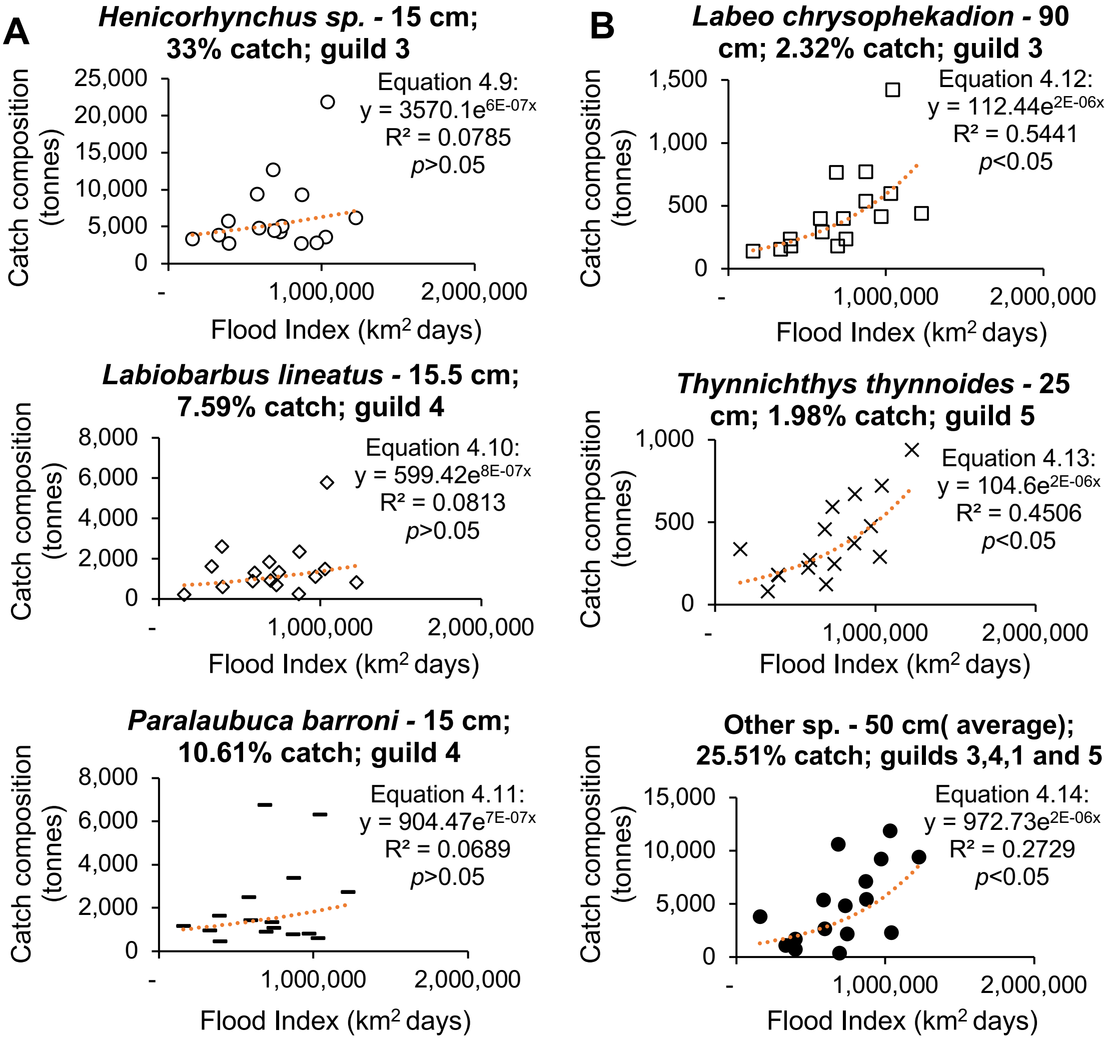

FIGURE 4.17 - THE FLOOD INDEX (KM^2 DAYS) INFLUENCE ON THE SPECIES COMPOSITION (TONNES) OF THE *DAI* FISHERY FROM 1997-2013. STATISTICALLY SIGNIFICANT DIFFERENCES WERE FOUND FOR: *LABEO CHRYSOPHEKADION, THYNNICHTHYS THYNNOIDES* AND OTHER SP. (B; $P<0.05$). NON-STATISTICALLY SIGNIFICANT DIFFERENCES WERE FOUND FOR: *HENICORHYNCHUS SP., LABIOBARBUS LINEATUS* AND *PARALAUBUCA BARRONI* (A; $P>0.05$). NOTE THE COEFFICIENT OF DETERMINATION (R^2), MAXIMUM SPECIES BODY-SIZE AND GUILDS.

4.6 CONCLUSIONS

The level of detail obtained from the Landsat flood model in assessing seasonal (dry and wet seasons) modifications in the lake area, produces the same findings performed by the Digital Bathymetric Model. This is one of the most significant findings in this chapter, providing evidence of the usefulness and reliability of using remote earth observation tools for assessing the historical importance of the predictability and seasonality of the flood pulse in driving the observed variations in the catchment area of a complex freshwater system.

The spatio-temporal analyses of the flood balance show that during the 19-years, the amount of water flowing into and out of the lake has not changed substantially, which contributed to the historical low variations in the flood level. Additionally, the influence of strong El Niño (1997-1998) and La Niña (2011-2012) events might have had a role in the low and high observed flood levels, influencing the *dai* catch. Yet, limitations in the available data constrained on the quantification.

The *dai* fishery total catch has not changed substantially during the 20-years period analysis. Yet, the fish effort has increased. Moreover, the fishery is composed by over 50% of small body-size species that perform seasonal medium to long distance migrations from and into the lake. If counting with medium to large body-size species, the *dai* catches are composed by nearly 80% migratory fish species. Notably, the flood level variations in the lake did not significantly influence the observed total catch (1996-2014). Yet, historical variations in the flood index significantly influenced medium to large body-size fish species, which require longer flood duration and inundation extent to growth. For the small body-size species, the flood pulse does not significantly influence their annual catch. However, all species are likely to be affected by future variability in the timing of the flood, disruptions in the flood pulse and migratory barriers, associated with hydropower development, irrigation and climate change scenarios. The findings of this chapter help to fill the identified gap for the importance of quantifying the historical flood pulse dynamics and the eco-hydrologic interactions established in the seasonal flooded floodplains, for the fish dynamics and abundance supported, reflected in the historical *dai* fishery for the percentage contribution.

The results achieved in this chapter will be incorporated in the next chapter to predict the future changes in the catchment area and floodplain inundation pattern. Moreover, predictions will be made on the consequence to the *dai* fishery, species composition and contribution to the catch, and food security to future populations.

CHAPTER 5

Quantifying the impacts of hydropower, irrigation and climate change scenarios on the dynamics and productivity of the Tonle Sap Great Lake system

Overview of the chapter: This chapter focuses on quantifying future changes (2036 - 2064; see below) to the TSGL system's flood pulse and floodplain habitats inundation extent, as a result of scenarios of basin wide hydropower development, irrigation and climate change. Importantly, the consequences of such changes for the *dai* fishery and fish species guild composition and contribution to the catch are projected from the individual and combined impacts on the seasonal flood pulse-driven floodplain inundation dynamics. Such projections are vital in order to understand the potential future food security issues for the increasing populations of the Mekong basin.

5.1 INTRODUCTION

The Mekong river is undergoing rapid economic development, with increasing demands on energy, food and water from the rising populations that is expected to reach 100-145 million by 2050 (Varis *et al.,* 2012). This growth has already fuelled the development of hydropower dams and irrigation expansion schemes for energy production, water reservoir and agriculture (Grumbine and Xu, 2011; Kondolf *et al.,* 2018; Schmitt *et al.,* 2018; Hecht *et al.,* 2019; Hoang *et al.,* 2019). According to Kummu *et al.,* (2010) and Hecht *et al.,* (2019), the basin's hydropower reservoir storage may rise to over 90 km^3 in the next few decades, compared to the 8.6 km^3 in 2008, with over 120 dams projected to be built in the next 50 years (Keskinen *et al.,* 2012; Lauri *et al.,* 2012; Hoang *et al.,* 2016;2019; Hecht *et al.,* 2019). Moreover, irrigation is a major consumer of water in the basin, with a projected increase from 6.6 million hectares in 2010 to 8.2-9.7 million hectares in 2030 and a 65% increase in 2060 (Young, 2009; Hoanh *et al.,* 2010; MRC, 2011). Yet, the benefits of flood regulation, annual water provision and enhancement of energy and food provision often neglect the negative environmental consequences from the development of water infrastructures and diversion schemes (Hecht *et al.,* 2019). As such, the expected modifications in the annual and seasonal streamflow conditions, reductions in sediment flux and nutrient-bounded sediments, along with shifts in the predictability and seasonality of the historical flood pulse dynamics, and consequent alterations in floodplain habitats, are argued to significantly impact upon the productivity of the Mekong region and the ecosystem services provided (Kummu *et al*., 2010; Piman *et al.,* 2013; Pokhrel *et al.,* 2018; Hecht *et al*., 2019). Climate change scenarios in the region are projected to increase the variability of the precipitation patterns (4% increase), while increasing the annual temperature (1-2 ºC) and magnifying the uncertainty of extreme flood/drought events, having significant impacts particularly in the TSGL system and Vietnamese delta (Keskinen *et al.,* 2010; Västilä *et al*., 2010; Arias *et al.,* 2012;2014). Therefore, threats to the high fish diversity, particularly to the migratory fish guild species, and modifications in the vital inland fisheries have been argued to be a consequence of the multiple drivers of environmental change on the system. These impacts relate to changes upon the timing, duration and extent of flooded floodplains, migratory barrier effect and modifications in the life cycle, resulting from the shift in the long-term hydrologic regime, directly threatening the future fish protein provision for food security to people (Hortle, 2007;2009; Baran and Mychowoda, 2009; Ziv *et al.* 2012; Halls *et al.,* 2013a; Chea *et al.,* 2016).

Although efforts have been made to quantify the individual and combined impacts of the Mekong river's multiple drivers of environmental change on the streamflow regime, sediment flux and habitat inundation dynamics, particularly in the Vietnamese delta, much less attention has been paid to the TSGL system flood pulse, the associated area of seasonally inundated floodplains and the impacts upon the fishery resources. Kummu and Sarkkula (2008) predicted the impacts of hydropower development in the Mekong basin on the TSGL system's flood pulse characteristics, arguing that the area of open water could increase by 17-40% in the dry season, as a direct response of higher water levels. This is associated with dam impoundment operations on the seasonal flow conditions observed in the basin, which in the wet season would result in the storage of the higher flows, releasing it in the dry season, when lower flows are observed, particularly in downstream areas, resulting in higher flood levels (see Chapter 3; Lu and Siew, 2006; Kummu and Sarkkula, 2008). The study also mentioned that the area of seasonally inundated floodplains could decrease in the wet season by 7-16% as a direct consequence of the flow storage in the wet season and consequent decrease in water levels. Of note is that changes in flood duration and timing were also reported and associated with the mainstream dam's active storage of water during the wet season and release in the dry season. Through a combination of remote sensing tools and hydrological modelling scenarios, Arias *et al.,* (2012;2014) demonstrated that, when compared to climate change, a much greater impact was felt as a result of water infrastructure development (comprising 126 future dams and active storage greater than $2x10^6$ m^3, in both mainstream river and tributaries; MRC, 2009a; Piman *et al.,* 2013) on the lake's flood pulse characteristics and inundation pattern and thus the diversity of seasonally inundated habitats (e.g. gallery forests, flooded habitats dominated by shrublands and grasslands, agriculture rice fields and village crops, among others). The authors applied a baseline average, driest and wettest year approach to quantify and compare future scenarios of change, stating higher water levels during the dry season (+0.87±0.17m) and reduced water levels at the peak of the wet season in October (-0.4±0.5m), driven by hydropower scenarios. Moreover, the area of open water could increase by +18 to +21%, as well as the area of rainfed habitats (+10% to +14%). However, the area covered with seasonally flooded habitats and gallery forest would be reduced by -13 to -22% and -75% to -83%, respectively. The combined effect of climate change and hydropower development is projected to reduce the maximum flood extent by 529±619 km^2 and reduce the flood duration of habitats flooded for 9 months (e.g. gallery forest) by 33±44% in area.

These findings above are similar to the previous reported estimations by Kummu and Sarkkula (2008) and Västilä *et al.,* (2010). Pokhrel *et al.,* (2018) recently estimated the potential disruption on the TSGL system's floodplain flood dynamics, due to future projections on the upstream dams' flow regulation, using modelling simulated scenarios of the Mekong river's flow conditions and satellite imagery. The results suggested that the vital, unique bi-directional flow reversal in the Tonle Sap River could cease if the Mekong river's flood pulse observed in Stung Treng decreases by up to 50% and was delayed by one-month. Moreover, the increase in flood level in the dry season and reduction during the wet season are expected to impact upon the duration of the floodplain flooded areas by the time of the flow reversal, with expected decrease in flood duration by 2 to over 50 days, significantly impacting the areas flooded for longer than 6 months, with expected consequences to the productive fisheries. Although these studies have recognised the importance of the flood pulse dynamics to the predictable seasonal inundation established in floodplain habitats and mentioned the significance to fish dynamics, abundance and fishery yields, the analyses have only quantified changes on the flood pulse. Thus, key gaps are identified regarding the quantification of the consequences of these eco-hydrologic changes in the TSGL system to the *dai* fishery and fish composition of the catch, particularly for the medium to long distance migratory species that contribute nearly 80% to the catch (see Chapter 4). Given that migratory species contribute over 50% to the total yield and to the highest fish protein consumption in the system and basin (i.e. 70 kg person^{-1} year^{-1}; see Chapter 4; So, 2010), it is vital to address these issues, since modifications to the historical productive fishery and the composition and contributing role of the migratory fishes to the catch, will likely exacerbate future food security issues in a region marked by severe malnourishment (Hortle 2007;2009; Baran, 2010; So, 2010).

Consequently, in light of the relationships and findings established in Chapter 4 for a baseline period of 1996-2014, this chapter aims to quantify and compare the individual and combined impacts of the Mekong river's future (2036-2064) hydropower development, irrigation expansion and climate change scenarios on the flood pulse and floodplain flood dynamics of the TSGL system. These scenario-driven changes are then used to estimate the consequences to the *dai* fishery, migratory fish guilds composition and contribution to the catch, and food security, in light of the projected increase in the system and basin's population. Hence, a set of specific objectives are established to answer this chapter's aims:

1. Quantify and compare the individual and combined drivers' impacts on the future (2036-2064) annual and seasonal variations in the flood level of the lake, based on the baseline flood level conditions;
2. Applying the Landsat flood model (1996-2014), quantify and compare the individual and combined drivers' impact on the future annual and seasonal variations in the lake's permanent area, based on the baseline conditions;
3. Quantify and compare the individual and combined drivers' impacts on the future variations in the flood pulse-driven floodplain flood dynamics, and the influence on the *dai* fishery catch and migratory fish guild catch composition, following the flood index baseline relationships established;

4. Predict future food security challenges, driven by the modifications in the *dai* fishery resources and in light of the projected increase in the basin's population, following the baseline annual fish protein consumption.

5.2 METHODS

5.2.1 HYDROPOWER DEVELOPMENT, IRRIGATION EXPANSION AND CLIMATE CHANGE SCENARIOS

This study uses the VMod modelling results of the future (2036-2064) daily discharge conditions at Kratie's gauging station, under the individual and combined impacts of the multiple drivers of environmental change, obtained from Hoang *et al.,* (2016;2019)[12] to project the future daily water level conditions at the TSGL system. Hoang *et al.,* (2016;2019) provide the only available detailed daily projections of the flow conditions at the closest (i.e. nearly 250 km) gauging station to the TSGL system that is expected to influence the flood pulse dynamics in the lake (see sub-section 5.2.3). These detailed projections result from the assessment of the individual and combined impacts of the three drivers argued to impact the most the Mekong river's flow dynamics into the future (i.e. hydropower, irrigation and climate change; Hoang *et al.*, 2016;2019). Moreover, and for the TSGL system, other studies (e.g. Arias *et al.*, 2012;2014) not only have only focused in two drivers, but also the methodological approach applied (i.e. projection of changes in the future flood pulse dynamics based on the simulation of the mean, wettest and driest year, from a baseline observed mean, wettest and driest year) did not provide daily estimations of the lake's water level and, therefore, could not allow the future analyses of the annual and seasonal changes in the flood level conditions and consequent effect on the catchment area and floodplain flood dynamics of the system here assessed. Thus, Hoang *et al.* (2016;2019) modelled scenarios of daily discharge offered the only reliable approach to extrapolate the future daily water levels at the TSGL system. This allowed detailed assessment of the individual and combined impacts of the three drivers to the annual and seasonal flood pulse dynamics, floodplain flooding and

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[12] Although Hoang *et al.,* (2016;2019) report analyses for the 2036-2065 period, for this study the simulated scenarios of daily discharge data for Kratie, only has data until 2064. Therefore, all analyses in this chapter report to the 2036-2064 period.

quantified effect on the *dai* fishery resources. A brief description of the individual drivers' scenarios used by the authors, the VMod model operation module, calibrations, validations and scenarios setups, are mentioned below. For full technical descriptions of the VMod hydrological model operation, parameterization and applied studies for the Mekong basin, see Lauri *et al.,* (2006;2012), Räsänen *et al.,* (2012), Arias *et al.,* (2014), Darby *et al.,* (2016) and Hoang *et al.,* (2016;2019).

I) HYDROPOWER DEVELOPMENT SCENARIO

The hydropower dam database of the Mekong River Commission (MRC, 2009a) and the hydropower dam dataset from ADB (2004) were used by Hoang *et al.,* (2019) to establish the hydropower development scenario. This scenario included a total of 126 dams at different stages of development (i.e. in operation, under construction or at the planning phase), including 16 mainstream dams and 110 tributary dams, with a total active storage of 108 km^3. All hydropower dams are expected to be fully operational across the 2036-2064 period assessed.

II) IRRIGATION EXPANSION SCENARIOS

MIRCA dataset – "Global Dataset of Monthly Irrigated and Rainfed Crop Areas around the Year 2000" (Portmann *et al.,* 2010) was firstly used to establish a baseline irrigation scenario. This dataset provides data on irrigated area and cropping calendar for 26 crops at 5 arc-minutes resolution that according to the reported methodology by Hoang *et al*., (2019) is equivalent to nearly 9 km x 9 km at the Equator. Since the resolution is higher than the 5 km x 5 km resolution of the VMod hydrological model's grid (see sub-section 5.2.2), a new irrigation raster layer of 5 km x 5 km was resampled to keep consistency (Hoang *et al.,* 2019).

Irrigated rice is the most dominant crop in the Mekong basin, accounting to over 80% of the total irrigated land (Hoang *et al.,* 2019), with the remaining 20% linked to other crops (e.g. cassava, sugar cane, soybean and maize; Paiboonvorachat *et al.,* 2018). Therefore, the irrigation scenarios applied by the authors only focused on irrigated rice, with a baseline total irrigated rice area of 4.1 million hectares attributed to two cropping seasons (i.e. 2.04 million hectares in May and 2.07 million hectares at October). The future irrigation scenarios (2036-2064) were established using the MIRCA dataset and the global projected irrigation expansion scenarios by Fischer *et al.,* (2007). Importantly, the authors applied spatially explicit irrigated land expansion factors, derived from Fischer *et al.,* (2007) on the baseline MIRCA dataset, to calculate the future irrigated rice area.

III) CLIMATE CHANGE SCENARIOS

The downscaling of baseline climate data (1971-2000) is detailed in Hoang *et al.,* (2016), from the WATCH (Water and Global Change) forcing data for historic temperature (covering the 1958-2001 period; Weedon *et al.,* 2011) and the precipitation-based APHRODITE (Asian Precipitation – Highly-Resolved Observational Data Integration Towards Evaluation) dataset (i.e. a gridded observation-based precipitation dataset developed from a high-density network of rain gages over Asia; Yatagai *et al.,* 2012). Four input climatic variables are required for the VMod setup; daily mean, maximum and minimum temperatures, and precipitation. Future projections of these variables were derived from the Coupled Model Intercomparison Project 5 (CMIP5) for five GCMs (i.e. Global Circulation Models: ACCESS-1.0; CCSM4; CSIRO-Mk3.6.0; HadGEM2-ES and MPI-ESM-LR). Each GCM's climate data was individually extracted under two RCPs (i.e. RCP 4.5 and RCP 8.5; see Chapter 1 for the definitions), for the period 2036-2064. These models were selected based on their reported performance regarding historic temperature reproduction (Huang *et al.,* 2014), seasonal precipitation (Hasson *et al.,* 2016) and climate extremes (Sillmann *et al.,* 2013). The GCM's data, following Piani *et al.,* (2010), were downscaled using bi-linear interpolation to a 0.5ºx0.5º spatial resolution and statistically bias corrected through quantile mapping (Hoang *et al*., 2016;2019).

5.2.2 VMOD HYDROLOGICAL MODEL

VMod is a distributed hydrological model extensively applied to simulate the Mekong river's flow conditions under changing environmental scenarios (Lauri *et al.,* 2006;2012; Arias *et al.,* 2014; Hoang *et al.,* 2016;2019). The model uses a square grid representation of river basins, with the grids using multiple raster layers containing data for flow direction, river network, soil and land use (Hoang *et al.,* 2016). The VMod setup covered the entire Mekong basin and was contains data pertaining to soil, land use and elevation datasets, allowing daily hydrologic simulations at 5 km x 5 km spatial resolution (see Hoang *et al.,* 2016;2019 for information on the soil, land and elevation datasets). The observed river's flow data for model calibration and validation was collected from the MRC (2011) hydro-meteorological database for Chiang Saen, Vientiane, Nakhon Phanom, Mukdahan, Pakse, Stung Treng and Kratie gauging stations. For this study, only the simulated scenarios of daily flow conditions at Kratie were used, for the closest distance to the TSGL system (see sub-section 5.2.1).

I) INDIVIDUAL DRIVERS' OPERATION AND SIMULATION MODULES

The VMod simulation process begins by interpolating climate inputs for each grid cell from climate input data, which require a minimum of four daily climate forcing variables (see sub-section 5.2.1 for the climate change scenarios). Using an inverse distance weighted-interpolation, climate forcing data was calculated for each grid cell. Subsequently, soil surface processes and the soil water balance are calculated, so that river flow is routed from cell to cell and finally into the river network (see Lauri *et al.,* 2006 and Hoang *et al.,* 2016 for further descriptions).

The impact of hydropower dam operation was assessed by simulating monthly outflow for each dam, since actual hydropower dam operation data, although available for the current dams in operation (MRC, 2009a), are not available for those projected to be in operation in the future period. Moreover, the dams' simulation was based on the optimisation scheme by Lauri *et al.,* (2012). According to Hoang *et al.,* (2019), the optimisation process uses active storage, monthly inflow, minimum outflow and designed optimal outflow set of parameters, to calculate the monthly outflow for each individual dam. Constraining aspects were added to the dam's operation (i.e. maintaining dry season flow constant and reservoirs filling and emptying during the wet and dry seasons, respectively). The operation rules developed for each dam followed the upstream-to-downstream flow pattern, to account for the upstream existing and planned flow regulation from the cascade dam development (Lu and Siew, 2006; Lauri *et al.,* 2012; Fan *et al.,* 2015). For further information on the technical aspects of the hydropower dam operation module see Hoang *et al.,* (2019).

The irrigation impact on the Mekong river's flow regime was simulated following the crop and irrigation water use module developed by Hoang *et al.,* (2019), based on the AquaCrop developed model by FAO (2012). The AquaCrop-based module simulates crops growth over a step-wise procedure, starting from measuring the soil water balance, followed by canopy developments, crop water use, irrigation demand, biomass and crop yields, as stated in Hoang *et al.,* (2019) methodology. This module, which is fully integrated into the VMod modelling framework, operates by 24 hours simulation time-steps, in which the irrigation water is extracted from the river and applied to the corresponding irrigated model grid cells. The water balance is calculated in the crop model and runoff is directly linked to the routing module of VMod. Further technical aspects of the crop and irrigation module can be seen in Hoang *et al.,* (2019).

II) MODEL CALIBRATIONS, VALIDATIONS AND SCENARIOS' SETUP

The VMod hydrological model was calibrated (1981-1991) and validated (1991-2001) by Hoang *et al.,* (2016) against observed daily discharge at Chiang Saen, Vientiane, Nakhom Phanom, Mukdahan, Pakse, Stung Treng and Kratie mainstream gauging stations. The individual stations' daily river discharge plots and flow duration curves (Vogel and Fennessey, 1995) were used to visually check the goodness of fit between observed and simulated data. Additionally, the Nash-Sutcliffe Efficiency (ranging between $-\infty$ and 1, where values closer to 1 demonstrate a good fit between observed and simulated time series; Nash and Sutcliffe, 1970) and relative biases indices (Normalized Root Mean Square Error) were used to assess the model's performance during the calibration and validation process. The model showed overall good performance in reproducing the historical river flow conditions, with the assessment of the individual's gauging station Nash-Sutcliffe Efficiency always higher than 0.5, and an average value of 0.67 across the stations, during the calibration and validation periods. The hydropower simulation module was also calibrated and validated by Hoang *et al.,* (2019) against the observed impacts of the Chinese dam cascade (Lauri *et al.,* 2012; Räsänen *et al*., 2017). A two-step procedure was applied to calibrate and validate the irrigation simulation module, by first comparing the simulated crop outputs with reference data from the AquaCrop model (World Bank, 2012), followed by calibrating the simulated yields, crop water use and irrigation water demand for wet rice in two sub-catchments (i.e. Xebang Fai in Lao PDR and Yom in Northern Thailand; World Bank, 2012; ICEM, 2015). Reliable simulation performances were obtained for both modules (see Hoang *et al.,* 2019).

Since VMod showed good performance in simulating the historical river's flow conditions and based on Hoang *et al.,* (2016;2019) scenarios setups to obtain the simulated daily discharge under the individual and combined drivers' scenarios, Table 5.1 shows the drivers' scenarios assessed in this study to quantify and compare the individual and combined drivers' impacts on the historical flood pulse, floodplain flood dynamics and *dai* fishery resources.

TABLE 5.1 - INDIVIDUAL AND COMBINED DRIVERS' SCENARIOS ASSESSED IN THIS STUDY FOR HYDROLOGICAL IMPACT ASSESSMENT, BASED ON HOANG *ET AL.,* (2016;2019) VMOD MODELLING SCENARIOS SETUP TO OBTAIN FUTURE DAILY DISCHARGE DATA.

Scenarios	***Future (2036-2064)***
Climate Change	Hoang *et al.,* (2016) individual RCP4.5 and RCP8.5 daily discharge at Kratie.
Hydropower development	Hoang *et al.*, (2019) individual RCP4.5+Hydropower daily discharge at Kratie and RCP8.5+Hydropower daily discharge at Kratie.
Irrigation expansion/ Combined impacts	Hoang *et al.,* (2019) individual RCP4.5+Hydropower+Irrigation daily discharge at Kratie and RCP8.5+Hydropower+Irrigation daily discharge at Kratie
Notes	The individual drivers' impacts on the annual and seasonal variations in the TSGL system's flood pulse-driven flood level, permanent area, flood index, *dai* catch and fish species guild composition (see sub-sections 5.2.4 and 5.2.5) were assessed by comparing each set of scenarios' results (i.e. comparing the Climate Change RCP4.5 results to the RCP4.5+Hydropower results, for the mentioned variables, this way assessing the individual hydropower's impact. The same was applied to the individual irrigation assessment and to the RCP8.5 scenarios).

5.2.3 THE MEKONG RIVER'S HISTORICAL (1996-2014) FLOW AND RELATION TO THE TSGL SYSTEM FLOOD LEVEL: FUTURE WATER LEVEL EXTRAPOLATIONS

The VMod hydrologic model applied by Hoang *et al.,* (2016;2019) simulated daily future (2036-2064) discharge conditions at Kratie's gauging station, under the individual and combined impacts of the Mekong river's hydropower development, irrigation expansion schemes and climate change scenarios. Importantly, no future projections of the daily water level at the TSGL system, under the individual and combined multiple drivers of environmental change, are available (see sub-section 5.2.1). Therefore, the daily water level projections at the lake were extrapolated from the VMod's simulated daily discharge conditions at Kratie, under the individual and combined drivers' scenarios. The need to extrapolate the future daily water level conditions at the TSGL system, was to allow the quantification of the future changes in the flood pulse, floodplain flood dynamics and *dai* fishery resources, based on the relationships established in Chapter 4 (see sub-sections 5.2.4 and 5.2.5). For that, a baseline (1996-2014) relationship was established between the observed one-day delay[13] daily discharge at Kratie's gauging station and the observed daily water level at Kampong Luong's gauging station (see Chapter 4; Kummu *et al.,* 2014).

Kummu and Sarkkula (2008), Campbell *et al.,* (2009) and Kummu *et al.,* (2014) described the unique bi-directional flow reversals observed between the TSGL system and the Mekong river. The complexity of these hydrologic shifts means that at the onset of the wet season, the rise in the Mekong river flow and the low flood level observed at the TSGL system, results in the Mekong's upstream-downstream unidirectional flow pattern to be reversed into the TSGL system at the Tonle Sap river junction (see Chapter 4 for the geographical location). The fall in the Mekong river flow (i.e. decrease in the observed peak flow), marking the end of the wet season and the onset of the dry season, results in the flow reversal, where the waters from the lake flow out to the mainstream river (Kummu and Sarkkula, 2008; Kummu *et al.,* 2014).

Assessing the rise and fall dynamics of the Mekong river and relating it with the complexity of the bi-directional flow reversals at the TSGL system is a challenging process, since there are inter-annual variations in the transitional lag-times for the rise and fall of the Mekong's streamflow that result in changes in the flood levels in the TSGL system (Kummu *et al.,* 2014). Therefore, the following baseline metrics for the period 1996-2014 were assessed to establish the best option to extrapolate the future (2036-2064) daily water level at the Kampong Luong's gauging station, from the VMod's simulated daily discharge at Kratie's gauging station.

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[13] The geographical distance (i.e. nearly 250 km) of Kratie's gauging station to the TSGL system, leads to the inference of a one-day delay for the observed flow conditions at Kratie to result in a flood level change inside the lake (i.e. at Kampong Luong gauge). This inference was based on individual time-series (1996-2014) regression analyses performed assessing the daily flow at Kratie and same-day, 1-day delay, 2-day delay and 3-day delay water level conditions at Kampong Luong.

- <u>Baseline (1996-2014) observed Kratie's one-day delay daily discharge relation to the observed daily Kampong Luong water level</u>: daily observed flow at Kratie and correspondent daily water level at Kampong Luong one day later were gathered from the MRC Data Portal[14] for the baseline period. Annual assessments of Kratie's flow hydrograph (i.e. for the minimum and peak flow) were made to define the rise (i.e. the day after the minimum flow date until the peak flow date) and fall (i.e. the day after the peak flow date until the minimum flow date) periods. The corresponding individual periods lake's water level was gathered. The combined baseline period's rise and fall relationships were established to assess the importance of the flow conditions at Kratie to the observed water level variations at Kampong Luong (Figure 5.1). From Figure 5.1 it is possible to see a hysteresis loop associated with the rise and fall of the Mekong river. The hysteresis effect in unsteady flows has been argued to be a challenge in hydrometry, where the standard application of a single-valued rating curve to the available stage-discharge measurements is considered inappropriate (Petersen-Øverleir, 2006; Muste and Lee, 2013; Huang *et al.,* 2017). Thus, individual linear and non-linear regression analyses have been applied to account for the counter-clockwise effect (Petersen-Øverleir, 2006). In Figure 5.1, the linear regression fit (Equation 5.1) best describes the rise relationship established ($R^2=0.83$; ANOVA; $F_{(1,2853)}=13595.92$; $p=0.000$) between the daily Kampong Luong water level and discharge at Kratie lagged by one day. With respect to the falling limb relationship, a logarithmic regression fit (Equation 5.2) was applied ($R^2=0.77$; ANOVA; $F_{(1,3720)}=12915.78$; $p=0.000$) to the daily lake's water level, driven by the one-day delay daily discharge conditions at Kratie.

RISE EQUATION: Y=0.0001434X + 1.107 **(EQUATION 5.1)**

FALL EQUATION: Y=2.59LN(X) - 17.09 **(EQUATION 5.2)**

The future (2036-2064) daily water level extrapolations from the simulated daily discharge, were performed by applying the previous reported approach to define

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[14] http://portal.mrcmekong.org/
Kratie gauging station ID: 014901;
Kampong Luong gauging station ID: 020106.

the rise and fall periods. Then, by applying the baseline's individual rise and fall equations (Equation 5.1 and Equation 5.2, respectively), the daily future water level at the lake was extrapolated. The individual rise and fall relationships established showed an averaged 3-meter gap in the future water level extrapolations. Therefore, modifications on those relationships are described below, in an attempt to reduce this seasonal water level gap, which is not observed in reality.

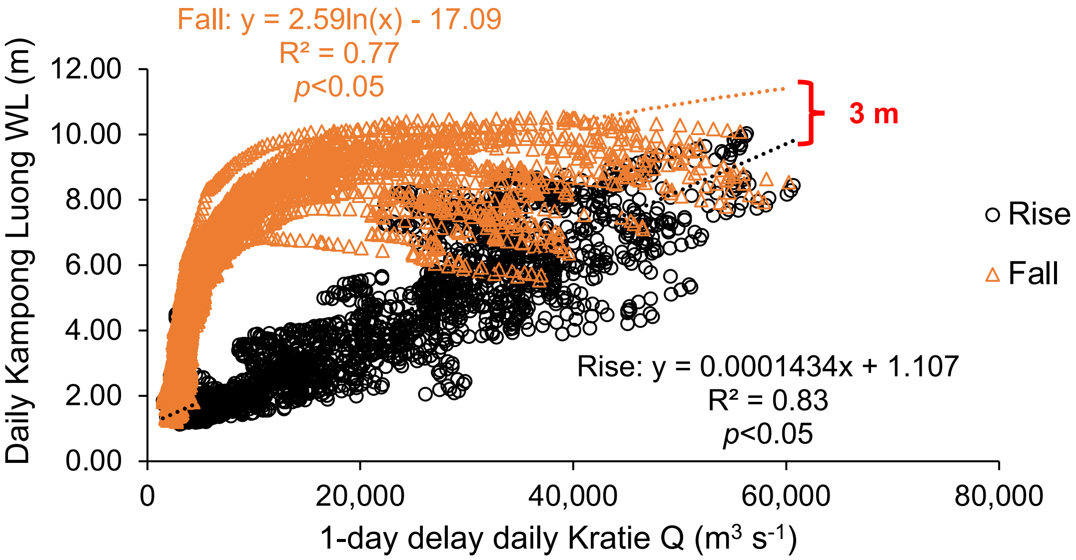

FIGURE 5.1 - HISTORICAL (1996-2014) RELATIONSHIPS ESTABLISHED BETWEEN THE OBSERVED ONE-DAY DELAY FLOW ($M^3\ S^{-1}$) AT KRATIE'S GAUGING STATION AND THE OBSERVED DAILY WATER LEVEL (M) AT KAMPONG LUONG'S GAUGING STATION, TO EXTRAPOLATE FUTURE DAILY WATER LEVEL (M). BLACK CIRCLES REPRESENT THE RISE RELATIONSHIP (R^2=0.83; *P*=0.000). ORANGE TRIANGLES REPRESENT THE FALL RELATIONSHIP (R^2=0.77; *P*=0.000). THE RED BRACKET SHOWS THE MEAN 3-M WATER LEVEL GAP IN THE FUTURE WATER LEVEL.

- <u>Baseline (1996-2014) observed Kratie's one-day delay daily discharge relation to the observed daily Kampong Luong water level, with a plateau stage:</u> as previously described, there are transitional lag-time periods from the rise and fall flow dynamics of the Mekong river to induce a change in the flood levels of the TSGL system (Kummu and Sarkkula, 2008; Kummu *et al.,* 2014). Yet, the definition of those transitional periods is a challenging aspect on the complex TSGL system flood pulse dynamics. Nevertheless, the establishment of a baseline plateau stage for the rise and fall previously described relationships was attempted. The plateau stage was yearly defined based on the transitional rise and fall periods in the one-day delay discharge at Kratie, with the correspondent water level values at Kampong Luong. Furthermore, the same procedure was applied to the yearly future simulated daily discharge scenarios, to define a rise, fall and plateau relationships. Despite an expected improvement in the water level extrapolations (i.e. reduction in the 3-meter water level gap), the application of the baseline three equations established (i.e. for the rise, fall and plateau; see Appendix 6) to the future water level extrapolations, only showed a marginal, averaged 0.5-meter, decrease in the water level gap (see Appendix 6). In addition, the resulting low explanatory influence of the defined plateau stage (R^2=0.20), reflected the difficulty in establishing the transitional lag-time periods at the TSGL system, for the complexity of the hydrologic conditions at the TSGL-Mekong river system. For this reason, and although significant efforts were applied to improve the plateau relationship, this approach was discarded for the future projections.

- <u>Baseline (1996-2014) observed Kratie's one-day delay daily discharge relation to the observed daily Kampong Luong water level, following a water level peak flood:</u> the previous rise and fall relationships were established based on the minimum and peak flow values at Kratie. The possibility that the flood level inside the TSGL system would continue to rise after the Mekong's peak flow was reached was assessed based on the measurement of the lake's minimum and peak flood level, to define the rise and fall metrics. The aim here was to address the importance of the bi-directional flow reversals in continuing to provide inflow water to the lake, and this way rising the flood level, while the peak flow in the mainstream river started to decrease. As such, yearly assessments of the minimum and peak water levels were measured to delimit the baseline (1996-2014) rise and fall periods, with the correspondent Kratie's one-day delayed daily discharge values. The application of the defined baseline's rise and fall metrics to the future extrapolations of the Kampong Luong's daily water level, from the simulated daily discharge scenarios (2036-2064), not only did not improve the previously described water level gap, but it increased the gap to an average of 5.2 meters (see Appendix 6). Therefore, although expected for the flood levels of the lake to continue to rise over a short-period of time, despite the decrease in the mainstream river flow (Kummu *et al.,* 2014), from the established relationships this was not observed, and those metrics were not applied to the future projections.

- Baseline (1996-2014) observed Kratie's one-day delay daily discharge relation to the observed daily Kampong Luong water level, applying a single annual linear and non-linear regression fit: the previous relationships acknowledged the hysteresis loop and consequent importance of individually addressing the rise and fall dynamics of the flow. In the current attempt to reduce the observed water level gap, the previous rise and fall relationships were replaced by the application of a single-annual linear and non-linear regression analyses. A baseline (1996-2014) linear regression fit analysis was established to assess the importance of the Kratie's one-day delay daily discharge in driving the observed daily water level variations at Kampong Luong. The estimated equation was applied to the simulated future daily discharge scenarios to extrapolate the future daily water level at the TSGL system. Importantly, although no water level gap was observed, the application of a single-annual linear fit equation under-estimated the future lake's water level values (averaged of 3.3-meter decrease; see Appendix 6), when compared to the rise and fall-based future water level extrapolations. The same methodology was applied but employing a single-annual non-linear regression fit analyses (first by a logarithmic and then by a power fit; see Appendix 6), which resulted in an averaged 2.7-meter (for the logarithmic fit) and 2.9-meter (for the power fit) under-estimations of the lake's water level, compared to the rise and fall-based future water level extrapolations. Consequently, although recognising that no water level gap was present on the future daily water level extrapolations, the water level under-estimation undermined the application of the single-annual metrics for the further analyses. Notably, it highlighted the importance of addressing the rise and fall flow dynamics to predict the flood level variations at the TSGL system.

- Baseline (1996-2014) observed Kratie's one-day delay daily discharge relation to the observed daily Kampong Luong water level, following the hydrologic year approach: the final attempt to reduce the water level gap focused on applying the hydrologic year approach (i.e. 1st May – 30th April; Kummu *et al.*, 2014) to define the baseline rise and fall relationships. The application of the hydrologic year approach is described in the literature to account for the pulsing of water into and out of the lake (Kummu and

Sarkkula, 2008; Kummu *et al.,* 2014). Additionally, the baseline relationships established to understand the historical importance of the TSGL system's flood pulse to the annual and seasonal variations in the lake's flood level, followed the hydrologic year approach (see Chapter 4). Thus, after defining the simulated daily discharge's rise and fall periods, following the hydrologic year approach, by measuring the minimum and peak flow, the baseline hydrologic year's rise and fall metrics were applied to extrapolate the lake's water level. An averaged 3.2-meter gap in the water level extrapolations was observed (see Appendix 6). Therefore, the application of the hydrologic year approach to extrapolate the future water level was discarded for the future projections.

Following the assessment of the methodologies applied for the establishment of the baseline rise and fall metrics, to accurately extrapolate the lake's future (2036-2064) daily water level, from the VMod's simulated daily discharge scenarios, the rise-fall relationships in Figure 5.1 were chosen. It is recognised that there are potential implications of the averaged 3-meter water level gap to the further quantifications of the individual and combined drivers' impacts on the annual and seasonal variations in the TSGL system's future flood level, permanent lake area and floodplain flood dynamics (see the Discussion section). As such, a curve fit using the MATLAB toolbox, with a 95% confidence prediction boundary equations (Table 5.2), were applied to predict the water level's mean, upper and lower variations, which were then applied to measure the variations in the water level-based estimations of the variables under study (i.e. permanent lake area, flood index, *dai* catch and individual fish species composition), by applying the baseline relationships established in Chapter 4 (see sub-sections 5.2.4 and 5.2.5).

TABLE 5.2 - PREDICTION BOUNDARY EQUATIONS FOR THE RISE AND FALL METRICS, TO ESTIMATE FUTURE DAILY WATER LEVEL VARIATIONS (M) AT 95% CONFIDENCE LEVEL.

	Mean boundary	***Upper boundary***	***Lower boundary***
Rise equation	Y=0.0001434x+1.107	Y=0.0001459x+1.16	Y=0.000141x+1.053
Fall equation	Y=2.59ln(x)-17.09	Y=2.635ln(x)-16.69	Y=2.545ln(x)-17.48

5.2.4 Quantifying the individual and combined drivers' impacts on the TSGL system flood pulse-driven flood level, permanent lake area and floodplain inundation profile

Annual and seasonal variations in the TSGL system flood pulse-driven flood level (following Kummu *et al.,* 2014 hydrologic year approach regarding Julian days) and permanent lake area (following a Julian days calendar year approach) were quantified using the daily extrapolations of the lake's water level, under the individual and combined impacts of hydropower, irrigation and climate change scenarios. The results are reported as means for the entire future period for each individual and combined drivers, for the mean, upper and lower boundaries, with the quantified errors based on the differences between the RCPs scenarios (i.e. RCP4.5 and RCP8.5). The results were compared, for the percentage variation, to the baseline conditions in Chapter 4, to assess the individual and combined impacts of the driver's scenarios on the future (2036-2064) dry (November – April) and wet seasons' (May – October) flood level, permanent area, annual flood level variations and peak flood timing. The quantification of the daily permanent area was performed using the baseline Landsat flood model's break slope equations established for the dry and wet seasons (Equations 4.5 and 4.6, respectively), to quantify future seasonal changes in the catchment area, reflecting the variations in the lake's flood pulse-driven flood level. The future changes in the flood pulse-driven seasonal floodplain inundation profile were assessed by applying the flood index methodology described in Chapter 4 (see Equation 4.3), assessing the timing, duration and extent of the floodplains annual flood events. The individual and combined impacts of the drivers' scenarios on the floodplain inundation profile were quantified for the future period and compared with the baseline conditions, for the percentage change. Notably, the baseline period's threshold area defining a flood event (i.e. 6,093 km^2) was here applied to define a flood event, to allow for the comparison of changes. Particularly, changes at the timing (start/end), duration and extent of the flood from each individual and combined drivers will be further discussed (see Discussion section) for the expected future disruptions on the historical high productivity of the system and consequences to the fish abundance supported and ecosystem services provided (Sarkkula *et al.,* 2003; Campbell *et al.,* 2006;2009; Halls *et al.,* 2013a).

5.2.5 PREDICTING THE FUTURE OF THE *DAI* FISHERY CATCH RESOURCES FROM THE QUANTIFIED IMPACTS OF THE INDIVIDUAL AND COMBINED DRIVERS' SCENARIOS ON THE FLOOD PULSE-DRIVEN FLOODPLAIN INUNDATION PROFILE

The baseline (1996-2014) non-linear regression fit equation (Equation 4.8) established in Chapter 4, assessing the importance of the floodplains' flood index to the observed variations in the *dai* fishery catch, was used to estimate the future (2036-2064) annual *dai* fishery catch and averaged period catch. The estimation was based on the quantified annual future flood index for the individual and combined drivers' scenarios. The averaged period catch was compared (i.e. for the % change) to the baseline averaged catch, to assess the individual and combined influence of the drivers to the historical productive *dai* fishery (Halls *et al.,* 2013a). Furthermore, the baseline individual non-linear regression fit equations established, assessing the importance of the floodplains' flood index to each individual top-5 fish species guild composition of the catch (*Henycorhynchus* sp. – Equation 4.9; *Labiobarbus lineatus* – Equation 4.10; *Paralaubuca barroni* – Equation 4.11; *Labeo chrysophekadion* – Equation 4.12; *Thynnichthys thynnoides* – Equation 4.13; Other sp. – Equation 4.14), were used to estimate the individual future annual top-5 fish species composition of the *dai* fishery and the percentage contribution of each species and guilds belonged to the future *dai* catch (i.e. 2036-2064). The comparison of the individual top-5 species contribution to the future catch with the baseline species contribution (see Chapter 4), was performed for the percentage change, to assess the influence of the individual and combined drivers' effects on the floodplains' flood index, on the quantified changes. The *dai* catch and top-5 fish species composition of the catch are reported as means for the entire future period for each individual and combined drivers, for the mean, upper and lower boundaries, with the quantified errors based on the differences between the RCPs (i.e. RCP4.5 and RCP8.5).

5.2.6 STATISTICAL ANALYSES

The future changes in the TSGL system's flood level, permanent lake area, floodplains' flood index, *dai* catch and fish species guild composition and contribution to the catch, driven by the individual and combined impacts of the Mekong river's drivers of environmental change, were quantified and compared to the baseline conditions in Chapter 4, to assess significant differences. The homogeneity of variance and normality assumptions for the one-way ANOVA test failed to be accomplished, thus non-parametric Kruskal-Wallis statistical analyses followed by the Dunn's post hoc test were applied to understand the individual and combined effects of the drivers' scenarios on the future changes in the flood pulse characteristics, floodplain flood dynamics and *dai* fishery resources, with the upper, lower and mean boundary variations assessed. All statistical analyses were performed in SPSS 24.0 and interpreted with reference to the 95% confidence level (Zar, 2010).

5.3 RESULTS

5.3.1 THE INDIVIDUAL AND COMBINED IMPACTS OF THE MEKONG RIVER'S HYDROPOWER, IRRIGATION AND CLIMATE CHANGE SCENARIOS ON THE TSGL SYSTEM FLOOD LEVEL AND PERMANENT LAKE AREA

The Mekong river's individual and combined scenarios of hydropower, irrigation and climate change profoundly modified the flood pulse characteristics of the TSGL system. In Figure 5.2 it is possible to see that individual climate change scenarios for both RCP's (i.e. Figure 5.2; **A – a)** and **B - d**), for the three boundary predictions, resulted in a mean 24-28±3 days (i.e. 140-144 days, corresponding to 17th-21st September) early peak flood, compared to the baseline mean 168±3 days (i.e. 15th-18th October) peak flood (black line). Regarding the individual hydropower scenarios, under the RCP 4.5 scenario (Figure 5.2; **A- b**), and for the three boundaries, a mean 14±3 days (i.e. 154±3 days) delay compared to the individual climate change results is detected, resulting in an early mean 14±3 days (i.e. 1st-3rd October) peak flood level compared to the baseline conditions. Regarding the RCP 8.5 scenario (Figure 5.2; **B- e**), and for the three boundaries, a marginal mean 4±3 days (i.e. 144±3 days) delay compared to the individual climate change results is detected, resulting in a mean 24±3 days early peak flood level (i.e. 21st-23rd September) compared to the baseline condition. The individual irrigation scenarios and combined drivers' scenarios followed the same mean flood peak changes detected for the hydropower individual effects, under RCP 4.5 scenarios (Figure 5.2; **A- c**) and RCP 8.5 scenarios (Figure 5.2; **B- f**).

A - RCP 4.5: **a)** Climate change; **b)** Climate change+hydropower; **c)** Climate change+hydropower+irrigation
B - RCP 8.5: **d)** Climate change; **e)** Climate change+hydropower; **f)** Climate change+hydropower+irrigation
Baseline: —; Mean boundary: — Upper boundary: — Lower boundary: - -

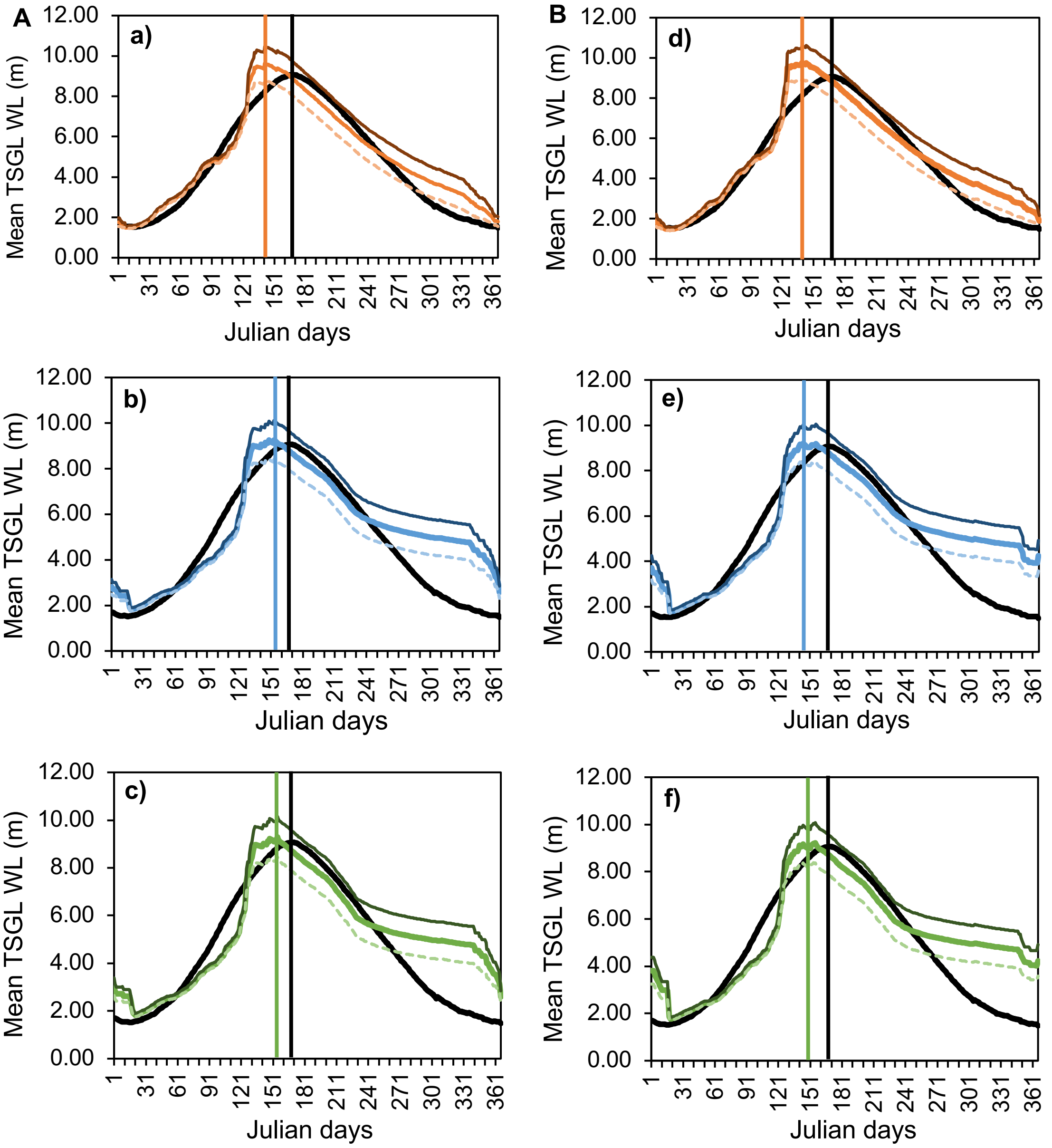

FIGURE 5.2 - FUTURE (2036-2064) MEAN DAILY VARIATIONS (HYDROLOGIC YEAR: 1ST MAY – 30TH APRIL) IN THE WATER LEVEL (M) AND PEAK FLOOD (VERTICAL LINES) OF THE TSGL SYSTEM UNDER THE INDIVIDUAL AND COMBINED DRIVERS. A) SHOWS THE INDIVIDUAL CLIMATE CHANGE **(A)**, CLIMATE CHANGE+HYDROPOWER **(B)** AND IRRIGATION/COMBINED DRIVERS' IMPACTS **(C)** UNDER THE RCP 4.5 SCENARIO. B) SHOWS THE INDIVIDUAL (**D**-CLIMATE CHANGE; **E**-CLIMATE CHANGE+HYDROPOWER) AND COMBINED (**F**-IRRIGATION/COMBINED) DRIVER'S IMPACTS UNDER THE RCP 8.5 SCENARIO. UPPER (THIN LINE) AND LOWER (DASHLINE) BOUNDARIES ARE SHOWN. BASELINE (1996-2014) CONDITIONS ARE PRESENTED BY THE BLACK LINE.

The findings shown in Figure 5.2 and synthesized in Table 5.3, Table 5.4 and Table 5.5 for the mean, upper and lower boundary variations, respectively, show that individual climate change impacts on the mean boundary flood level resulted in major changes in the water levels, which are predicted to increase by +0.32±0.03m (+7±1%) in the dry season (November – April) mean flood level and increase by +0.24±0.05m (+5±1%) in the wet season (May – October; Table 5.3) mean flood level. The upper (lower boundary) predictions showed a +24±1% (-9±0%) dry season increase (decrease) in the mean flood level and a +11±2% (-2±1%) wet season increase (decrease) in the mean flood level, respectively (Table 5.4 and Table 5.5, respectively).

The individual hydropower scenarios (i.e. by the quantified differences from the individual climate change scenarios' results; see Table 5.1) resulted in the highest modifications to the flood levels, with a mean boundary prediction of +0.84±0.00m (+20±1%) increase in the dry season mean flood level and -0.30±0.01m (-6±0%) decrease in the wet season mean flood level (Table 5.3). The upper and lower boundary predictions showed a +20±0% and +18±1% dry season increase in the mean flood level and a -6±1% upper and lower boundary wet season decrease in the mean flood level, respectively (Table 5.4 and Table 5.5, respectively).

The individual irrigation scenarios caused marginal mean boundary -0.04±0.00m (-1±0%) decrease in the dry season mean flood level and -0.03±0.01m (-2±1%) decrease in the wet season mean flood level (Table 5.3), with the upper and lower boundary predictions showing a -1±0% dry season decrease in the mean flood level for both boundaries and no modifications in the wet season mean flood level, compared to the mean boundary predictions (Table 5.4 and Table 5.5, respectively). Overall, the combined drivers resulted in a mean boundary +1.12±0.03m (+26±0%) increase in the dry season mean flood level and in a -0.09±0.01m (-2±1%) decrease in the wet season mean flood level (Table 5.3), with the upper and lower boundaries showing a +43±1% and +8±1% increase in the dry season mean flood level and a +5±2% (-8±1%) increase (decrease) in the wet season mean flood level, respectively (Table 5.4 and Table 5.5, respectively).

The annual flood level variations (i.e. for the averaged future period maximum and minimum water level), for the mean, upper and lower boundary predictions, showed no marked variations between the individual and combined drivers' scenarios (Table 5.3, Table 5.4 and Table 5.5).

In Figure 5.3, a mean boundary monthly analysis of the percentage change in the mean flood level of the TSGL system, under the RCP 4.5 individual and combined scenarios **(A)** and RCP 8.5 individual and combined scenarios **(B)** is performed by comparing to the monthly baseline mean flood level conditions. Importantly, this analysis follows the synthetized findings in Table 5.3 and helps to understand the early and delay pattern in the mean flood level curves of Figure 5.2, showing that individual hydropower scenarios comprised 2% to 70% flood level increase in the dry season months (November-April), for both RCP's, with the highest % change, compared to baseline, seen in April, the historical driest month in the basin. Also, in March climate change individual scenarios show a 54±1% increase in the flood level, being nearly 10% higher than the individual hydropower scenarios. Notably, under both RCP's scenarios, hydropower individual scenarios resulted in 38-60% increase in the flood level of the wet season (May), yet for the remaining wet season months (June-October) it shows the highest decreases (-5-20%). Furthermore, individual climate change resulted in increases in both dry and wet season months' flood levels, with exception to August-08, October-10, November-11 and December-12. No upper and lower boundary individual monthly analyses were performed, since these are described in Table 5.4 and Table 5.5, respectively and would only reflect higher flood level variations, according to the individual and combined scenarios.

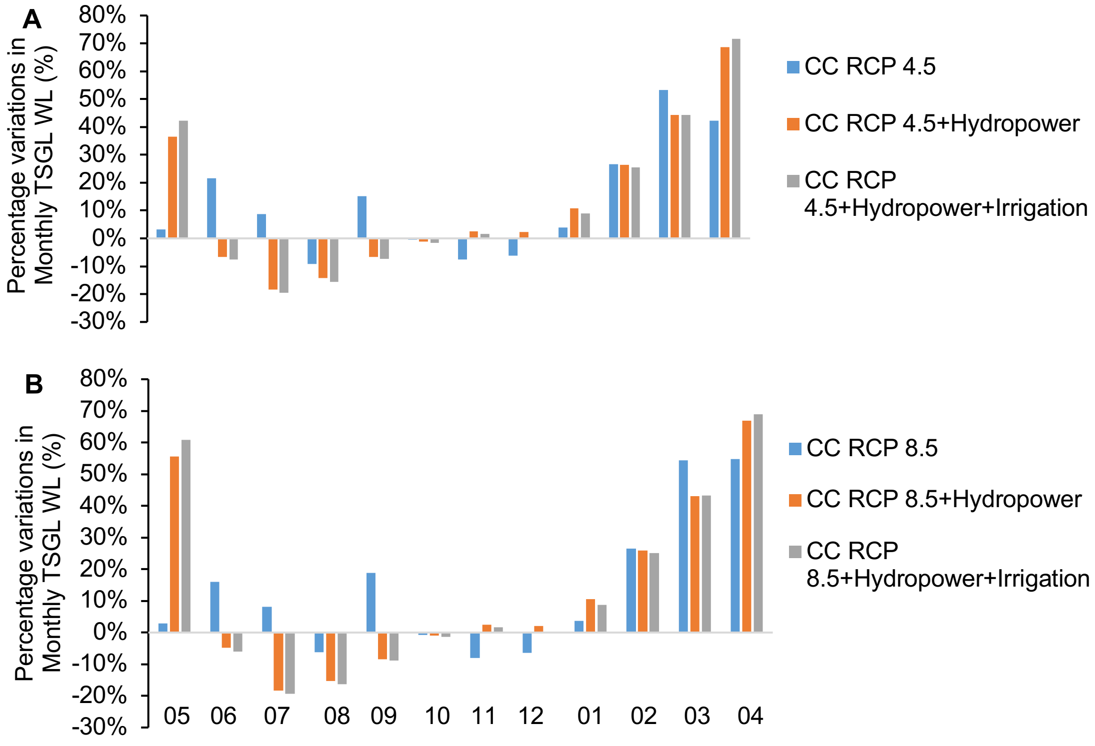

FIGURE 5.3 - ANALYSIS OF THE % VARIATION IN THE FUTURE MONTHLY MEAN FLOOD LEVEL MEAN BOUNDARY ESTIMATIONS, COMPARED TO THE BASELINE CONDITIONS, UNDER THE INDIVIDUAL AND COMBINED DRIVERS' SCENARIOS, FOR THE RCP 4.5 (A) AND RCP 8.5 (B). INDIVIDUAL MONTHS ARE REPRESENTED BY THE CORRESPONDING CALENDAR NUMBERS.

The daily quantifications of the lake's permanent area followed the baseline Landsat flood model (sub-section 5.2.4) and the daily flood level estimations, under the individual and combined drivers of environmental change. From Figure

5.4 it is possible to see that the described variations in the timing of the peak flood (Figure 5.2) resulted in the approximately same timing of variations in the peak area. Individual climate change scenarios, under both RCP's and for the three boundaries (Figure 5.4; **A- a); B- d**), resulted in a mean 27-28±3 days (i.e. 261-262 corresponding to 17th-18th September) early peak area, compared to the baseline mean 289±3 days (i.e. 15th-18th October) peak area.

Individual hydropower scenarios for both RCP's (Figure 5.4; **A- b); B- e**), and for the three boundaries, resulted in a mean 11±3 days (i.e. 273±3 days) delay in the peak area, compared to the individual climate change scenarios, resulting in a mean 15±3 days earlier peak area (i.e. 30th September-2nd October), compared to the baseline peak area. The irrigation/combined scenarios (Figure 5.4; **A- c); B- f**) followed the same variation as reported by individual hydropower.

The findings shown in Figure 5.4 and synthetized in Table 5.3, Table 5.4 and Table 5.5 for the mean, upper and lower boundaries, respectively, show that individual hydropower scenarios caused the highest mean boundary changes in the mean permanent area, with a +1,718±34 km^2 (+26±0%) increase in the dry season and -668±27 km^2 (-5±0%) decrease in the wet season (Table 5.3). The upper and lower boundaries showed a +36±0% and +9±0% dry season increase in the mean permanent area and a -9±1% and -10±0% wet season decrease in the mean permanent area, respectively (Table 5.4 and Table 5.5, respectively).

The individual irrigation scenarios caused the least impact on the mean permanent area, with a mean boundary quantified -96±46 km^2 (-1±0%) decrease in the wet season and -90±6 km^2 (-2±1%) decrease in the dry season. Also, the upper and lower boundary predictions showed a -2±1% and -3±0% dry season decrease in the mean permanent area and a -1±0% wet season decrease in the mean permanent area for both boundaries, respectively (Table 5.4 and Table 5.5, respectively). Overall, the combined drivers resulted in a mean boundary +1,467±7 km^2 (+27±1%) increase in the dry season mean permanent area and in a -538±144 km^2 (-8±2%) decrease in the wet season mean permanent area (Table 5.3), with the upper (lower boundary) predictions showing a +56±1% (-15±1%) increase (decrease) in the dry season mean permanent area and a +4±3% (-17±2%) increase (decrease) in the wet season mean permanent area, respectively (Table 5.4 and Table 5.5, respectively).

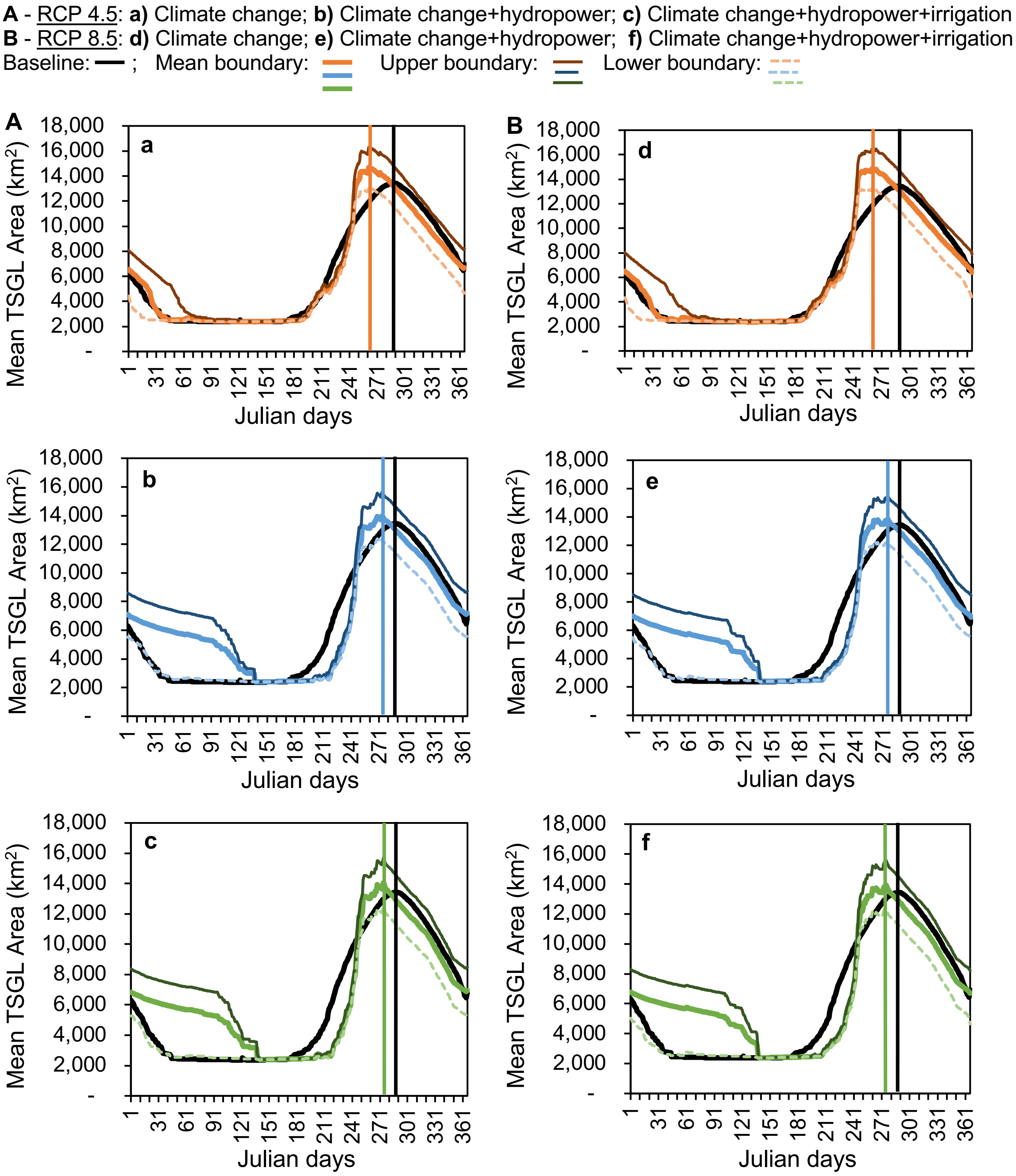

FIGURE 5.4 - FUTURE (2036-2064) MEAN DAILY VARIATIONS (JULIAN CALENDAR: 1ST JANUARY – 31TH DECEMBER) IN THE TSGL AREA (KM²) AND PEAK AREA (VERTICAL LINES) UNDER THE INDIVIDUAL AND COMBINED DRIVERS. A) SHOWS THE INDIVIDUAL CLIMATE CHANGE **(A)**, CLIMATE CHANGE+HYDROPOWER **(B)** AND IRRIGATION/COMBINED DRIVERS' IMPACTS **(C)** UNDER THE RCP 4.5 SCENARIO. B) SHOWS THE INDIVIDUAL (**D**-CLIMATE CHANGE; **E**-CLIMATE CHANGE+HYDROPOWER) AND COMBINED (**F**-IRRIGATION/COMBINED) DRIVER'S IMPACTS UNDER THE RCP 8.5 SCENARIO. UPPER (THIN LINE) AND LOWER (DASHLINE) BOUNDARIES ARE SHOWN. BASELINE (1996-2014) CONDITIONS ARE PRESENTED BY THE BLACK LINE.

The mean boundary analysis of the percentage change in the mean monthly permanent lake area, under the RCP 4.5 individual and combined scenarios **(A)** and RCP 8.5 individual and combined scenarios **(B)** is performed by comparing to the baseline monthly mean permanent lake area conditions (Figure 5.5). Importantly, the individual hydropower scenarios (i.e. for the quantified difference between climate change+hydropower and the combined scenarios) comprised a 2% to 120% increase in the mean permanent lake area regarding the dry season months (November-April), for both RCP's scenarios, with the three highest percentages of change seen from February to April, compared to the baseline conditions, which are the historical driest months observed in the basin. Notably, under both RCP's scenarios, hydropower individual scenarios resulted in the highest decreases in the TSGL system mean permanent lake area (-26 – 33%) in the wet season. Yet, in May a relatively higher influence of a combined hydropower and irrigation scenarios (grey bar), resulted in a 20-29% increase in the mean permanent lake area. The same higher influence of the hydropower and irrigation scenarios is observed for the increased % variation in the mean permanent lake area in April and % decrease in July-August. No upper and lower boundary individual monthly analyses were performed, since these are described in Table 5.4 and Table 5.5, respectively and would only reflect higher permanent lake area variations, according to the individual and combined scenarios.

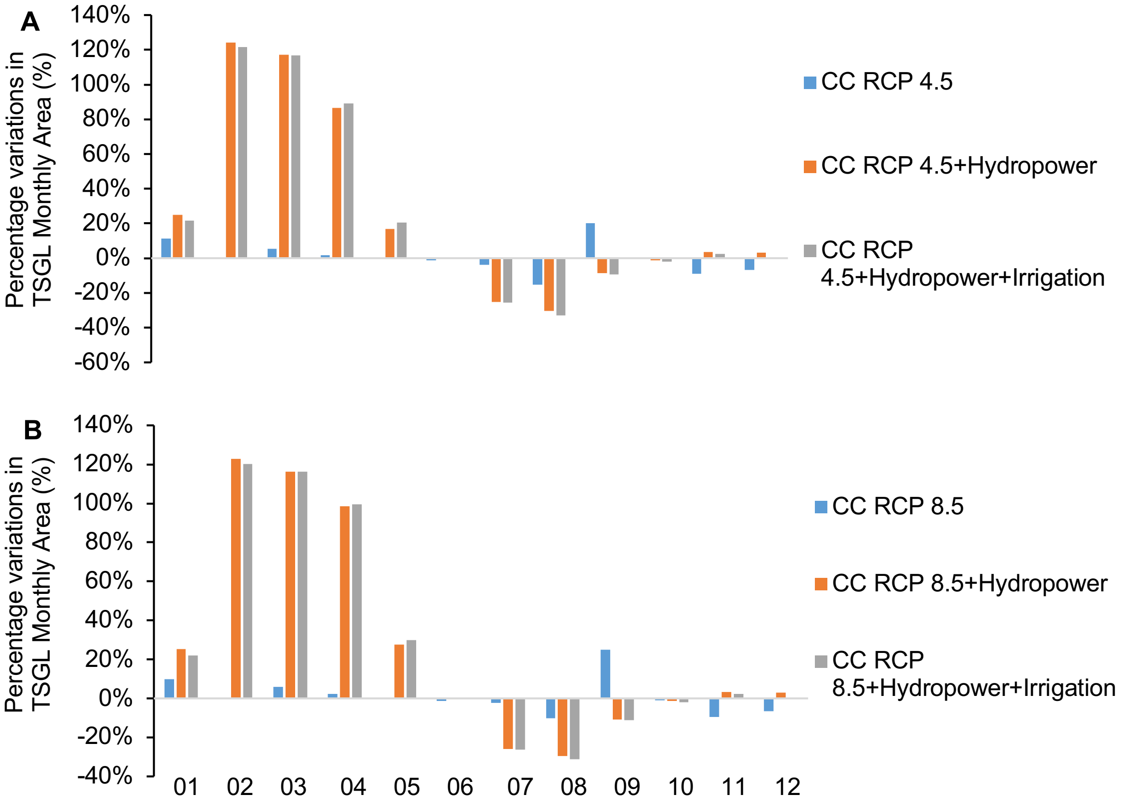

FIGURE 5.5 - ANALYSIS OF THE % VARIATION IN THE FUTURE MEAN MONTHLY PERMANENT LAKE AREA MEAN BOUNDARY ESTIMATIONS, COMPARED TO THE BASELINE CONDITIONS, UNDER THE INDIVIDUAL AND COMBINED DRIVERS, FOR THE RCP 4.5 (A) AND RCP 8.5 (B). INDIVIDUAL MONTHS ARE REPRESENTED BY THE CORRESPONDING CALENDAR NUMBERS.

5.3.2 THE INDIVIDUAL AND COMBINED IMPACTS OF THE MEKONG RIVER'S HYDROPOWER, IRRIGATION AND CLIMATE CHANGE SCENARIOS ON THE FLOOD PULSE-DRIVEN FLOODPLAIN INUNDATION PROFILE

The changes in the flood pulse-driven floodplain inundation dynamics, driven by the individual and combined Mekong river's hydropower, irrigation and climate change scenarios are assessed in Figure 5.6 and synthetized in Table 5.3, Table 5.4 and Table 5.5 for the mean, upper and lower boundary predictions of variations in the timing, duration and extent of the flood (i.e. the flood pulse variables), respectively. The flood index proposed by Halls *et al.,* (2013a) for the TSGL system, assesses both flood extent and duration, being key to understand modifications in the long-term floodplain flooding dynamics, also evidencing changes in the timing of the flood events (see Chapter 4).

Notably, all individual and combined drivers' scenarios resulted in a mean and lower boundary (upper boundary) decrease (increase) in the mean flood index, compared to the baseline conditions (Table 5.3, Table 5.4, Table 5.5 and Figure 5.6). Individually, climate change resulted in a -7±3% decrease (-47,855±21,719 km^2 and -9±3 days decrease of mean flood duration) in the mean flood index, compared to the baseline conditions. Also, a -51,202±29,702 km^2 (-7±2%) decrease in the mean flood index (with a +16±2 days (+11±0%) increase in the mean flood duration) is observed under individual hydropower scenarios, while individual irrigation scenarios resulted in a -23,471±2,431 km^2 (-3±0%) decrease in the mean flood index (with a -7±1 days decrease in the mean flood duration), with these quantifications resulting from the assessed differences regarding the individual climate change scenarios' results.

The combined hydropower and climate change scenarios showed the highest modifications in the mean flood index, with a -10±1% decrease (-69,355±7,983 km^2 and +7±1% increase in the mean flooding days). Noteworthy, the results here presented are for the mean boundary predictions (Table 5.3). A +28±3%; +10±4% and +31±0% increase in the mean flood index (with +14±2%; +50±5% and +64±6% increase in the mean flood duration) is predicted for the individual climate change, hydropower and combined drivers' scenarios, respectively, for the upper boundary predictions. Also, the individual irrigation scenarios resulted in a marginal -4±3% decrease in the upper boundary mean flood index prediction (with a +1±0% increase in the mean flood duration; Table 5.4). Moreover, a -

33±3%; -8±4%; -2±0% and -40±1% decrease in the mean flood index (with a -21±3%; -7±2%, -4±1% and -29±0% decrease in the mean flood duration) is predicted for the individual climate change, hydropower, irrigation and combined drivers' scenarios, respectively, for the lower boundary predictions (Table 5.5).

Overall, individual climate change scenarios caused the highest impacts on the upper and lower boundaries mean flood index, particularly on the mean flood extent and lower boundary mean flood extent and duration (Table 5.4 and Table 5.5). However, individual hydropower scenarios caused the highest impact on the mean boundary quantified mean flood index, particularly showing shifts in the flood timing and duration (Table 5.3). This is further observed in the upper boundary flood timing and duration estimations (Table 5.4).

Hydropower development is expected to significantly increase (χ^2 =69.143; df=9; *p*=0.002) the number of floodplain inundation days, with a predicted 1-month delay of the flood start (i.e. middle August start) and 1-4 months delay in flood ending (i.e. February-May for the mean, upper and lower boundary predictions; Table 5.3, Table 5.4 and Table 5.5), resulting in an overall 8±1 months of floodplain flooding, 2 months more compared to the baseline 6 months of floodplain flooding (see Chapter 4).

From Figure 5.6 it is possible to see the presence of outliers in both RCP 4.5 individual and combined drivers' scenarios (except for the lower boundary of the combined drivers and hydropower scenario; **A)** and RCP 8.5 individual and combined drivers' scenarios (except for the upper boundary of the hydropower scenario; **B)**. These outliers show distant values from the remaining observations and were kept for their importance in showing the variations (i.e. for the mean, upper and lower boundary predictions) in the individual and combined drivers' scenarios predicted effect on the floodplain flood index.

The non-parametric Kruskal-Wallis statistical analysis followed by the Dunn's post-hoc test showed statistically significant differences in the mean flood index of the lower boundary climate change+hydropower scenarios and the mean flood index of the lower boundary combined drivers' scenarios for both RCP's, compared to the baseline mean flood index (χ^2=97.34; df=4; *p*=0.02 and χ^2=103.55; df=4; *p*=0.01 for RCP 4.5 and χ^2=103.41; df=4; *p*=0.01 and χ^2= 109.02; df=4; *p*=0.01 for RCP 8.5; respectively). This evidenced the significant impacts of both climate change scenarios and hydropower development on the resulting lower boundary prediction of the flood index. Also, statistically significant differences ($p<0.05$) were detected between and within the upper and lower mean flood index prediction boundaries and the mean boundary mean flood index, for the individual and combined drivers' scenarios, under the two RCP's. No statistically significant differences ($p>0.05$) were detected between the mean boundary mean flood index of the individual and combined drivers' scenarios, under the two RCP's, and the baseline mean flood index.

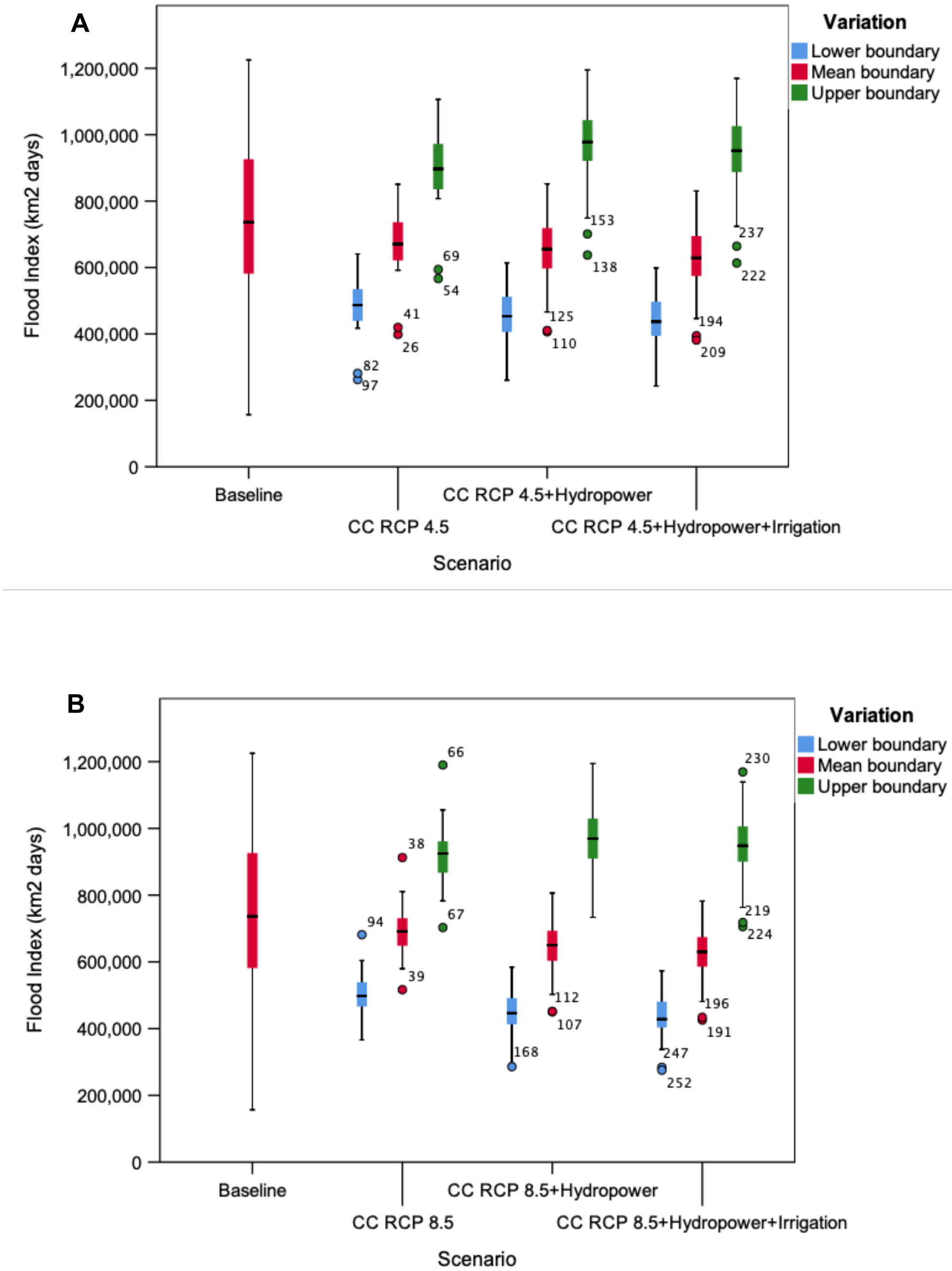

FIGURE 5.6 - BOXPLOT ANALYSIS OF THE BASELINE (1996-2014) AND FUTURE (2036-2064) PREDICTIONS IN THE FLOOD INDEX (KM^2 DAYS), UNDER THE INDIVIDUAL AND COMBINED DRIVERS' SCENARIOS. A) REPRESENTS THE PREDICTIONS UNDER THE RCP 4.5 SCENARIO AND B) REPRESENTS THE PREDICTIONS UNDER THE RCP 8.5 SCENARIO. LOWER (BLUE), MEAN (RED) AND UPPER (GREEN) 95% CONFIDENCE PREDICTION BOUNDARIES ARE SHOWN

TABLE 5.3 - SYNTHESIS ANALYSIS OF THE INDIVIDUAL AND COMBINED IMPACTS OF THE MEKONG RIVER'S DRIVERS OF ENVIRONMENTAL CHANGE ON THE FLOOD PULSE VARIABLES, FLOODPLAIN HABITATS FLOOD DYNAMICS AND *DAI* FISHERY RESOURCES. THE RESULTS ARE PRESENTED AS MEANS OF THE ENTIRE BASELINE (1996 – 2014) AND FUTURE (2036-2064) PERIODS, FOR THE MEAN BOUNDARY. THE % VARIATION COMPARED TO THE BASELINE CONDITIONS IS SHOWN IN BRACKETS FOR THE GAINS (+; BLUE) AND LOSSES (-; RED).

Variables	Baseline	Climate Change		Hydropower development		Irrigation expansion	
		RCP 4.5	RCP 8.5	RCP 4.5	RCP 8.5	RCP 4.5+Hydropower	RCP 8.5+Hydropower
Dry season WL (m)	4.38	4.70 *(+7%)*	4.73 *(+8%)*	5.54 *(+27%)*	5.57 *(+27%)*	5.50 *(+26%)*	5.53 *(+26%)*
Wet season WL (m)	5.07	5.31 *(+5%)*	5.36 *(+6%)*	5.01 *(-1%)*	5.07 *(+0%)*	4.98 *(-2%)*	5.06 *(-1%)*
Annual WL variation (m)	4.21±0.98	±0.54	±0.55	±0.55	±0.57	±0.55	±0.57
Peak flood level	October	September	September	October	September	October	September
Dry season permanent lake area (km^2)	5,366	5,205 *(-3%)*	5,184 *(-3%)*	6,923 *(+29%)*	6,936 *(+29%)*	6,833 *(+27%)*	6,840 *(+28%)*
Wet season permanent lake area (km^2)	6,813	6,966 *(+2%)*	7,123 *(+5%)*	6,325 *(-7%)*	6,455 *(-5%)*	6,275 *(-8%)*	6,419 *(-6%)*
Flood Index (km^2 days)	721,626	673,771 *(-7%)*	695,490 *(-4%)*	652,271 *(-10%)*	644,288 *(-11%)*	628,800 *(-13%)*	623,248 *(-14%)*
Timing of the flood (start-end)	July (late) – January (middle)	August (middle) – January (early)	August (middle) – January (early)	August (late) – February (middle)	August (late) – February (early)	August (late) – February (early)	August (late) – January (late)
Duration (days)	156	147 *(-6%)*	150 *(-4%)*	163 *(+5%)*	164 *(+5%)*	157 *(+0%)*	157 *(+0%)*
Catch (tonnes)	19,532	16,930 *(-13%)*	17,232 *(-12%)*	16,614 *(-15%)*	16,465 *(-16%)*	16,266 *(-17%)*	16,155 *(-17%)*
Henycorhynchus sp. (tonnes)/contribution to total catch (%) – Guild 3	6,453/ 33%	5,359/ 32% (-1%)	5,425/ 32% (-1%)	5,291/ 32% (-1%)	5,262/ 32% (-1%)	5,217/ 32% (-1%)	5,196/ 32% (-1%)
Labeo chrysophekadion (tonnes)/ contribution to total catch (%) – Guild 3	449/ 2%	442/ 2% (+0%)	457/ 3% (+1%)	424/ 1% (-1%)	414/ 1% (-1%)	405/ 1% (-1%)	397/ 1% (-1%)
Labiobarbus lineatus (tonnes)/ contribution to total catch (%) – Guild 4	1,470/ 8%	1,031/ 6% (-2%)	1,048/ 6% (-2%)	1,014/ 6% (-2%)	1,006/ 6% (-2%)	995/ 6% (-2%)	989/ 6% (-2%)
Paralaubuca barroni (tonnes)/ contribution to total catch (%) – Guild 4	2,055/ 11%	1,453/ 9% (-2%)	1,474/ 9% (-2%)	1,432/ 9% (-2%)	1,423/ 9% (-2%)	1,409/ 9% (-2%)	1,402/ 9% (-2%)
Thynnichthys thynnoides (tonnes)/ contribution to total catch (%) – Guild 5	384/ 2%	411/ 2% (+0%)	425/ 3% (+1%)	395/ 2% (+0%)	385/ 2% (+0%)	376/ 2% (+0%)	367/ 2% (+0%)
Other sp. (tonnes)/ contribution to total catch (%) – Guilds 1,3, 4 and 5	4,944/ 26%	3,820/ 23% (-3%)	3,956/ 23% (-3%)	3,669/ 22% (-4%)	3,579/ 22% (-4%)	3,500/ 22% (-4%)	3,430/ 21% (-5%)

TABLE 5.4 - SYNTHESIS ANALYSIS OF THE INDIVIDUAL AND COMBINED IMPACTS OF THE MEKONG RIVER'S DRIVERS OF ENVIRONMENTAL CHANGE ON THE FLOOD PULSE VARIABLES, FLOODPLAIN HABITATS FLOOD DYNAMICS AND *DAI* FISHERY RESOURCES. THE RESULTS ARE PRESENTED AS MEANS OF THE ENTIRE BASELINE (1996 – 2014) AND FUTURE (2036-2064) PERIODS, FOR THE UPPER BOUNDARY. THE % VARIATION COMPARED TO THE BASELINE CONDITIONS IS SHOWN IN BRACKETS FOR THE GAINS (+; BLUE) AND LOSSES (-; RED).

Variables	Baseline	Climate Change		Hydropower development		Irrigation expansion	
		RCP 4.5	RCP 8.5	RCP 4.5	RCP 8.5	RCP 4.5+Hydropower	RCP 8.5+Hydropower
Dry season WL (m)	4.38	5.44 (+24%)	5.49 (+25%)	6.31 (+44%)	6.35 (+45%)	6.27 (+43%)	6.31 (+44%)
Wet season WL (m)	5.07	5.65 (+11%)	5.71 (+13%)	5.34 (+5%)	5.42 (+7%)	5.32 (+5%)	5.41 (+7%)
Annual WL variation (m)	4.21±0.98	+0.54	+0.55	+0.55	+0.57	+0.55	+0.57
Peak flood level	October	September	September	October	October	October	October
Dry season permanent lake area (km^2)	5,366	6,493 (+21%)	6,479 (+21%)	8,402 (+57%)	8,435 (+57%)	8,316 (+55%)	8,346 (+56%)
Wet season permanent lake area (km^2)	6,813	7,567 (+11%)	7,747 (+14%)	6,920 (+2%)	7,078 (+4%)	6,869 (+1%)	7,051 (+4%)
Flood Index (km^2 days)	721,626	898,694 (+25%)	923,593 (+28%)	972,862 (+35%)	965,063 (+34%)	947,493 (+31%)	942,460 (+31%)
Timing of the flood (start-end)	July (late) – January (middle)	August (late) – January (late)	August (late) – January (late)	August (late) – April (late)	August (late) – May (middle)	August (late) – May (middle)	August (late) – May (middle)
Duration (days)	156	175 (+12%)	177 (+14%)	246 (+57%)	255 (+64%)	246 (+58%)	256 (+64%)
Catch (tonnes)	19,532	20,761 (+6%)	21,182 (+8%)	22,212 (+14%)	22,009 (+13%)	21,715 (+11%)	21,566 (+0%)
Henycorhynchus sp. (tonnes)/contribution to total catch (%) – Guild 3	6,453/ 33%	6,137/ 30% (-3%)	6,223/ 30% (-3%)	6,419/ 29% (-4%)	6,383/ 29% (-4%)	6,323/ 29% (-4%)	6,297/ 29% (-4%)
Labeo chrysophekadion (tonnes)/ contribution to total catch (%) – Guild 3	449/ 2%	697/ 3% (+1%)	726/ 3% (+1%)	812/ 4% (+2%)	792/ 4% (+2%)	773/ 4% (+2%)	757/ 4% (+2%)
Labiobarbus lineatus (tonnes)/ contribution to total catch (%) – Guild 4	1,470/ 8%	1,236/ 6% (-2%)	1,258/ 6% (-2%)	1,312/ 6% (-2%)	1,302/ 6% (-2%)	1,286/ 6% (-2%)	1,279/ 6% (-2%)
Paralaubuca barroni (tonnes)/ contribution to total catch (%) – Guild 4	2,055/ 11%	1,703/ 8% (-3%)	1,730/ 8% (-3%)	1,794/ 8% (-4%)	1,782/ 8% (-3%)	1,763/ 8% (-3%)	1,754/ 8% (-3%)
Thynnichthys thynnoides (tonnes)/ contribution to total catch (%) – Guild 5	384/ 2%	649/ 3% (+1%)	675/ 3% (+1%)	756/ 3% (+1%)	737/ 3% (+1%)	719/ 3% (+1%)	704/ 3% (+1%)
Other sp. (tonnes)/ contribution to total catch (%) – Guilds 1, 3, 4 and 5	4,944/ 26%	6,033/ 29% (+3%)	6,276/ 30% (+4%)	7,027/ 32% (+6%)	6,849/ 31% (+5%)	6,686/ 31% (+5%)	6,546/ 30% (+4%)

TABLE 5.5 - SYNTHESIS ANALYSIS OF THE INDIVIDUAL AND COMBINED IMPACTS OF THE MEKONG RIVER'S DRIVERS OF ENVIRONMENTAL CHANGE ON THE FLOOD PULSE VARIABLES, FLOODPLAIN HABITATS FLOOD DYNAMICS AND *DAI* FISHERY RESOURCES. THE RESULTS ARE PRESENTED AS MEANS OF THE ENTIRE BASELINE (1996 – 2014) AND FUTURE (2036-2064) PERIODS, FOR THE LOWER BOUNDARY. THE % VARIATION COMPARED TO THE BASELINE CONDITIONS IS SHOWN IN BRACKETS FOR THE GAINS (+; BLUE) AND LOSSES (-; RED).

Variables	Baseline	Climate Change		Hydropower development		Irrigation expansion	
		RCP 4.5	RCP 8.5	RCP 4.5	RCP 8.5	RCP 4.5+Hydropower	RCP 8.5+Hydropower
Dry season WL (m)	4.38	3.98 (-9%)	3.98 (-9%)	4.79 (+9%)	4.80 (+10%)	4.75 (+8%)	4,76 (+9%)
Wet season WL (m)	5.07	4.99 (-2%)	5.01 (-1%)	4.68 (-8%)	4.73 (-7%)	4.66 (-8%)	4.71 (-7%)
Annual WL variation (m)	4.21±0.98	-0.53	-0.55	-0.55	-0.56	-0.55	-0.56
Peak flood level	October	September	September	September	September	September	September
Dry season permanent lake area (km^2)	5,366	4,240 (-21%)	4,213 (-22%)	4,712 (-12%)	4,658 (-13%)	4,550 (-15%)	4,490 (-16%)
Wet season permanent lake area (km^2)	6,813	6,378 (-6%)	6,506 (-5%)	5,717 (-16%)	5,809 (-15%)	5,661 (-17%)	5,762 (-15%)
Flood Index (km^2 days)	721,626	485,900 (-33%)	504,196 (-30%)	452,827 (-37%)	447,027 (-38%)	438,728 (-39%)	434,847 (-40%)
Timing of the flood (start-end)	July (late) – January (middle)	August (late) – December (middle)	August (late) – December (late)	August (late) – December (late)	August (late) – December (late)	August (late) – December (middle)	August (late) – December (middle)
Duration (days)	156	123 (-21%)	128 (-18%)	115 (-26%)	116 (-25%)	110 (-29%)	111 (-29%)
Catch (tonnes)	19,532	14,278 (-27%)	14,496 (-26%)	13,860 (-29%)	13,773 (-29%)	13,685 (-30%)	13,621 (-30%)
Henycorhynchus sp. (tonnes)/contribution to total catch (%) – Guild 3	6,453/ 33%	4,785/ 34% (+1%)	4,835/ 33% (+0%)	4,691/ 34% (+1%)	4,672/ 34% (+1%)	4,651/ 34% (+1%)	4,638/ 34% (+1%)
Labeo chrysophekadion (tonnes)/ contribution to total catch (%) – Guild 3	449/ 2%	302/ 2% (+0%)	311/ 2% (+0%)	282/ 2% (+0%)	277/ 2% (+0%)	274/ 2% (+0%)	271/ 2% (+0%)
Labiobarbus lineatus (tonnes)/ contribution to total catch (%) – Guild 4	1,470/ 8%	886/ 6% (-2%)	898/ 6% (-2%)	863/ 6% (-2%)	858/ 6% (-2%)	853/ 6% (-2%)	850/ 6% (-2%)
Paralaubuca barroni (tonnes)/ contribution to total catch (%) – Guild 4	2,055/ 11%	1,273/ 9% (-2%)	1,289/ 9% (-2%)	1,244/ 9% (-2%)	1,238/ 9% (-2%)	1,232/ 9% (-2%)	1,228/ 9% (-2%)
Thynnichthys thynnoides (tonnes)/ contribution to total catch (%) – Guild 5	384/ 2%	281/ 2% (+0%)	289/ 2% (+0%)	263/ 2% (+0%)	258/ 2% (+0%)	255/ 2% (+0%)	252/ 2% (+0%)
Other sp. (tonnes)/ contribution to total catch (%) – Guilds 1,3,4 and 5	4,944/ 26%	2,608/ 18% (-8%)	2,688/ 19% (-7%)	2,442/ 18% (-8%)	2,400/ 17% (-9%)	2,373/ 17% (-9%)	2,342/ 17% (-9%)

5.3.3 THE FUTURE OF THE *DAI* FISHERY CATCH AND SPECIES GUILD COMPOSITION AND CONTRIBUTION TO THE CATCH

The individual and combined impacts of the Mekong river's hydropower, irrigation and climate change scenarios on the floodplain flood dynamics (see sub-section 5.3.2) resulted in pronounced decrease in the *dai* fishery catch, compared to the baseline (1996-2014) average of 19,532 tonnes (see Chapter 4). Individual climate change scenarios resulted in a -13±1% (16,930±302 mean tonnes) decrease in catch. Whereas, individual hydropower and irrigation scenarios resulted in a mean loss of -767±451 tonnes (-4±2%) and -348±111 tonnes (-2±1%), respectively, quantified from the differences between the climate change individual results. Of note, the combined drivers resulted in the highest decrease in the mean catch (-17±0%; -3,377±111 tonnes).

Table 5.4, Table 5.5, and Figure 5.7 show the 95% confidence upper (lower) predicted variations in the *dai* catch. The individual drivers' scenarios resulted in +8±2% (-27±1%), +5±3% (-3±1%) and +3±0% (-1±0%) increase (decrease) in the mean catch, by climate change, hydropower and irrigation scenarios, respectively. Outliers were identified in the boxplot analysis (Figure 5.7), for the three prediction boundaries, under RCP 4.5 **(A)** and RCP 8.5 **(B)** individual and combined scenarios' impact on the *dai* fishery, reflecting values that were distant from the remaining observations (e.g. the baseline outlier represented the 8,560 tonnes minimum observed catch for the entire period).

The non-parametric Kruskal-Wallis analysis followed by the Dunn's post hoc test showed statistically significant differences ($p<0.05$) for the upper and lower boundary mean catch estimations between and within all drivers' scenarios and the mean boundary estimations. Non-statistically significant differences were detected between the baseline and the mean boundary mean catch of the individual scenarios, under the two RCP's ($\chi^2_{(\text{climate change})}=-14.78$; df=3; $p=0.53$; $\chi^2_{(\text{climate change+hydropower})}=-4.79$; df=3; $p=0.84$; $\chi^2_{(\text{climate change+hydropower+irrigation})}=5.45$; df=3; $p=0.82$ for RCP 4.5 and $\chi^2_{(\text{climate change})}=-23.68$; df=3; $p=0.32$; $\chi^2_{(\text{climate change+hydropower})}=-2.68$; df=3; $p=0.91$; $\chi^2_{(\text{climate change+hydropower+irrigation})}=6.79$; df=3; $p=0.77$ for RCP 8.5). However, statistically significant differences ($\chi^2=80.04$; df=3; $p=0.001$ for RCP 4.5 and $\chi^2=85.47$; df=3; $p=0.002$ for RCP 8.5) were observed between the baseline mean catch and the mean boundary mean catch, under the combined drivers' scenarios.

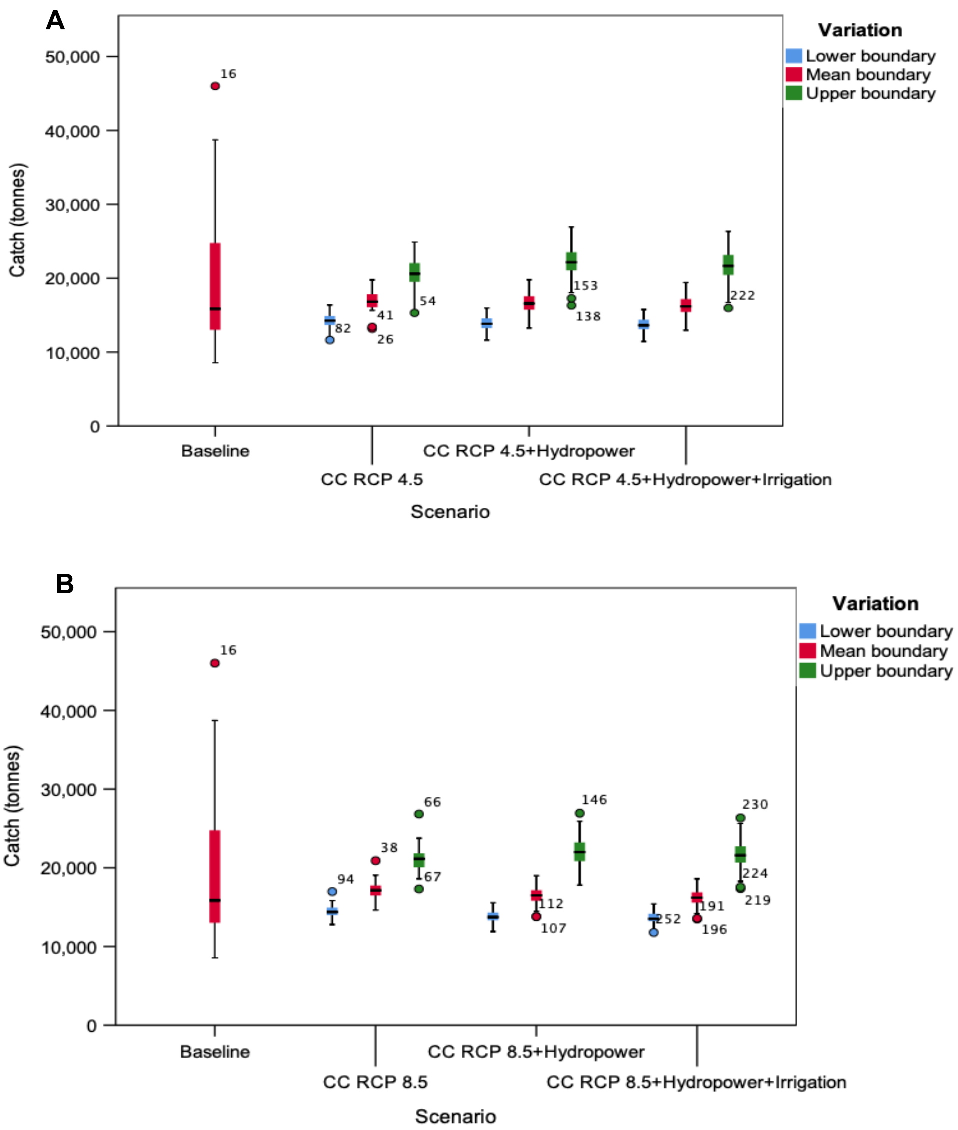

FIGURE 5.7 - BOXPLOT ANALYSIS OF THE BASELINE (1996-2014) AND FUTURE (2036-2064) PREDICTIONS IN THE *DAI* CATCH (TONNES), UNDER THE INDIVIDUAL AND COMBINED DRIVERS' SCENARIOS. A) SHOWS THE PREDICTIONS UNDER THE RCP 4.5 SCENARIO AND B) THE PREDICTIONS UNDER THE RCP 8.5 SCENARIO. LOWER (BLUE), MEAN (RED) AND UPPER (GREEN) 95% CONFIDENCE PREDICTION BOUNDARIES ARE SHOWN.

The comparison between the individual mean top-5 fish species composition of the *dai* catch for the baseline period with the changes in the mean individual species composition caused by the RCP 4.5 individual and combined scenarios **(A)** and RCP 8.5 individual and combined scenarios **(B)** is present in Figure 5.8. Also, the % contribution of the individual top-5 fish species to the future catch, the guilds belonged and % of change in contribution, compared to the baseline, resulting from the individual and combined drivers' impact are shown in Table 5.3 for the mean boundary, and Table 5.4 and Table 5.5 for the upper and lower boundary predictions, respectively. The predictions in the top-5 mean species composition of the *dai* fishery for the individual and combined drivers, under the

RCP’s scenarios, did not show marked differences between RCP’s (Figure 5.8). Yet, apart from *Labeo chrysophekadion* (guild 3) for the mean boundary climate change predictions and *Thynnichthys thynnoides* (guild 5) for all individual and combined drivers’ scenarios mean boundary predictions, predicted losses in the mean species composition and 1-5% decrease in the contribution compared to the baseline, are evidenced in Table 5.3 and Figure 5.8, with the highest % decrease seen for the Other sp. group, under the combined drivers’ scenarios. Under the upper boundary prediction, a higher variation in the individual species % contribution to the individual and combined drivers’ scenarios *dai* fishery, compared to the baseline, is observed (2-4% decrease for *Henycorhynchus sp., Labiobarbus lineatus* and *Paralaubuca barroni,* and 1-6% increase for *Labeo chrysophekadion, Thynnichthys thynnoide*s and Other sp.; Table 5.4), with the individual hydropower scenarios displaying the highest changes, particularly for the Other sp. group. Under the lower boundary prediction, a 1% increase in the *Henycorhynchus sp.* contribution to the catch, compared to the baseline, is predicted, while no change is predicted for the *Labeo chrysophekadion* contribution to the catch and a 2% contribution decrease is predicted for the medium distance migratory species *Labiobarbus lineatus* and *Paralaubuca barroni* (guild 4). Importantly, the lower boundary shows a 7-9% decrease in the contribution of the Other sp. group to the *dai* catch compared to the baseline, under the individual and combined drivers, with the highest decrease predicted under the combined drivers’ scenarios (Table 5.5). Notably, the Other sp. group is mainly composed by medium to long distance migratory fish species (guilds, 4 and 3, respectively; see Chapter 4). Therefore, it is likely that it will be the most impacted group under the individual hydropower and combined drivers’ scenarios. Consequently, regarding future changes in the migratory fish guilds composition of the *dai* catch, it is predicted that small (e.g. *Henycorhynchus sp., Paralaubuca barroni* and *Labiobarbus lineatus*) to large (e.g. *Pangasius sp. Puntioplites proctozysron, Botia modest*a and *Labiobarbus siamensis* present in the Other sp. group and *Labeo chrysophekadion*) body-size (see Chapter 4 for revising information on each species body-size) medium to long distance migratory fish species (i.e. guilds 4 and 3, respectively) will be the most impacted from the combined drivers’ scenarios, comprising a 1-9% decrease in the contribution to catch, compared to the baseline conditions. Also, the baseline analysis showed that guilds 3 and 4 contributed nearly 80% to the *dai* catch, yet following the combined drivers' analysis on the individual guilds 3 and 4 contribution to the future catch, the contribution is 69%, (78% and 68% for the upper and lower boundary predictions, respectively) reflecting a 11% (2% and 12%) decrease for the three boundaries (Table 5.3, Table 5.4 and Table 5.5).

Although non-statistically significant differences were detected between the baseline species composition of the catch and the mean boundary estimated mean species composition of the catch, under the individual and combined drivers, upper and lower variations in the mean species composition showed statistically significant differences *($p<0.05$)* between and within the mean boundary composition estimations for the individual and combined drivers.

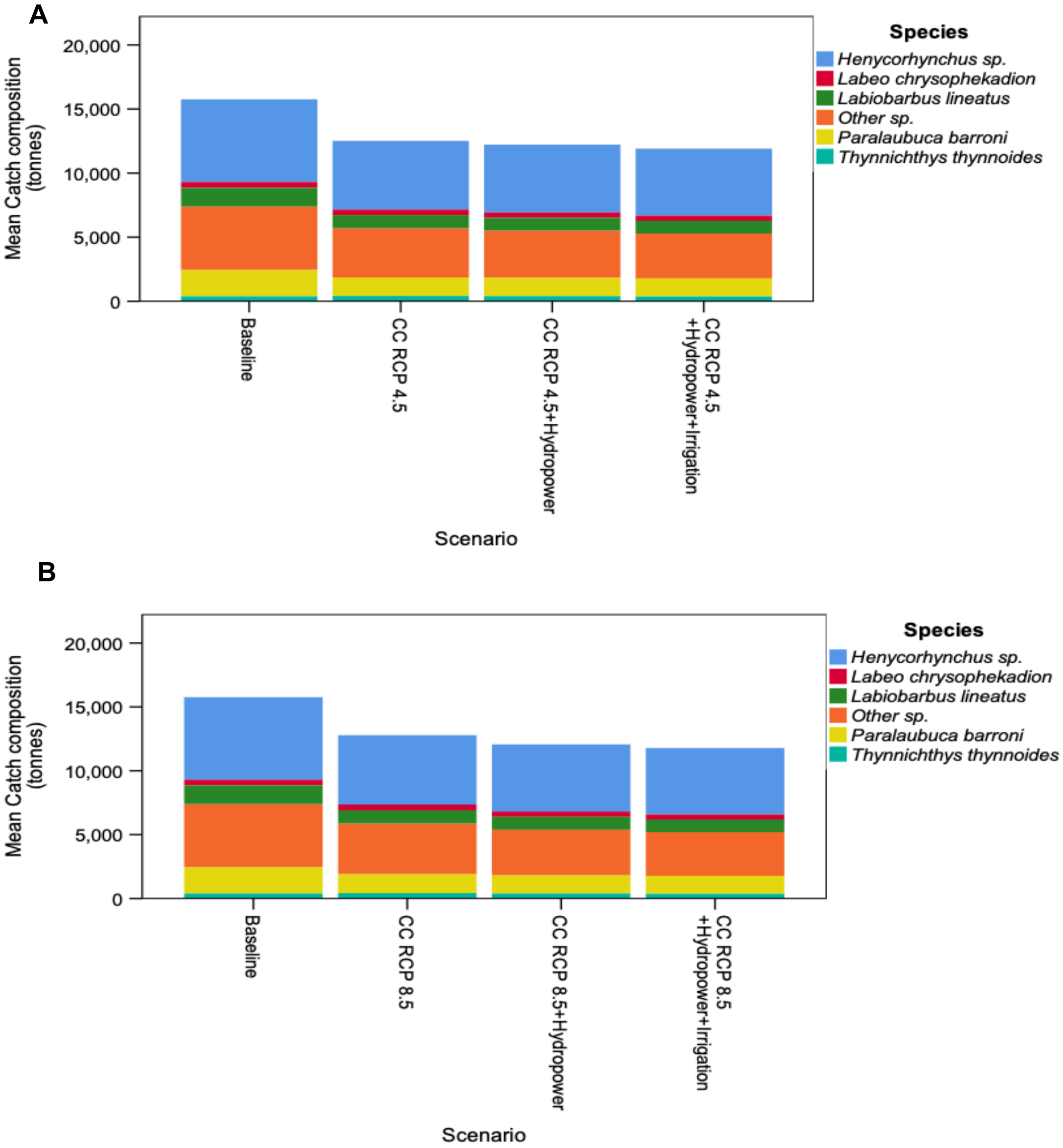

FIGURE 5.8 - BOXPLOT ANALYSIS OF THE BASELINE (1996-2014) AND FUTURE (2036-2064) PREDICTIONS IN THE TOP-5 MEAN SPECIES COMPOSITION OF THE *DAI* CATCH (TONNES), UNDER THE INDIVIDUAL AND COMBINED DRIVERS' SCENARIOS. A) REPRESENTS THE PREDICTIONS UNDER THE RCP 4.5 SCENARIO AND B) THE PREDICTIONS UNDER THE RCP 8.5 SCENARIO.

5.4 DISCUSSION

5.4.1 LIMITATIONS IN THE TSGL SYSTEM FUTURE WATER LEVEL EXTRAPOLATIONS

The VMod modelling analyses by Hoang *et al.,* (2016;2019) provided the only available projections of the daily hydrologic conditions at the Mekong basin (i.e. daily discharge for the Kratie's gauging station), under the individual and combined Mekong river's scenarios of hydropower, irrigation and climate change (see sub-section 5.2.2). Since no available projections on the daily water level at the TSGL system exist, and to quantify and compare the future changes in the flood pulse dynamics, floodplain inundation profile and *dai* fishery resources, driven by the individual and combined Mekong river's drivers of environmental change, it was necessary to extrapolate the daily water level at the lake from the daily simulated discharge scenarios at the mainstream gauge station (see sub-section 5.2.3). The two individual baseline metrics established (i.e. rise and fall) resulted in an average 3-meter water level gap on the rise-fall breakdown period. Although efforts were applied to reduce the water level gap (see sub-section 5.2.3), none resulted in a substantial decrease. In addition, identified gaps in the literature for the Mekong river (and to other tropical river systems under unsteady flow conditions), regarding the hysteresis behaviour, for the application of individual metrics to capture the discharge/water level relationships, while acknowledging the complexity of the bi-directional flow reversals in the TSGL–Mekong system, constrained further improvements on the metrics applied to extrapolate the future daily water level at the lake. The water level gap was observed in the rise to fall periods. More precisely, at the end of the rise period and next day start of the fall period. Consequently, and knowing that the rise to fall period delimits the wet season peak for the Mekong river, the quantified variations in the wet season predictions in the flood level, peak flood, permanent area and floodplain's flood index, under the individual and combined drivers' scenarios and for the three 95% confidence prediction boundaries, are expected to have been influenced by the averaged 3-meter gap. The level of influence is linked to the higher/lower wet season estimations on the variables under study, compared to other studies (see later). However, the different methodological approaches applied by other studies (e.g. the historical average, driest and wettest years approach in Arias *et al.,* 2012;2014) is likely to have resulted in the comparable differences in the wet season flood level, permanent area and floodplain flood dynamics estimations from those studies and the findings here reported (see sub-section 5.4.2), making it difficult to address if the water level gap resulted in a under or over-estimation in the quantified wet season variables. Thus, although providing important insights of the expected impacts of the individual and combined Mekong river's hydropower, irrigation and climate change scenarios on the wet season quantified changes in the flood level, peak flood, permanent lake area and floodplain flood dynamics, these results should be considered under the potential influence of the water level gap.

5.4.2 FUTURE DISRUPTIONS ON THE FLOOD PULSE AND FLOODPLAIN FLOOD DYNAMICS: CONCERNS TO THE PRODUCTIVITY OF THE TSGL SYSTEM

The baseline analyses in Chapter 4 demonstrated the importance of maintaining the historical flood pulse characteristics of the TSGL system for the role in driving the predictable and seasonal variations in the flood level and riparian floodplain inundation timing, duration and extent, key for the historical high productivity associated to the system, particularly for the outstanding fish abundance supported and inland fisheries (Sarkkula *et al.,* 2003; Campbell *et al.,* 2006;2009; Lamberts, 2006;2008; Kummu and Sarkkula, 2008; Arias *et al.,* 2012;2013;2014). The historical low level of variations in the predictability and seasonality of the flood pulse in the TSGL system, reflect the long-term relatively unchangeable flow conditions of the Mekong river, for the role in the unique bi-directional flow reversal (Kummu *et al.,* 2014). Therefore, although historically the Mekong river's flow has been increasingly regulated in the extensive network of lateral tributaries and in the upstream sections of the basin, by the Chinese cascade dams since 1993 (see Chapter 3), the downstream effect of the regulated flow has not significantly modified the historical flood pulse dynamics in the TSGL system. Subsequently, the yearly predictable seasonal inundation extent and duration in floodplain habitats has sustained and driven the migratory fish guilds species that seasonally seek these areas for spawning, feeding and sheltering, which are then reflected in the annual inland fishery yields, vital for economical income and fish protein to the riparian population (see Chapter 4; Campbell *et al.,* 2006;2009; Lu and Siew, 2006; Arias *et al.,* 2012;2013;2014; Halls *et al.,* 2013a; DHI, 2015).

However, the next 20-50 years projections of water infrastructure development (i.e. hydropower dams, reservoirs and irrigation schemes) and changing climate scenarios, are threatening the Mekong river's flow conditions, with expected disruptions on the TSGL system historical flood pulse characteristics, bringing negative consequences to the timing, duration and extent of the floodplain flooded areas and to the vital fishery resources supported (Kummu and Sarkkula, 2008; Arias *et al.,* 2012;2013;2014; Piman *et al.,* 2013; Pokhrel *et al.,* 2018).

Hoang *et al.,* (2016) showed that under solely climate change projections (2036-2065), the downstream flow conditions at Kratie would result in an annual 5% increase, a 25% increase in the driest month (i.e. April) and an 8% reduction in the wet season month of June. Furthermore, according to a later projection incorporating hydropower development and irrigation expansion schemes (Hoang *et al.,* 2019), annual flows would remain similar to the baseline conditions, under individual hydropower scenarios, yet a 63% flow increase is projected to Kratie during the dry season, with up to 133% increase projected in April. Also, a smaller, yet noticeable 15% reduction in the flow conditions at Kratie is projected, being most substantial during the first half of the wet season (i.e. June-September). Under individual irrigation scenarios the annual flow conditions at Kratie are projected to see a marginal 3.2% decrease, while the higher changes are projected during the dry season, with a 9% decrease. Finally, the combined impacts of the three drivers on the Kratie's flow regime projected a up to 150% increase in the dry season flow and a 10-25% decrease in the wet season flows. Importantly, hydropower scenarios were argued to impact the most the flow conditions in the mainstream river at Kratie, also impacting upon the timing of the rise and fall changes, with the baseline flow peak in October projected to be shifted to an earlier peak in August-September.

The findings from Hoang *et al.,* (2016;2019) help to explain the results achieved in sub-sections 5.3.1 and 5.3.2, for the role of the seasonal flow dynamics in the mainstream river is driving the complex bi-directional flow reversals in the lake, influencing the flood level and floodplain flood dynamics. Under individual climate change projections, the mean peak flood level in the lake was projected to be shifted to an earlier nearly 1-month (i.e. 140-144 days, corresponding to 17th-21st September), compared to the baseline conditions (i.e. 168±3 days corresponding to 15th-18th October; Figure 5.2). Regarding hydropower scenarios, marked differences were observed between the RCPs scenarios, with a mean peak flood level observed at 1st October±3 days, corresponding to a 14±3 days early flood peak, compared to the baseline conditions, under RCP 4.5. For the RCP 8.5, the mean peak flood level was projected to 21st September, corresponding to 4 days delay compared to the RCP 8.5 climate change projection and resulting in a 24 days earlier shift, compared to the baseline conditions (Figure 5.2). These results follow Hoang *et al.,* (2016) findings regarding a reduction in the wet season flow conditions in June, under climate change projections, with the same level of flow reduction projected from June-September, under hydropower scenarios (Hoang *et al.,* 2019). The earlier peak flood combined with the reported % changes in the flow conditions would result in an earlier flow reversal to the lake, potentially around February/March, which help to understand the +0.32±0.03 m (+7±1%; with +24±1% upper and -9±0% lower boundary variations, respectively) dry season increase in the mean flood level of the lake and a comparable slighter decrease in the mean flood level in the wet season of +0.24±0.05 m (+5±1%; with +11±2% upper and -2±1% lower boundary variations, respectively), under climate change projections, with the dry (wet) season percentage increase (decrease) being similar to Hoang *et al.,* (2016) reported projections. Yet, a higher +0.84±0.00 m (+20±1%; with +20±0% upper and +18±1% lower boundary variations, respectively) dry season increase in the mean flood level of the lake and a -0.30±0.01m (-6±0%; with -6±1% upper and -6±1% lower boundary variations, respectively) wet season decrease in the mean flood level, are projected under individual hydropower scenarios. Of note, the percentages here reported, although lower than Hoang *et al.,* (2019) projections, show the influence of the reported 63% dry season increase in the mainstream flow in driving the 20% projected increase in the flood level of the lake. Likewise, Hoang *et al.,* (2019) projection of 15% decrease in the wet season flows help to understand the projected 6% decrease in the flood levels of the lake.

The monthly analysis of the % change in the flood level of the lake (Figure 5.3) corroborates the argument of an earlier flow reversal in the lake and consequent increase in the dry season mean flood level, with climate change resulting in a 54% increase in the flood level in the lake in March. Also, hydropower scenarios resulted in 2-70% increase in the dry season flood levels, with the highest percentage occurring in April. The 5-20% decrease in the monthly flood levels of the lake, under hydropower scenarios, during June-October, explain the 24-14 days (according to the different RCP scenarios) earlier peak flood occurrence in the lake and the timing of the flow reversal (i.e. the water flowing out of the lake into the mainstream river), given the earlier Kratie's peak flow projected in September. Of note, these changes in the timing of the peak flood and modifications in the bi-directional flow reversals follow the same trend reported by Piman *et al.,* (2013), which projected that under different scenarios of dam development, the reduced peak flow in Kratie in September would result in 3-13 days earlier (with +/- 19-25 days variation) flow reversal in the TSGL system.

The reduced annual variations in the TSGL system flood level, under the individual and combined scenarios is in accordance to Hoang *et al.,* (2016;2019) projected low variations in the annual flow conditions at Kratie, under the individual and combined scenarios. For the RCP 4.5 scenario, the peak flood under individual hydropower scenarios is projected to be near the baseline (i.e. 1st October compared to the baseline 15th October; Figure 5.2), yet caution should be taken, since the 3-meter water level gap potentially resulted in this variation, compared to the RCP 8.5 scenario (i.e. 24 days early shift; Figure 5.2).

The modelled changes in the seasonal and annual flood level and permanent lake area show that hydropower is projected to impact the flood pulse conditions and permanent lake area of the TSGL system more than solely climate change (Figure 5.4 and Figure 5.5 for the mean daily permanent lake area and peak lake area, and the % change analysis compared to the baseline, respectively, and Table 5.3, Table 5.4 and Table 5.5 for the synthetized analyses on the mean, upper and lower boundary variations in the annual and seasonal flood level, peak flood and permanent area, respectively). Kummu and Sarkkula (2008), following the three Cumulative Impact Assessment (CIA) studies performed by the Mekong River Commission for the Mekong river's hydropower impacts on the TSGL system flood pulse (see Kummu and Sarkkula, 2008 for detailed information) projected that the dry season flood level would increase from 0.15-0.60 meters, while the wet season flood level would decrease from 0.36-0.54 meters. Notably, the findings reported in this chapter are similar to the projections from Kummu and Sarkkula (2008), which also stated a permanent lake area increase between 400-1,000 km^2 (17-40%) during the dry season. The quantified seasonal variations in the TSGL system mean flood level, under the individual and combined drivers' scenarios resulted in the same level of change in the mean permanent lake area, with individual hydropower development projected to increase in +26±0% the dry season mean lake area (+1,718±34 km^2; with +36±0% and +9±0% upper and lower boundary variations, respectively; Table 5.3, Table 5.4 and Table 5.5). However, a -5±0% wet season decrease is projected (-668±27 km^2; with -9±1% and -10±0% upper and lower boundary variation, respectively, Table 5.3, Table 5.4 and Table 5.5). The quantified increase in the dry season mean permanent lake area from the individual hydropower scenarios is under the range of variation reported by Kummu and Sarkkula (2008), showing the same direction and level of change from the Mekong River Commission CIA studies. Also, the study reported a 1-month earlier peak flood (i.e. September), under hydropower scenarios, evidencing, with the Piman *et al.,* (2013) findings, that the RCP 4.5 individual hydropower scenarios' projection of only 14 days earlier peak flood (i.e. 1st October compared to the RCP 8.5 peak flood result) resulted from the 3-meter water level gap. Therefore, the projected increase in the hydropower development in the Mekong in the next 20-50 years will profoundly change the flood pulse characteristics of the TSGL system.

The hydropower scenarios assessed in this study contemplated the planned three largest mainstream hydropower developments in the Lower Mekong Basin (i.e. Xayaboury and Don Sahong in Lao PDR and Sambor in Cambodia), which are expected to be fully operational during the future period of this study. These water infrastruture developments are projected to have an installed capacity of over 4,000 MW and storage capacity of over 5x10^6 m^3, with the Sambor dam projected to have the maximum installed and storage capacity (MRC, 2009a; Grumbine and Xu, 2011). Worryingly, the Sambor dam will be located in Kratie's province (see Chapter 4; Baird, 2011; Baran *et al.,* 2011; Sithirith, 2016). Given the proximity of Kratie's province to the TSGL system, the mainstream flow importance for the bi-directional flow reversals and the resulting quantified changes in the flood pulse dynamics of the lake, it is likely that this dam (along with the other 125 dams assessed under the hydropower scenarios; see sub-section 5.2.1) will not only impact Kratie's flow regime, but also the TSGL system, impacting upon the flood pulse dynamics, floodplain seasonal inundation pattern, the migratory corridors and suitable habitats for the migratory fish guilds, which will then affect the fishery yields and fish protein to a projected 2 million people (see later; Lamberts, 2008; Campbell *et al.,* 2009; DHI, 2015).

The combined hydropower and climate change scenarios of the Mekong river are projected to impact the floodplain inundation profile, by decreasing in

69,355±7,983 km^2 (-10±1%) the mean flood extent and increasing the mean flood duration in 7±1% (Figure 5.6). This apparent opposite direction of change results from the individual action of each driver. Climate change is projected to impact (i.e. for the upper and lower boundary prediction of variations; see Table 5.4 and Table 5.5, respectively) the mean flood extent the greatest, while significant increases in the mean flood duration are quantified for the mean and upper boundary prediction of variations, under the individual hydropower scenarios (see Table 5.3 and Table 5.4, respectively). Notably, hydropower development will significantly shift the timing of the flood start by 1-month delay (i.e. delay until the middle of August), also delaying the timing of the flood end by 1-4 months (i.e. February-May; according to Table 5.3, Table 5.4 and Table 5.5 for the mean, upper and lower boundary predictions, respectively). This will result in a total of 8 months of floodplain inundation, compared to the baseline 6 months of inundation assessed in Chapter 4. Kummu and Sarkkula (2008) projected a decrease in the areas of inundation by 7-16%, under hydropower development, which is similar to the findings here stated (-51,202±29,702 km^2 and -7±2%; with +10±4% and -8±4% upper and lower boundary variation, respectively).

Arias *et al.,* (2014) distinguised five habitats types in the TSGL system and projected that water infrastructure developments would cause a 13-22% decrease in the area of seasonally flooded habitats (mainly composed by floodplains). Although no specific quantification was made regarding the changes in inundation duration in each of the referred habitats, which would allow a comparison with the here projected increases in the inundation duration under hydropower development, the authors, following the earlier findings by Arias *et al.,* (2012;2013), stated that projected seasonal variations in floodplain flooding driven by hydropower development, both individually and combined with climate change projections, will profoundly change the vegetation type ocurring in sections of floodplains that are historically flooded only 1-3 months (e.g. shrubland). Pokhrel *et al.,* (2018), recently projected that habitats flooded for over 6 months are likely to be severely impacted by hydropower development, with expected death of the vegetation cover, driven by the seasonal disruption in the timing and duration of the inundation dynamics of the riparian floodplain areas of the TSGL system. Consequently, these changes are expected to highly impact upon the historical high productivity of the system, with Arias *et al.,* (2014), projecting a 33-39% decrease in the net primary production (i.e. carbon production from the primary producers phytoplankton, periphyton and seasonally flooded rooted vegetation) by the combined hydropower and climate change projections (with individual climate change projections resulting in a 9-15% decrease in the net primary production, while 31% reduction is expected under hydropower development).

The decrease in the net primary production of the lake will likely affect the trophic interactions for food sources, affecting fishes regarding the migratory behaviour to recently inundated floodplain areas to feed, with particular impact upon juveniles and larvae early life-stages, and likely to influence negatively the fish stocks (Poulsen *et al.,* 2002;2004). Moreover, the quantified modifications in the timing, duration and flood magnitude observed in both the permanent lake area and connected floodplains, and blockage of key migratory corridors (i.e Mekong river-TSGL system) will likely modify the migratory dynamics and fish abundance, with expected negative impacts to the historical inland fisheries and fish species composition, posing severe challenges to the food security in a system relying heavily on fish protein (see sub-section 5.4.3).

5.4.3 LOSSES IN THE *DAI* FISHERY: NATURAL, SOCIAL AND POLITICAL CONCERNS

The findings in Chapter 4 demonstrated the historical importance of the flood pulse-driven seasonality of the floodplain inundation profile in influencing the annual *dai* fishery catch, for the supporting role on the top-5 small to large body-size medium to long distance migratory fish species (i.e. guilds 4 and 3, respectively) that compose the fishery and contribute nearly 80% to the annual yields. Importantly, it demonstrated that the low level of variations in the predictable seasonal inundation pattern in floodplains, linked to the seasonal variations in the flood level and free connected migratory pathways, significantly triggered the migratory behaviour and high fish abundance, which was reflected in the *dai* fishery (Baran, 2006;2010; Campbell *et al.,* 2009; Halls *et al.,* 2013a). Consequently, the quantified future changes in the historical floodplain flooding profile by both climate change and hydropower development resulted in a predicted 16% (-3,067±149 tonnes mean boundary prediction) loss in the mean *dai* fishery catch, with the combined action of all drivers amplifying the loss to -3,377±111 tonnes mean boundary prediction (-17±0%; see sub-section 5.3.3; Figure 5.7; Table 5.3). Furthermore, regarding the migratory species composition and contribution to the catch, the Other sp. group (with the majority of the species belonging to guilds 3 and 4; see Chapter 4; Halls, 2010; Halls *et al.,* 2013a) is projected to be the most impacted group under both hydropower development and combined drivers' impacts, with 7-9% expected decrease in the contribution to the future catch, compared to the baseline conditions, comprising the mean, upper and lower boundary predictions (Table 5.3, Table 5.4 and Table 5.5). Additionally, and under the individual and combined scenarios for the mean, upper and lower boundary predictions, all top-5 (except *Labeo chrysophekadion* for the individual climate change scenarios and *Thynnichthys thynnoides*; see sub-section 5.3.3) migratory species of the catch are expected to decrease both in mean tonnes (i.e. composition) and contribution (i.e. %), varying from 1-5% the loss in contribution, compared to the baseline. Thus, under the combined drivers' scenarios the alarming 11% (i.e. 69% contribution; 78%-68% for the upper and lower boundary predictions) projected decrease in guilds 3 and 4 contribution to the catch, compared to the nearly 80% baseline contribution, evidenced the negative consequences of hydropower, irrigation and climate change scenarios to the *dai* fishery resources, particularly for the migratory fish guild species.

The top-5 migratory fish guild species of the *dai* fishery are mainly medium to long distance migratory species, which have been argued, regarding the migratory behaviour, to be triggered by the timing of the seasonal variations in the flood level of the lake and consequent seasonal inundation pattern in floodplains, to migrate to these recently inundated areas to spawn, feed and find shelter during the wet season, migrating back to the permanent lake and out to the Mekong river, during the dry season, when the flood level and flooded floodplains start to recede (Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009; Halls, 2010). Importantly, the spawning behaviour of these species has been argued to have evolved based on these flood pulse dynamics, with the combination of extensive inundation and duration pattern providing the optimal conditions for larvae to growth and adults to feed and find shelter, being then captured in the *dai* fishery that has an historical fishery open season between October-March, targeting the fishes that have been growing inside the lake in the extensive inundated floodplains and migrate out of the system at the onset of the dry season (Baran *et al.,* 2001; Baran, 2006;2010; Campbell *et al.,* 2006;2009; Halls, 2010; Halls *et al.,* 2013). Consequently, hydropower development, by delaying in 1-month floodplain flood start (i.e.

middle August) and delaying in 1-4 months the flood end (i.e. February-May), while also decreasing the flooded areas (i.e. extent; see sub-section 5.4.2), is expected to profoundly modify the migratory triggers of the medium to long distance migratory fish species to migrate in and out of the lake, which helps to explain the decrease in the projected catch yields, composition and contribution to the *dai* catch. Also, these migratory guild species perform basinwide migrations both in the mainstream river and extensive network of tributaries (Poulsen *et al.,* 2002;2004; Baran, 2006; Valbo-Jorgensen *et al.,* 2009; Halls, 2010).

The future projections of mainstream (particularly the Sambor dam since it will be the closest to the TSGL system; Sithirith,2016) and tributary dams will likely block the migratory pathways for these species, disrupting the access to the floodplain areas of the TSGL system to complete the life cycles, resulting in a decrease in fish stocks and catch. Yet, and as argued in Chapter 4, the migratory blockage is expected to mainly affect large body-size migratory species (e.g. *Labeo chrysophekadion* and the majority of the species composing the Other sp. group), for the inefficacy of the fish passages, with the modelling analyses of Halls and Kshatriya (2009) predicting a mortality rate of 80% of large body-size species, while 75% survival is expected for small body-size species (e.g. *Henycorhynchus* sp.). For small body-size migratory species the impacts are expected to be significantly felt regarding the flood pulse-driven disruptions on the timing of the migratory pattern (i.e. disruptions in the timing of the flood level variations and consequent effect on floodplain habitats inundation extent), which help to understand the observed predictions in the decrease of *Henycorhynchus sp; Labiobarbus lineatus* and *Paralaubuca barroni* (i.e. three small body-size medium to long distance migratory species), both in mean composition and contribution to catch (Figure 5.8, Table 5.3, Table 5.4 and Table 5.5). Additionally, the *dai* fishery activities have been historically operating from October-March (Halls *et al.,* 2013a). Hence, the increase in floodplain flooding duration to a maximum of May (Table 5.5), although the flooded area decreases, driven by hydropower scenarios, might also explain the decrease in *dai* catch and the individual mean species composition and contribution, since fish will tend to spend more time in the flooded areas, only migrating out of the system at the end of April/beginning of May, when the *dai* fishery operation season has already ended.

The projected decrease in the mean catch yields and migratory fish species guild composition and contribution to the *dai* fishery, driven by the combined drivers' scenarios, with a profound influence of the hydropower development on the historical floodplain flooding profile, reflect a synergistic effect of disruptions on the historical migratory triggers, blockage of the migratory pathway, loss in the suitable floodplain habitats (i.e. although an increase in flood duration is observed, the likely decrease in the primary production and therefore decrease in the food sources (see sub-section 5.4.2), combined with a decrease in extent, might compromise the area and food available for fish to growth, particularly for large body-size migratory species that require longer flooded areas, duration and food to growth; see Chapter 4) and longer permanence of fish species (particularly small body-size species, more capable of occupying reduced flooded areas; Baran *et al*., 2001; Baran, 2006; Halls, 2010) in flooded floodplains by the time of the *dai* fishery open season. Of note, the projected decrease in the fishery resources will bring alarming social concerns, for the importance the inland fisheries place to the economical income and food security to people (see below; Hortle, 2007;2009; So, 2010). Also, it reflects the political views of the unbalanced importance of the fishery resources, compared to the priority given to water infrastructure development to seek economical income and foreign investment, compromising the natural and social security (i.e. from a food security perspective, but also from the worringly displacement of people and threats to human life from dam collapse; Latrubesse *et al.,* 2017; Olson and Morton, 2018; Hecht *et al.,* 2019) of a region expected to sustain high population numbers in the future (see later; Varis *et al.,* 2012; Winemiller *et al.,* 2016). Notably, this study is the first to project the individual and combined impacts of future hydropower, irrigation and climate change on the fishery resources, demonstrating again the low importance given to fish stocks dynamics and role on the fisheries by the policy makers.

The DHI (2015) study shows the only available projection of the consequences to the *dai* fishery resources from hydropower development. The study, stated that under SC1 (i.e. 11 mainstream dams operating at the same time) the TSGL system fish yield would increase in 232 tonnes. Yet, under SC2 (i.e. mainstream hydropower cascade dams plus tributary dams) and SC3 (i.e. mainstream hydropower cascade plus water diversions) a 290-601 tonnes decrease are projected, respectively. These projections are lower than the reported in this study (i.e. mean 767±451 tonnes decrease; -4±2%, with +5±3% and -3±1% upper and lower boundary predictions, respectively; Table 5.3, Table 5.4 and Table 5.5), for the individual hydropower projections, which is likely to result from a projection based in the historical fishery state, while the projections here reported combined the historical fishery assessment and the importance of habitat flood dynamics.

5.4.4 FUTURE FOOD SECURITY CHALLENGES IN THE REGION

According to So (2010) the inland fisheries developed in the TSGL system provide the highest historical fish protein consumption observed in the region (i.e. 70 kg person^{-1} year^{-1}) and entire Southeast Asia. In fact, no other region has an estimated annual fish protein consumption per person as great as that observed in the TSGL system when looking at the fish protein consumption along the Lower Mekong Basin countries (i.e. from a minimum of 43 kg capita^{-1} year^{-1} in Lao PDR to a maximum of 52.4 kg capita^{-1} year^{-1} in Cambodia, with the TSGL system's annual protein consumption per person likely to contribute over half to the observed estimate; see Chapter 3, Hortle, 2007;2009). The *dai* fishery, although only contributing 14% to the inland fisheries developed in the system (see Chapter 4) is likely to entirely contribute to this high estimated consumption, linked to the long-term record of the catch and for providing nearly 80% of small to large body-size medium to long distance migratory species, highly appreciated for household consumption and trade market (Hortle, 2007;2009; So, 2010). Thus, and knowing that over 1.2 million people inhabit the TSGL system, the fact that the inland fisheries (including the *dai* fishery) are able to provide 70 kg of fish protein to each person each year, shows the historical productivity of the fisheries, connected to an outstanding annual fish production, with the flood pulse-driven seasonal flooded floodplains being critical to support (i.e. for spawning, feeding and sheltering purpose; Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Campbell *et al.,* 2006;2009; Valbo-Jorgensen *et al.,* 2009) nearly 80% of migratory fish species that compose the *dai* fishery each year (Lamberts, 2006;2008).

A 17% (+10% -30% for the upper and lower boundary prediction variations, respectively) decrease in the future annual fish protein consumption per person (i.e. 58 kg person^{-1} year^{-1}, with a 77-49 kg person^{-1} year^{-1} upper and lower boundary prediction variations, respectively, assuming a total contribution of the *dai* fishery to the baseline 70 kg person^{-1} year^{-1}) is projected based on the quantified combined drivers' scenarios highest impact on the *dai* fishery (i.e. averaged 16,155 tonnes and -17% compared to the baseline 19,532 averaged tonnes). This projected decrease in the fish protein consumption demonstrates the alarming negative consequences of the combined action of hydropower, irrigation and climate change scenarios in the *dai* fishery yields, species composition and contribution to the fishery, ensuing a significant decrease in the annual fish protein to the future populations inhabiting the region. Although effective estimations in the future population numbers inhabiting the TSGL system are scarce, only with an expected increase to 2 million people, without referring the exact timeframe; the projected increase in the Mekong basin's population until 2050 to 100-145 million people, compared to the baseline 70 million people (i.e. 43-107% increase, respectively; Varis *et al.,* 2012), brings distressing food security issues not only to the TSGL system, but also basinwide, linked to the projected 17% decrease in the annual fish protein provision, under a baseline knowledge of 1.2 million people inhabiting the TSGL system and the assumption of the total *dai* fishery contribution to the annual fish protein consumption per person, while contributing 14% and 7% to the TSGL system and Cambodia's annual landings, respectively (Campbell *et al.,* 2006;2009; Lamberts, 2006;2008; Ziv *et al.,* 2012; Halls *et al.,* 2013a; DHI, 2015). Also, considering the upper boundary prediction of a 10% increase in the annual fish protein consumption per person (i.e. 77 kg person^{-1} year^{-1}), caution needs to be taken as this estimation is again based on a baseline TSGL system population number of 1.2 million people, while the future expected increase to 2 million people will most likely reflect in a % decrease. Thus, under the upper, mean and lower boundary predictions, it is expected a future decrease in the annual fish protein consumption per person, assuming the total contribution of the *dai* catch.

Although not assessed in this thesis, the combined climate change and hydropower scenarios quantified disruptions on the floodplain flooding dynamics (see sub-section 5.3.2) is expected to severely impact upon the rice farming and livestock farming activities developed not only in the Vietnamese delta (see Chapter 3; Kondolf *et al.,* 2018), but also in the TSGL system, which potentially will not be able to compensate for the fish protein loss, and therefore will amplify future food security issues in a region marked by high poverty and severe malnourishment (Hortle, 2007;2009; So, 2010). Orr *et al.,* (2012) by applying two hydropower dam scenarios for the Lower Mekong Basin at 2030 (Scenario 1 - 11 mainstream dams and Scenario 2 - 77 dams), suggested that basic food security is potentially at high risk, connected to the Scenario 1 29-64% increase in water use for food production in Cambodia and an alarming 42-150% increase, under

Scenario 2. Also, a Scenario 1 25-55% increase in land change for pasture land in Cambodia and 36-129% increase, under Scenario 2 are projected, to replace the expected fish protein loss from a Scenario 1 78,200-173,400 tonnes decrease in the Cambodian total fishery catch (based on an estimated 340,000 tonnes per year) and a Scenario 2 113,850–403,920 decrease in the Cambodian total fishery catch (based on an estimated 495,000-792,000 tonnes per year in 2030).

Hence, combining Orr *et al.,* (2012) findings to the TSGL system projections on the decrease in *dai* fishery catch and fish protein loss, driven by the combined drivers' impacts on the flood pulse-driven floodplain flood dynamics, and knowing the 7% baseline contribution of the *dai* fishery to Cambodia's annual landings, alarming future food security issues are projected to the region and basinwide, urging the needs to establish effective environmental conservation strategies, aiming to preserve the basin's historical flood dynamics, floodplain seasonal predictable inundation and migratory pathways, to sustain the fish abundance, fishery and food protein provision to the increasing populations, while addressing the water infrastructure developments in the basin, under changing climate.

5.5 CONCLUSIONS

The results here described provide distressing evidence of the expected future disruptions on the TSGL system flood pulse and floodplain inundation dynamics, and the consequences to the *dai* fishery resource, driven by the individual and combined effects of hydropower development, irrigation and the broader impacts of climate change scenarios. Individual hydropower scenarios are predicted to impact the flow conditions in the Mekong river most significantly, altering the bi-directional flow reversal into the lake system, notably in terms of the timing of the reversal and pulse, which will modify the seasonal flood level in the TSGL system. The increase in the mean flood level in the dry season and decrease in the wet season, will change the timing and flood magnitude of the permanent lake area. Importantly, both climate change and hydropower development will disrupt the floodplain inundation pattern, by decreasing the mean flooded area (i.e. extent) and increasing the mean flood duration. Particularly, individual hydropower scenarios are projected to impact the most, by delaying in 1-month the flood start and delaying in 1-4 months the flood end, with an overall 8-months of flood duration, compared to the baseline 6-months. Consequently, the quantified disruptions on the flood pulse historical dynamics and effect on the previous predictable floodplain inundation profile, key for sustaining fish stocks, were then reflected in the quantified losses in the productive *dai* fishery, driven by water infrastructure development and climate change scenarios. These resulted in an alarming 17% decrease in the *dai* fishery catch, 11% decrease in the contributing role of small and large body-size medium to long distance migratory species (i.e. guilds 4 and 3, respectively) and 17% decrease in fish protein availability to future populations. Hence, these findings provide the scientific background of the needs to prioritise environmental conservation actions of the Mekong river’s flood regime, for the key role in driving the TSGL system flood pulse and floodplain inundation profile that are vital to the inland fishery resources supported, the system’s productivity and to people for food security and livelihoods.

CHAPTER 6

Applying eDNA metabarcoding to monitor fish communities in a biodiversity hotspot under threat, the Mekong river

Overview of the chapter: This chapter presents a test of eDNA metabarcoding as an effective survey tool to detect the tropical river fish diversity, estimate relative abundance and characterise patterns of seasonal distribution. It explores the potential as well as identifies issues with the technique. It also provides critical inferences on the fundamental role of the seasonal flood regime and flood dynamics in driving the ecology and the resource-value for the *dai* fishery. The chapter highlights the importance of applying eDNA-based survey tools in order to prioritise environmental conservation actions in the basin.

6.1 INTRODUCTION

The Mekong river hosts the second highest freshwater fish species richness (i.e. 801 species according to the FishBase scientific online database[15]) in the World behind only the Amazon basin (Baran, 2010; DHI, 2015). The basin is home to a diversity of small, medium and large body-size species (see Table 6.1; Rainboth, 1996; Baran, 2006; Halls, 2010), exhibiting different migratory behaviour and adaptive strategies (see below; Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009). Four of the World's six largest freshwater species are known to inhabit the basin, including the Mekong giant catfish (*Pangasianodon gigas*; 3 meters body length) being endemic and their population classified as critically endangered, according to the IUCN Red List (Hogan, 2012). As extensively covered in earlier chapters (see Chapters 3-4), over 50% of the Mekong fish diversity are argued to perform migrations through the basin in completing their life cycles (Poulsen *et al.,* 2002;2004). These migrations have been linked to the long-term, relatively unchanging, seasonal flood regime (see the analyses in section 3.2 of Chapter 3) and consequent connectivity and predictability in the timing, duration and flood magnitude observed in productive

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[15] https://www.fishbase.se/search.php

habitats across the floodplain environments (see Chapters 3-4; Rainboth, 1996; Poulsen and Valbo-Jorgensen, 2000; Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Campbell *et al.,* 2006;2009; Valbo-Jorgensen *et al.,* 2009).

The mainstream river is key to all migratory species, acting as a “highway” where fish seasonally perform upstream and downstream migrations (longitudinal pattern). Moreover, according to Valbo-Jorgensen *et al.,* (2009), less than 20 species are known to permanently reside in the mainstream river, highlighting the migratory pathway role. The mainstream river is laterally connected to critical habitats (i.e. tributaries and lake with riparian floodplains) that seasonally host the migratory fish species, who enter into these habitats for feeding, sheltering and spawning (lateral pattern; Poulsen *et al.,* 2002;2004; Baran, 2010; Valbo-Jorgensen *et al.,* 2009). Also, these habitats host permanent species (residents), lacking migratory behaviour, but showing unique adaptations to the pronounced seasonal variations in the flood regime observed and consequent changes in water quality (e.g. dissolved oxygen and turbidity; see Chapter 3; Campbell *et al.,* 2006;2009). The guild concept, explored in Chapter 4, is an important ecological classification system, grouping fish species exhibiting the same behaviour regarding the interactions with the habitats explored, feeding habits and spawning cycle, gathering migratory and non-migratory species (Simberloff and Dayan, 1991; Welcomme *et al.,* 2006). A total of 11 guilds are used to classify the Mekong fish species, with species performing medium to long distance migrations (guilds 4 and 3, respectively), residents and non-migratory species (guilds 5 and 6, respectively), also described by whitefish, blackfish and greyfish (see Chapter 4; Halls and Kshatriya, 2009; Baran, 2010).

High uncertainty surrounds the number of species inhabiting the basin, the migratory behaviour, habitats explored and role of the flood regime and sediment flux in driving the migratory pattern (see Chapter 3; Baran, 2006; Valbo-Jorgensen *et al.,* 2009). Baran (2006) reported, for 30 migratory fish species (i.e. only 18% of the 165 species whose migratory behaviour is known), that seasonal variations in the flood regime (i.e. both water level and discharge) triggered (i.e. behaviour response) the migratory behaviour. The findings from Baran (2006), although important, left important open questions regarding the migratory behaviour for more than 700 species known to inhabit the basin. Therefore, although it is frequently argued that the majority of the Mekong fish communities are composed by migratory species, with the seasonal flood regime and sediment loads triggering the migratory behaviour for the habitats explored, these statements are hampered by the low number of species assessed (Poulsen *et al.,* 2002;2004; Baran, 2006) and the lack of long-term establishment of fish monitoring methodologies along the basin.

These identified gaps in knowledge urge to be tackled for the importance that the Mekong fishes represent to the natural system and to over 70 million people inhabiting the basin, who rely on fish for the fisheries as a primary source of protein intake (see Chapters 3-4; Hortle, 2007;2009; So, 2010). Future hydropower, irrigation and climate change scenarios in the basin are threatening fish communities, fishery yields and food security, by altering the predictability and seasonality of the flood regime and habitats' flood dynamics, with negative impacts upon the migratory patterns, fish production and fishery yields (see the Discussion section of this chapter for the findings of Chapter 5; Arias *et al.,* 2012;2013;2014; Hoang *et al.,* 2016;2019). In order to predict the consequences of environmental changes in the basin to fish abundance and migration, it is vital to apply effective methodologies for detecting the Mekong fish communities, while assessing the distribution patterns across the range of habitats.

Environmental DNA (eDNA) is the DNA present in environmental samples (e.g. freshwater, marine, and terrestrial samples) in the form of shed cells, urine, excretia, gametes and decaying material, released by organisms into their environment (Taberlet *et al.,* 2012;2018; Hoffmann *et al.,* 2016; Shaw *et al.,* 2016; Valentini *et al.,* 2016; Handley *et al.,* 2018). Through the application of molecular analyses (see Chapter 2 for the eDNA workflow) it is possible to assess the presence/absence of a single species (e.g. DNA barcoding; Keskin, 2014; Fukumoto *et al.,* 2015; Harper *et al*., 2018a) or the community structure (DNA metabarcoding; Thomsen and Willerslev, 2015; Hänfling *et al.,* 2016; Olds *et al.,* 2016; Handley *et al.,* 2018), the distribution (e.g. migratory behaviour; Laramie *et al.,* 2015; Bista *et al.,* 2017) and estimate the relative species' abundance found within habitats (i.e. site occupancy analysis; Hänfling *et al.,* 2016; Handley *et al.,* 2018), without the need to isolate the target organism (Shaw *et al.,* 2016; Deiner *et al*., 2017; Taberlet *et al.,* 2012;2018). Hence, eDNA-based approaches have revolutionised biodiversity monitoring and have been regarded as powerful tools for implementing conservation actions in different environments (Bohmann *et al.,* 2014; Janosik and Johnston, 2015; Hoffmann *et al.,* 2016). Also, the approaches are argued to be reliable in aquatic systems since eDNA in water has a low persistence time, compared to other environments (nearly 1 month; see Chapter 2), allowing near real-time species detection, even species present in low densities (Taberlet *et al.,* 2012;2018; Herder *et al.,* 2014; Hänfling *et al.,* 2016). Although still in its infancy, the current decade witnessed a rapid increase in the number of eDNA-based published studies applying different methodologies (e.g. differences in sampling design, DNA capture, DNA extraction and kits applied, preservation methods, PCR-based amplification conditions and primers tested, sequencing platforms and bioinformatic pipelines) for different purposes and targeted organisms (e.g. fishes, invertebrates, plants, mammals, among others), and from eDNA barcoding to eDNA metabarcoding (Kelly *et al.,* 2014; Deiner *et al.,* 2015;2017; Renshaw *et al.,* 2015; Boyer *et al.,* 2016; Elbrecht and Leese, 2016; Eichmiller *et al.,* 2016b; Spens *et al.,* 2016; Blackman *et al.,* 2017; Baker *et al.,* 2018; Sellers *et al.,* 2018; Taberlet *et al.,* 2018). However, and as recently pointed out by Cristescu and Hebert (2018), uncertainties exist regarding the eDNA dynamics in aquatic systems (e.g. dilution, transport and degradation rates, and abiotic and biotic interactions with the aquatic environment; see Chapter 2), which connected to the application of unstandardized methodologies, have influenced species detection (see Chapter 2; Taberlet *et al.,* 2012;2018; Herder *et al.,* 2014; Ficetola *et al.,* 2015;2016; Deiner *et al.,* 2017). Moreover, the eDNA studies have mainly focused on temperate environments (Kelly *et al.,* 2014; Keskin, 2014; Fukumoto *et al.,* 2015; Laramie *et al.,* 2015; Hänfling *et al.,* 2016; Port *et al.,* 2016; Bista *et al.,* 2017; Blackman *et al.,* 2017; Baker *et al.,* 2018; Handley *et al.,* 2018; Harper *et al.,* 2018a; Sellers *et al.,* 2018), which is in contrast with the paucity of studies carried out in the tropics, particularly for fish species detection (Hubert *et al.,* 2015; Bellemain *et al.,* 2016; Robson *et al.,* 2016). Thus, there is an urgent need to evaluate the potential of eDNA to monitor highly diverse tropical fish communities such as those found in the Mekong basin. This information is especially valuable given the additional socio-economic importance of this aquatic diversity and its increasing threat from environmental change.

This preliminary study aims to assess the capability of eDNA metabarcoding tools to detect the Mekong river fish diversity and characterise the patterns of seasonal species composition, relative abundance and distribution along the basin, for the habitats explored. Also, and in light of the findings from previous chapters, the significance of the seasonal flood regime and habitats' flood dynamics in driving the ecology and distribution dynamics of the eDNA identified species, the role on the *dai* fishery and the threats by environmental change scenarios of the basin, will be critically discussed for the needs to prioritise environmental conservation actions in the basin. The aims of this study will be accomplished by assessing a set of specific objectives:

1. eDNA metabarcoding analyses and high-throughput sequencing of the collected water samples along sites, to obtain target fish species' sequences and detect the Mekong river fish diversity and composition;
2. Assessment of the levels of cross-contamination present in the study;
3. Analysis of patterns of fish species diversity for the seasonal differences in composition, depth profile and relative abundance found along the basin;
4. Evaluation of seasonal differences in spatial distribution and guilds belonged of the species detected along individual sites of the basin;
5. Analysis of differences in the percentage of guilds present in each season.

6.2 MATERIAL AND METHODS

6.2.1 FIELDWORK AND SAMPLING DESIGN

The Asian monsoonal climate, by the delimitation of wet (May-October) and dry seasons (November-April), influences the seasonal variations in the flood regime and habitats available along the Mekong basin (Adamson *et al.,* 2009), with important implications for the abundance and migratory dynamics of the Mekong fish communities (Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009).

Water samples (one 2L surface and one 2L deep) were collected during both the wet (September 2016) and dry seasons (January 2017). In the wet season, samples were collected in both Lao PDR and Cambodia (see below), whereas in the dry season, fewer samples were obtained in Cambodia only.

- Cambodia (Figure 6.1 red pins, with description of individual sites, GPS coordinates and water samples depth in Appendix 7):
    - Stung Treng – mainstream Mekong river;
    - Se Prok, Se San and Se Kong Rivers (3S system) - tributaries;
    - Kratie – mainstream Mekong river;

  - TSGL system (Sites 1 and 2) – lake;
  - Tonle Sap River – tributary;
  - Phnom Penh – mainstream Mekong river.

- Lao PDR – **only wet season** (black pins of Figure 6.1, with description of individual sites, GPS coordinates and water samples depth in Appendix 7):
  - Luang Prabang – mainstream Mekong river;
  - Nam Ou River – tributary;
  - Vientiane – mainstream Mekong river;
  - Pakse – mainstream Mekong river.

Wet season total number of collected water samples: 26 - 13 sites x 2 water collections at each site (surface/deep).
Dry season total number of collected water samples: 18 - 9 sites x 2 water collections at each site (surface/deep).

In each site, two individual 2L plastic bottles with a lid were used. Before usage, each plastic bottle was immersed in 10% commercial bleach solution (containing <3% sodium hypochlorite) for 5 minutes, followed by 10% microsol detergent for 5 minutes and rinsed in purified water to assure complete sterilisation. For surface water collections (see Appendix 7), the open bottle was immersed, filled and emptied three times before complete fill, closed, labelled and preserved in ice until DNA capture (see below). For deep-water collections, a 5L Ruttner sampler was sterilised by the same process and placed into the water with the chambers open and heights attached, to guarantee deep immersion. The depth was recorded following the 1-meter marks in the line (see Appendix 7) and before reaching the maximum depth the open chambers were closed, pulled back into the boat and emptied in small quantities three times in the sterilised bottle, and then completely emptied into the bottle. After, the bottle was closed, labelled and preserved in ice until DNA capture. The process was repeated for all sites, with the plastic bottles and Ruttner sampler sterilised to avoid cross-contamination between sites and depth profile. Disposable latex gloves were used at all times and exchanged between depth profile and sites to avoid cross-contamination.

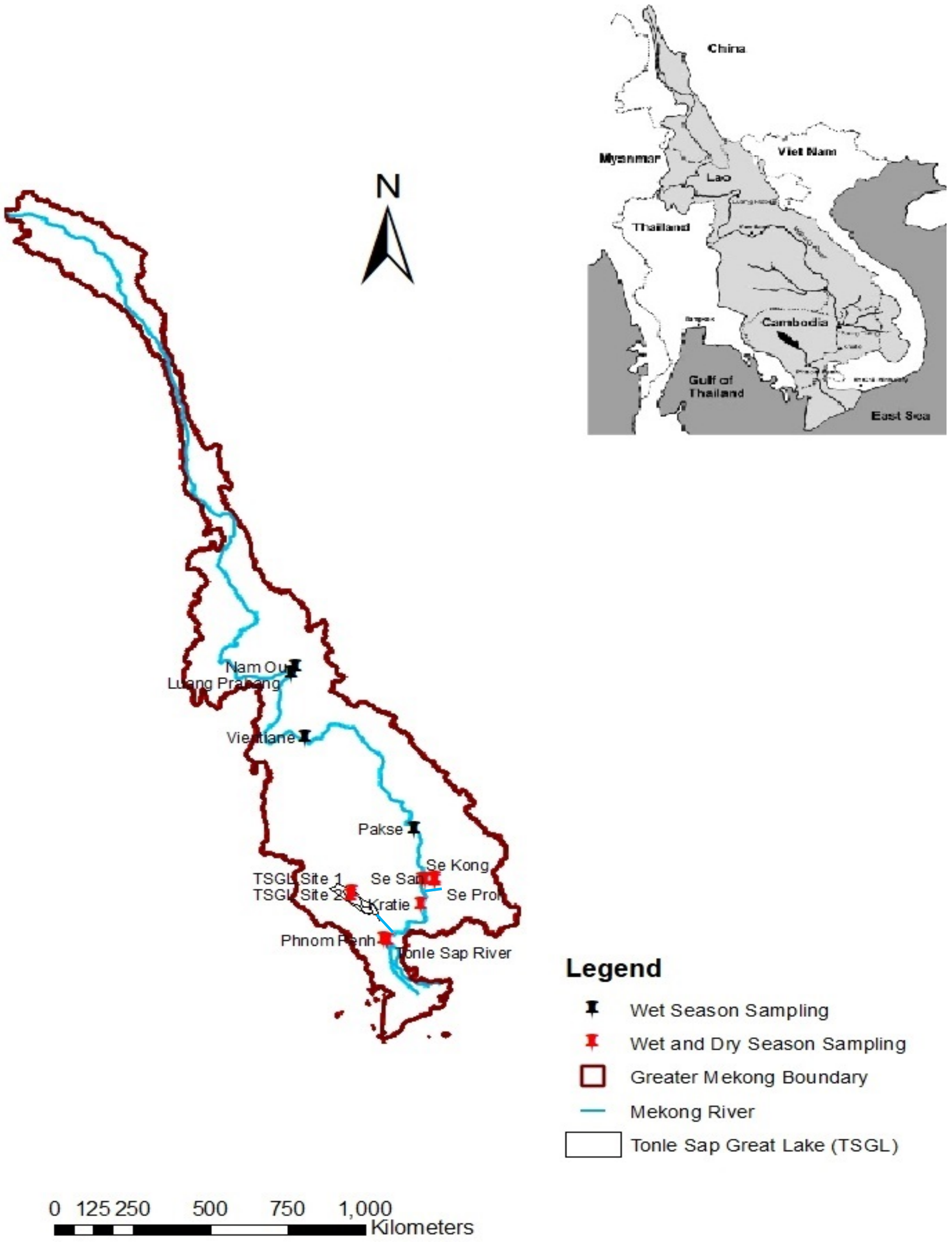

FIGURE 6.1 - MAP OF THE SITES WHERE INDIVIDUAL 2L WATER SAMPLES WERE COLLECTED. BLACK PINS REPRESENT SITES SURVEYED IN THE WET SEASON. RED PINS REPRESENT SITES SURVEYED IN BOTH SEASONS. THE MEKONG BASIN IS DELIMITED BY A DARK RED LINE, WITH THE MEKONG RIVER IN LIGHT BLUE LINE AND THE TONLE SAP GREAT LAKE (TSGL) SYSTEM DELIMITED BY A BLACK LINE. THE UP-RIGHT CORNER OF THE MAP SHOWS THE LOCATION OF THE MEKONG BASIN IN SOUTHEAST ASIA AND THE SHARING COUNTRIES (I.E. CHINA, MYANMAR, THAILAND, LAO PDR, CAMBODIA AND VIETNAM).

6.2.2 DNA CAPTURE AND PRESERVATION

The DNA found in freshwater environments is highly degraded and has limited persistence time, linked to biotic and abiotic factors affecting DNA molecules (see Chapter 2; Strickler *et al.,* 2015; Eichmiller *et al.,* 2016a;b; Li *et al.,* 2018; Tarbelet *et al.,* 2018). These factors are exacerbated in the tropics. Thus, to increase downstream species' detection, it is important to apply effective strategies aiming to concentrate and preserve the low DNA material found in the environment (Deinar *et al.,* 2015; Spens *et al.,* 2016; Yamanaka *et al.,* 2016a;b; Li *et al.,* 2018). Filtration of collected water samples and buffer preservation (e.g. RNAlater solution) of filter-papers are a reliable approach, particularly when filtration is performed in remote areas (Deiner *et al.,* 2015; Spens *et al.,* 2016; Yamanaka *et al.,* 2016b; Li *et al.,* 2018). Hence, in each site, the DNA from water samples was captured in less than 4 hours from sampling. Nalgene filtration units were attached to a vacuum pump, previously sterilised by the reported methodology. The water samples were filtered (herein referred as filter samples) through 0.45 μm cellulose nitrate filters and pads (47 mm diameter, Whatman, GE Healthcare, UK), following the study of Li *et al.,* (2018). In the wet season, because high suspended sediment is present, for each site and water collection according to depth profile, a total of 10 filters were used (each with a 200 ml of filtered volume). In the dry season, where waters are less turbid apart from the TSGL sites (10 filters used each with 200 ml of filtered volume), 5 filters (each with a 400 ml of filtered volume) were used for each site and water collection according to depth profile. Consequently, and in order to keep the filtered volume comparable between sites, seasons and depth profile, the DNA extractions (see below) of the filter samples with less than 400 ml per filter were performed combining 2 filters (200 ml + 200 ml). Filtration blanks were made to check for potential cross-contamination. These were performed for each site and depth profile (total n=44; n=26 in the wet season and n=18 in the dry season), after the sterilisation procedure of the Nalgene filtration units within (i.e. depth profile) and between sites, consisting in filtering 400 ml of purified water through the Nalgene filtration units and capturing in the 0.45 μm filter papers. All filter samples and blanks were individually placed inside a sterile 15 ml falcon tubes, filled with RNAlater buffer solution until complete immersion and labelled. The falcon tubes were kept inside a fridge (4 ºC) until transportation to the UK (less than 1 month after capture), and first kept in a cold chamber (4 ºC) in the Geography department of the University of Hull (UK) for 4 months, before transferred to the eDNA laboratory facility of the same University, for storage in a dedicated DNA-free freezer (-20 ºC) until DNA extraction.

6.2.3 DNA EXTRACTION TREATMENTS

Before filter samples were DNA extracted, a trial was carried out to compare different extraction methods and approaches (herein referred as pre-treatment analysis). A total of 96 samples were extracted: <u>3 sites</u> (TSGL Site 1, TSGL Site 2 and Se Kong) x <u>2 seasons</u> (dry and wet seasons) x <u>2 depth profile</u> (surface and deep) x 2 DNA extractions kits (see below) x 4 individual treatments (see below). The two DNA extraction kits employed Mu-DNA following Sellers *et al.,* (2018) protocol and PowerWater DNA Isolation Kit (MoBio Laboratories, Inc. Carlsbad, USA, now Qiagen) following the manufacturers' instructions. Regarding the 4 individual treatments (i.e. T1, RNA, T2 and T3) used for each DNA extraction kit (Figure 6.2 and Figure 6.3), a single filter out of 5 (400 ml filtered volume) or 2 filters out of 10 (200 ml + 200 ml of filtered volume) from each site, season and depth profile were used per kit and treatment (excluding RNA, see below). T1 consisted of the independent DNA extraction of the filter sample preserved in RNAlater buffer solution. RNA (short name adopted to refer to the RNAlater solution) consisted of the extraction, by the precipitation method following Spens *et al.,* (2016) protocol, of the remaining DNA potentially present in the buffer solution, where the filter sample, extracted by the T1 treatment, was preserved. T2 consisted of the DNA extraction of the filter sample and use of a new filter to filtrate (i.e. using the Nalgene equipment and vacuum pump) the potential DNA stored in the RNAlater buffer solution. The two filters were extracted together. T3 consisted of the filtration (i.e. using the Nalgene equipment and vacuum pump) of the RNAlater buffer solution in the same filter sample and subsequent DNA extraction. Of note, although T1 and RNA belong to the same sample, the DNA extraction, library preparation, PCR amplification and sequencing (see sub-section 6.2.4) were done separately to assess differences in DNA concentration (see sub-section 6.3.2).

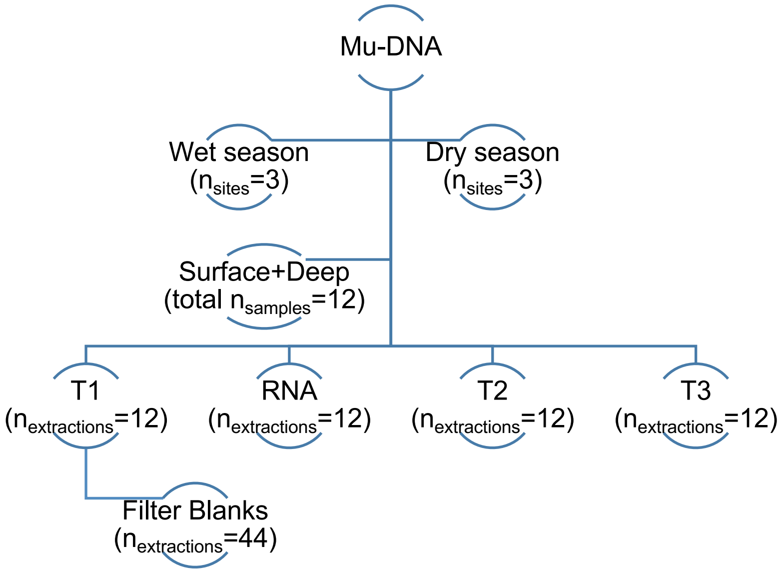

FIGURE 6.2 - MU-DNA EXTRACTION DESIGN COMPRISING THE NUMBER OF DNA EXTRACTIONS PERFORMED ACCORDING TO SEASONS, DEPTH PROFILE AND TREATMENTS APPLIED (T1, RNA, T2 AND T3). THE DNA EXTRACTIONS OF THE FILTRATION BLANKS WERE ONLY PERFORMED BY T1, TO ASSESS THE LEVEL OF CROSS-CONTAMINATION IN THE STUDY.

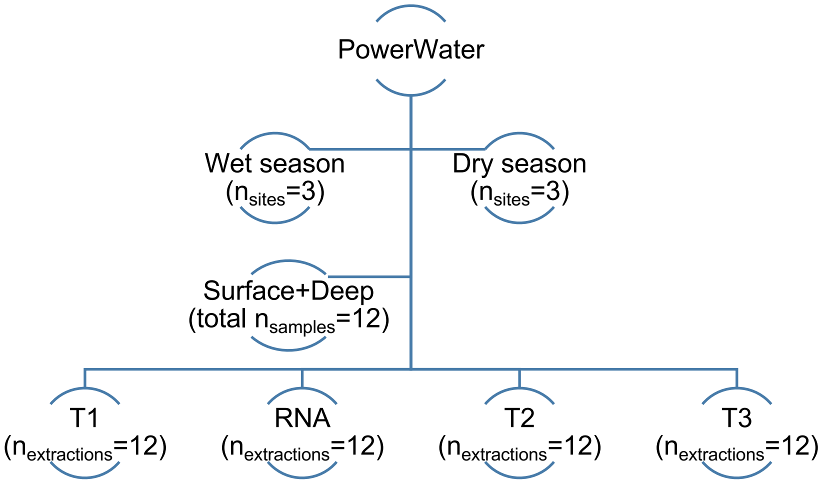

FIGURE 6.3 - POWERWATER DNA EXTRACTION DESIGN COMPRISING THE NUMBER OF DNA EXTRACTIONS PERFORMED ACCORDING TO SEASONS, DEPTH PROFILE AND TREATMENTS APPLIED (T1, RNA, T2 AND T3).

Following careful inspection of the results achieved in the pre-treatment analysis (see sub-section 6.3.2), the remaining sites according to both seasons (10 sites in the wet season and 6 sites in the dry season) and depth profile were extracted using the Mu-DNA kit and T1 and RNA treatments (n=64 DNA extractions, comprising 16 sites x 2 treatments x 2 depth profile). All filter blanks (n=44) were extracted using the same kit and T1 treatment (Figure 6.2). The extracted DNA was quantified using the Qubit HS DNA Quantification Kit (ThermoFisher, UK), following the manufactures protocol, and placed in a dedicated freezer (-20 ºC) inside the eDNA laboratory until library preparation and PCR-based amplification.

6.2.4 LIBRARY PREPARATION AND SEQUENCING

All extracted DNA samples (i.e. the pre-treatment analysis (n=96), remaining filter samples using only the Mu-DNA kit by the T1 and RNA treatments (n=64), and filter blanks (n=44)) were PCR-amplified following a two-step (double indexes) library preparation protocol according to Li *et al.,* (2019), for the 12S ribosomal RNA region, targeting a 106 bp vertebrate-specific fragment (Kelly *et al.,* 2014). The primer pair 12S_F1 (Sequence 5'-3': ACTGGGATTAGATACCCC) and 12S_R1 (Sequence 5'-3':TAGAACAGGCTCCTCTAG), combining 20 individually tagged forward and 20 individually tagged reverse primers (Li *et al.,* 2019), were used to create a unique individual combination of one tagged forward and one reverse primers for each individual filter samples (n=160), filter blanks (n=44) and PCR controls (i.e. Known template control or positive control (n=9) using the Lake Malawi endemic cichlid species, *Maylandia zebra* and No template control or negative control (n=9)). Three libraries, each containing 74 primer-tagged samples, were PCR-amplified with triplications made for each library (n=9).

The PCR reactions were performed in a dedicated sensitive sample laboratory at the University of Hull, to minimise the risk of contamination. Importantly, before any reaction took place, the PCR-hood and materials (including sets of eight-strip PCR tubes with individually attached lids, micropipettes and tips) were placed inside the hood and UV-light sterilised for 30 mins. The first PCR reaction was carried out in 25 μl volume containing: 12 μl of Q5® High-Fidelity 2X Master Mix (New England Biolabs), 2.5 μl of individual combination of tagged forward and tagged reverse primers (0.5 μM each primer), 5.5 μl of PCR water and 5μl of sample DNA[16] (<1000 ng DNA template). For the Known template control, minor modifications were made: Q5® High-Fidelity 2X Master Mix (17.5 μl), Primers (2.5 μl), PCR water (4μl) and *Maylandia zebra* DNA (1 μl). For the No template control the conditions were: Q5® High-Fidelity 2X Master Mix (17.5 μl), Primers (2.5 μl), PCR water (5μl). All PCRs were performed on an Applied Biosystems Veriti thermal cycler with modified cycling conditions from Li *et al.,* (2019): 98 ^{0}C (5 mins), 40 cycles: 98 ^{0}C (10 sec), 56 ^{0}C (15 sec) and 72 ^{0}C (20 sec). A final elongation step of 72 ^{0}C (5 mins) was performed. PCR products (including

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[16] The double volume of DNA used, from 2.5 μl reported in other studies (e.g. Hänfling *et al.,* 2016; Li *et al.,* 2019) to 5 μl, was associated to the low DNA concentration found in the DNA extractions (see Figure 6.4).

replicated libraries) were checked on ethidium bromide-stained agarose gel followed by pooling each replicated library to reduce potential bias during the PCR step (Hänfling *et al.,* 2016). The pooled libraries were cleaned using the Mag-Bind® RXNPure Plus Kit (Omega Bio-tek) using a dual bead-based size selection protocol (Li *et al.,* 2019). The ratios used for size selection were 0.9x and 0.15x magnetic beads to PCR product. The second PCR reactions were carried out in 50 μl volumes containing: 24 μl Q5® High-Fidelity 2X Master Mix, 2.5 μl of tagged primer pair, 5 μl of template DNA and 16 μl of molecular grade water. The thermal cycling conditions were modified from Li *et al.,* (2019): 95 ^{0}C (3 mins), 12 cycles: 98 ^{0}C (20 sec) and 72 ^{0}C (1 min). A final extension of 72 ^{0}C for 5 mins. The second PCR products were again cleaned using the Mag-Bind® RXNPure Plus Kit (Omega Bio-tek) using a dual bead-based size selection protocol (Li *et al.,* 2019). The ratios used for size selection were 0.7x and 0.15x magnetic beads to PCR product. The cleaned second PCR products were normalised following the Qubit HS DNA Quantification Kit (ThermoFisher, UK) and pooled. The final library concentration was quantified by qPCR using NEBNext® Library Quant Kit (New England Biolabs). The pooled quantified library was adjusted to 4 nM and denatured following the Illumina MiSeq library denaturation and dilution guide. The sequencing step was performed on the Illumina MiSeq platform using the MiSeq reagent kit V3 (2x300 bp chemistry), including a 10% addition of PhiX genomic library for improving the clustering during the initial sequencing cycles.

6.2.5 MEKONG FISH COMPILATION AND CURATED REFERENCE DATABASE, AND IN SILICO ANALYSIS

A custom phylogenetically curated reference database was compiled for the Mekong fish species, focusing on the 12S ribosomal RNA region, following the methodology applied in Hänfling *et al.,* (2016). The reason for compiling this custom reference database was to match the DNA sequences obtained from the MiSeq sequencing to the available fish DNA sequences compiled in the curated database and identify the fish species present in each of the sites. First, a list of the scientific names of the 801 target fish species known to inhabit the Mekong was made based on available literature (Rainboth, 1996; Poulsen *et al.,* 2002;2004; Baran, 2006; Halls, 2010) and the online scientific fish reference database (i.e. FishBase). Then, following the custom-made reproducible metabarcoding pipeline (metaBEAT v0.97.8), the available 12S sequences of the target species were retrieved from GenBank of NCBI (National Center for Biotechnology Information; an online public genetic repository containing all available sequences from different genetic markers (e.g. 12S) for diverse taxa, obtained from published studies) using E-utilities (Sayers, 2008). Using the ReproPhylo environment (Szitenberg *et al.,* 2015), the raw reference database (i.e. containing all sequences retrieved from GenBank) was processed for producing a non-redundant quality checked reference database (i.e. a curated reference database without misleading sequences, double sequences and other sources of potential errors). Using CD-hit-est v4.6.1 (Li and Godzik, 2006), sequences were extracted in FASTA format and clustered at 100% identity to remove redundancy. Following a quality control checkpoint, attention was paid to the sequence length, where short-sequence fragments (<50 bp) were removed and the remaining sequences aligned using MAFFT v7.0 (Katoh and Standley, 2013). Maximum likelihood tree of alignments was inferred with RAxML v8.0.2 (Stamatakis, 2006), following the GTR+ gamma model of substitutions. The resulting phylogenetic tree was carefully analysed for the potential presence of misleading sequences using SATIVA v0.9 (Kozlov *et al.,* 2016). After careful inspection for the presence of remaining misleading sequences, the curated reference database (comprising for the 12S: 449 species and representing 56.1% of all known species from the Mekong basin) was used in downstream analyses. In silico analysis were performed to infer the suitability of the chosen primers to the current tropical fish metabarcoding study. In this analysis the Kelly *et al.,* (2014) primer pairs (12S_F1 and 12S_R1) were evaluated against the Mekong fish curated reference database, using the ecoPCR v0.2 program (Ficetola *et al.,* 2010) of OBITools v1.01.22 (Boyer *et al.,* 2016), to evaluate the conservation of primer binding sites and species resolution of the resulting PCR amplicons. The maximum number of mismatches allowed per primer was set to three.

6.2.6 BIOINFORMATICS AND DATA ANALYSES

The bioinformatic analyses were performed on the custom reproducible metabarcoding pipeline (metaBEAT v0.97.8), using the 12S Mekong fish curated reference database and following the Hänfling *et al.,* (2016) methodology. The raw read data obtained from the Illumina Miseq sequencing platform were in FASTA format. The Trimmomatic 0.32 (Bolger *et al.,* 2014) program was used for quality trimming and removal of primer sequences from the raw reads. Following a phred quality control above 30, the average read quality was assessed in 5 bp sliding window, where starting from the 3'-end of the reads, reads were clipped and reads shorter than 90 bp read length were discarded. Using the FLASH 1.2.11 program (Magoč and Salzberg, 2011), the sequence pairs were merged into single high-quality reads. Then, by applying the 'uchime_ref' function and 100% clustering implemented in vsearch 1.1 (https://github.com/torognes/vsearch), the remaining reads were screened to identify chimeric sequences (i.e. when sequencing reads align to two distinct portions of the genome with little or no overlap) against the Mekong fish curated reference database, and redundant sequences were removed. Moreover, clusters represented by less than 3 sequences were defined as sequencing errors and discarded from downstream analyses. The non-redundant sequences were then compared against the Mekong fish reference database using BLAST (Zhang *et al.,* 2000), where two strategies were adopted following Hänfling *et al.,* (2016). Briefly, only the most significant matches to the reference database were recorded (i.e. the top 10% bit-scores), where if only a single taxon was present, the sequence was directly assigned to that taxon. On the other hand, if more than one taxon (i.e. taxa) was present, the sequence was assigned to the lowest taxonomic level that was shared by all taxa present for this sequence. Non-target sequences (i.e. sequences not belonging to any of the Mekong fish species present in the reference database) were defined based on sequences that had BLAST bit scores below 80 or less than 100% identity to any sequence present in the Mekong reference database. Additionally, to qualitatively assess the diversity of organisms present in the Mekong environment, non-target sequences were subject to a separate BLAST search against NCBI's complete nucleotide database, where using MEGAN 5.10.6 (Huson *et al.,* 2007), assignment of non-target sequences was obtained. The custom bioinformatics pipeline used is available on Github (https://github.com/HullUni-bioinformatics/metaBEAT).

Filtered data was summarized into: 1) the number of sequence reads (i.e. read counts) per species at each site, according to season and depth profile; 2) the proportion of individual species' read counts for all sites (i.e. site occupancy analysis). Importantly, and due to the high frequency of low species' read counts

(e.g. minimum of 3 reads) found, no low-frequency noise threshold, to account for false-positives, was applied as mentioned in other studies (e.g. Hänfling *et al.,* 2016; Handley *et al.,* 2018; Li *et al.,* 2019), for the risk of eliminating important species. Yet all sites, according to seasons and depth profile, whose total species' read count was zero were omitted from downstream analyses. Furthermore, and due to the higher number of samples whose DNA was extracted following the Mu-DNA kit by T1 and RNA treatments, T2, T3 and PowerWater DNA extracted filter samples were also omitted from downstream analyses. Of note, after inspection on the raw data, the sites where these two treatments and kit were applied had high frequency of zero read counts, thus no risk of omitting important species was present. Finally, and since T1 and RNA treatments, although extracted, PCR-amplified and sequenced separately, belong to the same site, the individual species' read counts were combined per individual site, season and depth profile.
To understand the best combination of DNA extraction kit and treatment to be applied for the remaining sites, for maximising the DNA concentration, in the pre-treatment analysis, a non-parametric Kruskal-Wallis one-way ANOVA followed by the post-hoc Dunn's test was conducted to test for statistically significant differences in read counts between individual DNA extraction kits and the four treatments applied. A 2-way ANOVA, after applying Tukey's ladder of powers transformation, was also applied to test for statistically significant differences in read counts combining both extraction kits and the four treatments, with interactions among treatments and kits considered. All analyses were considered statistically significant at p <0.05.

To assess the level of contamination present in the current study, which could potentially compromise downstream inferences, the individual and total target species' read counts found in filter blanks and PCR controls were compared to the individual and total target species' read counts found in target samples.

The patterns of eDNA species diversity found along the basin, according to seasons and depth profile, was investigated. This analysis was performed by applying a Non-metric Multidimensional Scaling (NMDS) with the analysis of similarities (ANOSIM), based on read counts, using the abundance-based Bray-Curtis dissimilarity index, with the *metaMDS* and *anosim* functions found in the Vegan v2.4-4 package on R (Oksanen *et al.,* 2017). The ANOSIM analysis measures the differences of mean ranks between seasons and within depth profile. Furthermore, the relative species abundance found in the basin was assessed, following the site occupancy analysis. The site occupancy analysis applied by Hänfling *et al.,* (2016) and Handley *et al.,* (2018) allows an assessment of the relative species abundance found within sites. Notably, the authors combined historical fish composition and abundance data from long-term established surveys (i.e. gill-net survey data) to establish rank abundance data and compare it with the eDNA read counts of species detected in each of the sampling sites, to assess the efficacy of eDNA-based site occupancy analysis in estimating the relative species abundance within sites. Yet, due to the lack of long-term historical records on fish abundance and low individual species' sequence reads within sites, the site occupancy analysis in the present study differed from Hänfling *et al.,* (2016) and Handley *et al.,* (2018), by assessing the relative individual species abundance detected for all sites, according to seasons. Independent Chi-square analysis (χ^2) was applied to assess differences in the individual species' relative abundance between seasons. The species composition (i.e. regarding the read counts) detected in individual sites, according to seasons, was further assessed, followed by an independent Chi-square analysis (χ^2) to test for statistically significant differences in the species composition according to seasons.

The analysis of the spatial distribution of individual species between sites and guilds between seasons was assessed to characterise the migratory dynamics and the habitats explored by each species. Also, the percentage of guilds (%) present in each season was assessed, by dividing the sum of the individual species' read counts detected for each guild by the total species' read counts detected for each season. Independent Chi-square analysis (χ^2) was applied to test for statistically significant differences in the guilds' composition per season.

All statistical analyses were made in R v3.5.1 (R_Core_Team, 2018) at 95% confidence interval (Zar, 2010) and graphs were plotted using *ggplot2* v2.2.1 (Wickham and Chang, 2016).

6.3 RESULTS

6.3.1 IN SILICO ANALYSIS

The in silico analysis showed that the Kelly *et al.,* (2014) 12S primer pair applied resulted in the amplification of the DNA sequences of 323 species (72%) out of the 449 Mekong river fish species' DNA sequences present in the curated reference database for the 12S genetic marker. Importantly, after careful inspection in the NCBI online platform for the 126 species whose DNA sequences were not amplified by the primer pair of this study, it was noted that those sequences, included in the 12S Mekong fish curated database, correspond to fragments amplified by a different primer (i.e. MIFISH primers; Myia *et al.,* 2015).

6.3.2 PRE-TREATMENT ANALYSIS

The DNA concentration obtained in the pre-treatment analysis (Figure 6.4) was low (average of <2 ng/μl for the 2 kits and 4 treatments applied) compared to other fish metabarcoding studies (e.g. Sellers *et al.,* 2018). The non-parametric Kruskal-Wallis one-way ANOVA (since normality was not accomplished for individual factors[17]: PowerWater's Shapiro-Wilk normality test: W=0.78; *p*= 6.11×10^{-7} and Mu-DNA's Shapiro-Wilk normality test: W=0.49; *p*=1.43×10^{-11}) identified statistically significant differences in the DNA concentration obtained from the PowerWater kit (Figure 6.4; χ^2=21.38; df=3; *p*=8.77×10^{-5}), with the post-hoc Dunn's test showing the detected significant differences in the T2 and T3 treatments (letter b in Figure 6.4). Non-statistically significant differences were found for the Mu-DNA kit for the treatments applied (Figure 6.4; letter a; χ^2=5.26; df=3; *p*=0.15). The Tukey's transformed 2-way ANOVA test, for the DNA concentration measured for the 2 kits and 4 treatments, with interactions considered, showed non-statistically significant differences (Treatments: F=1.27; df=3; *p*=0.28; Kit: F=1.77; df=1; *p*=0.45; Treatment x Kit: F=2.79; df=3; *p*=0.06). Although T2 and T3, for the PowerWater kit, yielded significantly higher DNA concentrations than T1 and RNA, when testing the combined effect of the 2 kits and 4 treatments (2-way ANOVA), non-statistically significant differences were observed. Importantly, T2 and T3 required higher laboratory handling than T1 and RNA (see sub-section 6.2.3 for the specificity of individual treatments). Consequently, T2 and T3 represented a higher risk of contamination than T1 and RNA. As so, the further DNA extractions for the remaining sites, according to the two seasons and depth profile (n=64), were performed following the T1 and RNA

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[17] Factors here refer to the two individual kits (PowerWater and Mu-DNA), followed by the 4 levels (i.e. treatments applied), to assess statistically significant differences in the DNA concentration measured by the Qubit HS DNA Quantification Kit.

treatments and applying the Mu-DNA extraction kit (Sellers *et al.,* 2018), since no significant differences were encountered between the two kits. Of note, the further DNA extractions (n=64) showed the same low DNA concentration found in the pre-treatment analysis (average of <1 ng/μl).

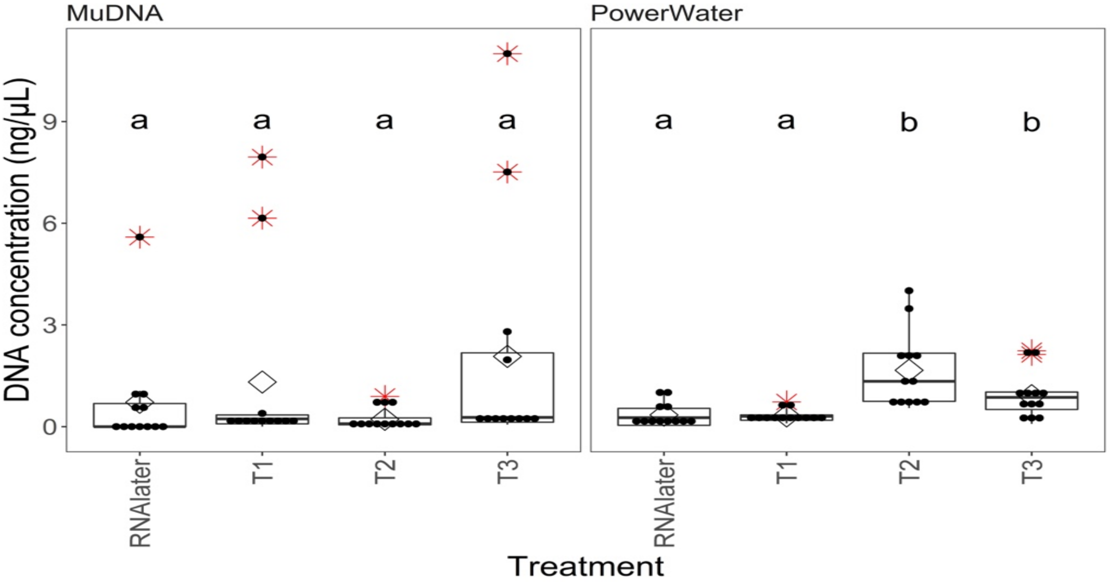


FIGURE 6.4 - PRE-TREATMENT ANALYSIS SHOWING THE DNA CONCENTRATION (NG/μL) MEASURED FOR THE 2 INDIVIDUAL KITS AND 4 TREATMENTS. BLACK POINTS REPRESENT INDIVIDUAL SAMPLES' DNA CONCENTRATIONS (N=48 FOR EACH KIT). DIAMONDS REPRESENT THE AVERAGE DNA CONCENTRATION AND RED STARS THE OUTLIERS FOUND. LETTERS REPRESENT THE STATISTICAL SIGNIFICANCE OF THE NON-PARAMETRIC KRUSKAL-WALLIS ONE-WAY ANOVA TEST PERFORMED, FOLLOWED BY THE POST-HOC DUNN'S TEST (A – NON-STATISTICALLY SIGNIFICANT DIFFERENCES; B – STATISTICALLY SIGNIFICANT DIFFERENCES).

6.3.3 THE TARGET FISH SPECIES DETECTED AND NON-TARGET SEQUENCES

The total raw sequence reads (i.e. the sum of the reads for the three libraries and before the bioinformatic quality filtering process took place) obtained from the Illumina MiSeq sequencing platform was 19,596,884 reads, with a 77% quality assessment made. After the bioinformatic filtering process and removal of chimeric sequences (see sub-section 6.2.6 for the definition), 14,284,162 total reads (Library 1: 5,286,325; Library 2: 5,158,688; Library 3: 3,839,149) were present. The Mekong curated reference database comprised 449 fish species' target sequences for the 12S genetic marker. After the filters applied during the bioinformatic analyses (see sub-section 3.2.6) and BLAST of the reference database with the MiSeq sequences, 25 Mekong fish species were detected (Table 6.1). Although only representing 3.12% of the known Mekong fish diversity (i.e. 801 total described species), from Table 6.1 it is possible to see the diversity of families (with 52% of the species belonging to the Cyprinidae family) and body sizes (ranging from 5 to 130 cm) present in this large transboundary river basin. Furthermore, *Henicorhynchus lobatus, Henicorhynchus siamensis, Labiobarbus leptocheilus, Pangasianodon hypophthalmus, Pangasius larnaudii, Paralaubuca typus, Thynnichthys thynnoides, Yasuhikotakia eos* and *Yasuhikotakia modesta*, have important economic value in Lao PDR and Cambodia, comprising an estimated 40% of the species found in the *dai* fishery (which will be further discussed; Baran *et al.,* 2005;2007; Halls *et al.,* 2013a; DHI, 2015).

TABLE 6.1 - THE MEKONG FISH DIVERSITY (SPECIES NAMES, FAMILIES AND AVERAGED BODY-SIZE; RAINBOTH, 1996; POULSEN *ET AL.*, 2002;2004; HALLS, 2010; FISHBASE ONLINE DATABASE).

Species	*Family*	*Averaged body-size (cm)*
Amblyrhynchichthys truncatus	Cyprinidae	30
Anabas testudineus	Anabandidae	13
Barbonymus gonionotus	Cyprinidae	35
Channa striata	Channidae	60
Clarias batrachus	Clariidae	25
Clarias gariepinus	Clariidae	90
Clupeoides borneensis	Clupeidae	5
Esomus metallicus	Cyprinidae	5
Hemibagrus spilopterus	Bagridae	25
Henicorhynchus lobatus	Cyprinidae	15
Henicorhynchus siamensis	Cyprinidae	15
Hypophthalmichthys molitrix	Cyprinidae	18
Labiobarbus leptocheilus	Cyprinidae	25
Lobocheilos melanotaenia	Cyprinidae	15
Mystacoleucus marginatus	Cyprinidae	10
Pangasianodon hypophthalmus	Pangasiidae	130
Pangasius larnaudii	Pangasiidae	125
Paralaubuca typus	Cyprinidae	15.5
Polynemus dubius	Polynemidae	15
Scaphognathops bandanensis	Cyprinidae	25
Schistura kaysonei	Nemacheilidae	5
Systomus orphoides	Cyprinidae	5
Thynnichthys thynnoides	Cyprinidae	25
Yasuhikotakia eos	Cobitidae	10
Yasuhikotakia modesta	Cobitidae	25

The separated BLAST search to the entire NCBI's nucleotide database (see sub-section 3.2.6) for the non-target sequences, identified different bacteria species (total read counts=200), a diversity of fish species not present in the Mekong river (e.g. Atlantic herring, freshwater bream, polar cod, Eurasian minnow, among others; total read counts=31,379), amphibian species (*Bufo bufo*: total read counts=5,573 and *Hoplobatrachus rugulosus*: total read counts=930), bird species (e.g. *Callohetta leucophrys*: total read counts= 56; *Gallus gallus*: total read counts=842; *Fulica atra*: total read counts=5,839; among others) and mammal species (e.g. *Canis lupus*: total read counts=9,683; *Homo sapiens*: total read counts=1,412,269; *Rattus nitidus*: total read counts=3; among others). The total read counts for the unassigned sequences was 478,169.

6.3.4 CONTAMINATION ANALYSIS

Although all efforts were taken to avoid potential sources of cross-contamination (i.e. the presence of the target fish species' read counts in the controls), this is unlikely to be 100% accomplished, due to the number of steps and factors involved in the eDNA workflow (see Chapter 2; Taberlet *et al.,* 2012;2018). However, if the total target species' read counts in the controls is considerably lower compared to the same observed in the target samples, the level of contamination is considered low and further inferences on species detection are not hampered by the level of contamination detected (Hänfling *et al.,* 2016). Figure 6.5 shows that although cross-contamination was present in the controls (i.e. Filter Blank, Known Template Control and No Template Control), the total read counts of individual target species detected are considerably lower than the total read counts detected for the same species in the target samples (i.e. Sample). The species used as positive (i.e. Known template control; *Maylandia zebra*) was omitted from the analysis, since it would significantly mask the total read counts detected for the target species. This is because total read counts are expected to be high for *Maylandia zebra*, since this is the known template control applied in the PCR step to effectively assess the performance of the PCR-amplification stage.

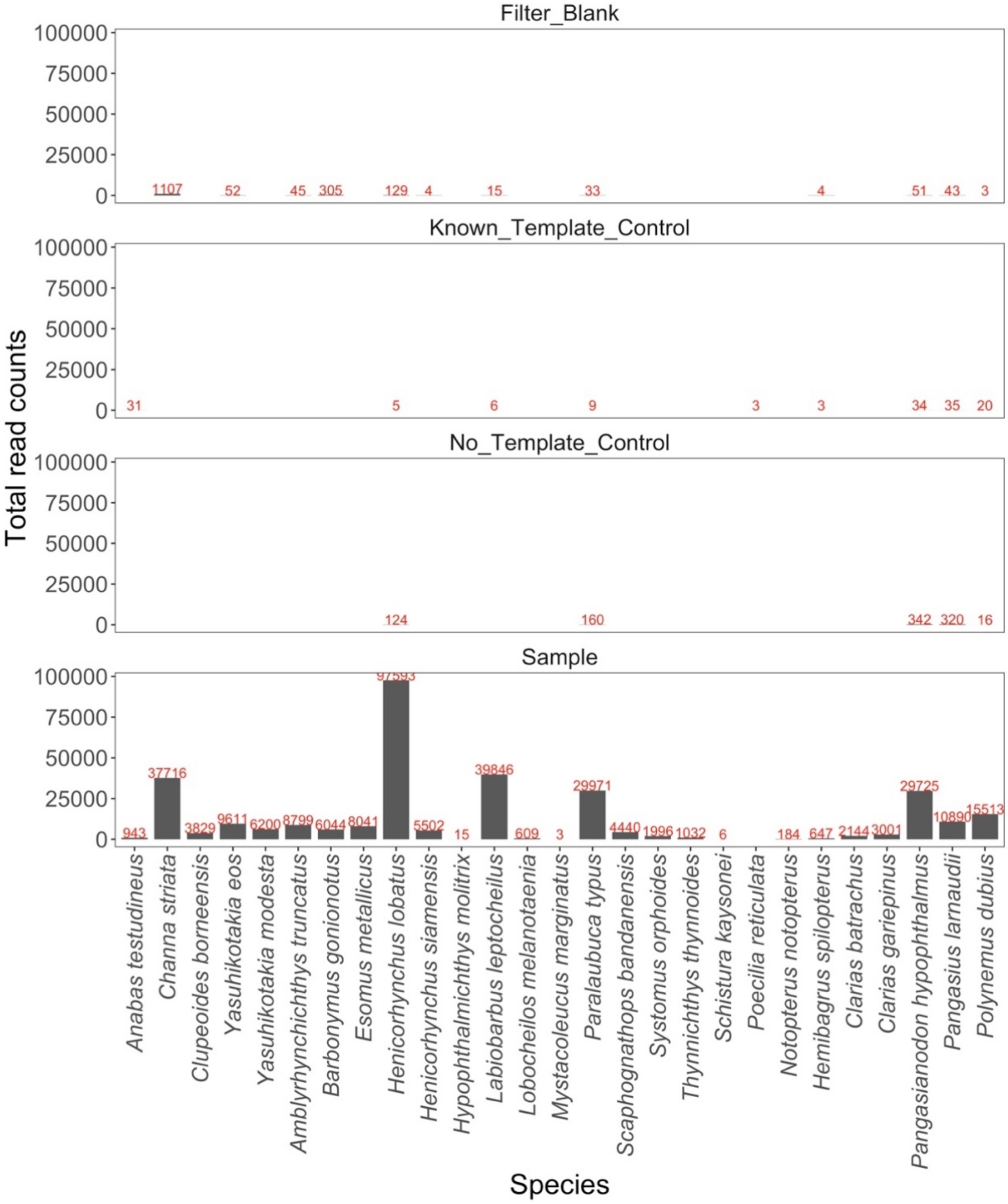


FIGURE 6.5 - INDIVIDUAL TARGET SPECIES' TOTAL READ COUNTS PRESENT IN THE CONTROLS (FILTER BLANK, KNOWN TEMPLATE CONTROL AND NO TEMPLATE CONTROL) AND TARGET SAMPLES (SAMPLE) TO ASSESS THE LEVEL OF CROSS-CONTAMINATION.

In order to understand the total read count distribution for all target species (excluding *Maylandia zebra*), in the controls and target samples, the individual species' total read counts were combined in Figure 6.6. Following the results mentioned above, it is possible to see a higher distribution of the total read counts of the target species in the target samples, compared to the same present in the controls. Likewise, considerably lower total read counts (<20,000) were present in the controls compared with the target samples (maximum of 60,000). This result further supports the evidence described in Figure 6.5 for the low level of contamination identified in this study.

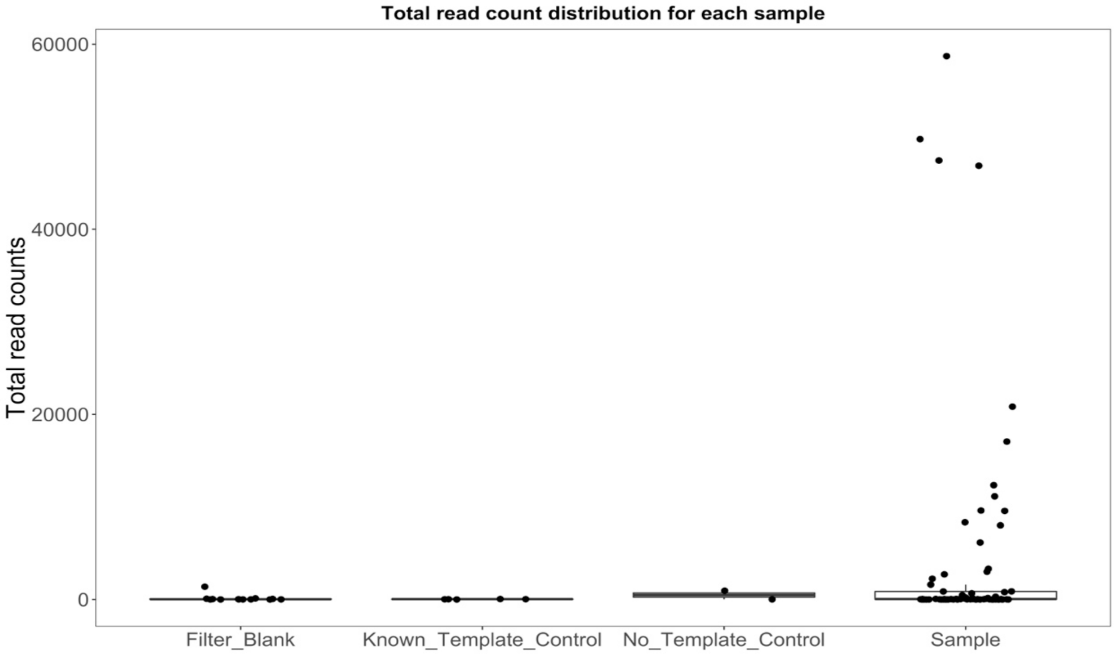


FIGURE 6.6 - TOTAL SPECIES' READ COUNT DISTRIBUTION FOR THE CONTROLS (I.E. FILTER BLANKS, KNOWN TEMPLATE CONTROL AND NO TEMPLATE CONTROL) AND TARGET SAMPLES.

6.3.5 PATTERNS OF EDNA IDENTIFIED SPECIES DIVERSITY: SEASONAL DIFFERENCES IN SPECIES ABUNDANCE AND COMPOSITION IN THE BASIN

The depth profile analysis for the species composition detected in surface and deep waters, in individual sites and across both seasons, did not show a clear distinctive pattern (see Appendix 8). This result is further explored in Figure 6.7, with the black and orange ellipses from the NMDS analysis not showing a clear discriminating pattern between the clustering of individual sites for the species composition detected in surface and deep water collected samples, respectively. Moreover, the ANOSIM analysis revealed non-statistically significant differences (R=0.006; *p*=0.377) in the depth profile analysis. Yet, a clear distinction between wet and dry seasons on the clustering of individual sampled sites where species composition was detected is visualised by the pink and blue ellipses, respectively. This result is further corroborated by the statistically significant differences detected between the two seasons from the ANOSIM analysis (R=0.292; *p*=0.001). Thus, for the downstream analyses only seasonal differences in the species composition found in individual sites will be assessed, with the read counts of the individual species detected in the depth profile analyses pooled, according to site and season.

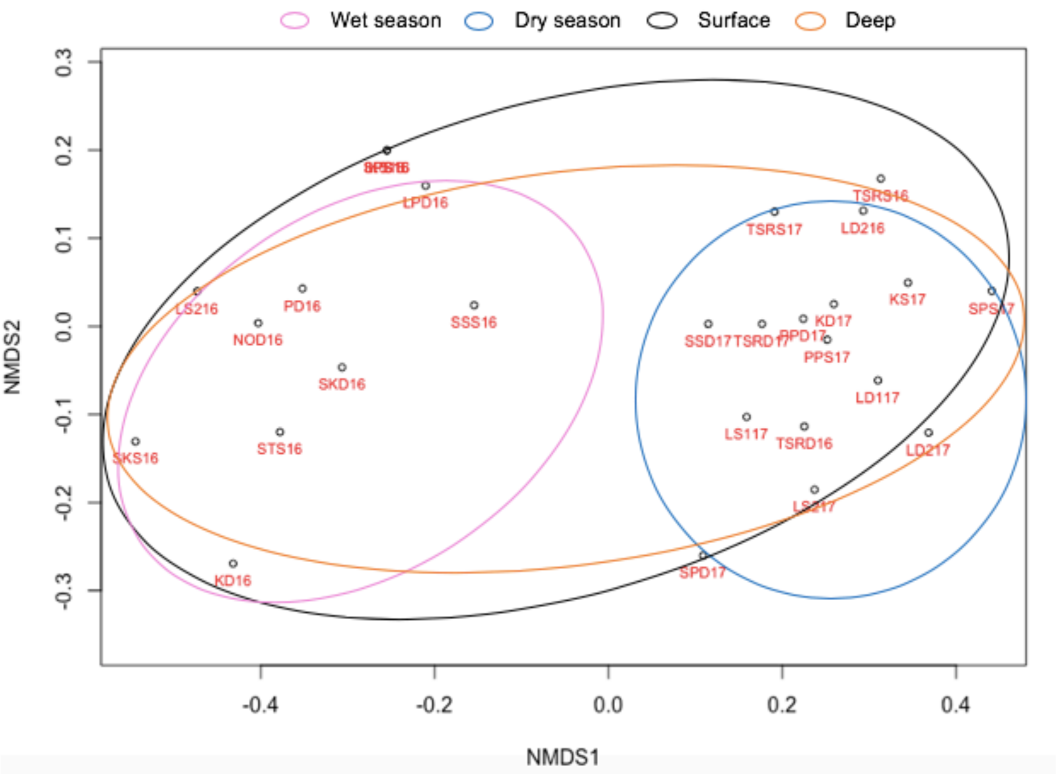


FIGURE 6.7 - NON-METRIC MULTIDIMENSIONAL SCALING (NMDS) TO IDENTIFY A CLUSTERING PATTERN ON INDIVIDUAL SITES WHERE SPECIES WERE DETECTED, ACCORDING TO SEASONS AND DEPTH PROFILE. ELLIPSES CORRESPOND TO THE SIMILARITY ANALYSIS (ANOSIM). RED LETTERS AND NUMBERS RELATE TO THE INITIALS OF EACH SITE, DEPTH PROFILE AND SEASON.

Figure 6.8 further summarises the seasonal differences in the species composition detected along the Mekong river, by assessing the relative abundance of individual species for all sampled sites, according to seasons (i.e. site occupancy analysis; see sub-section 6.2.6). The site occupancy analysis shows that in the dry season (grey bars; Figure 6.8), *Henicorhynchus lobatus, Labiobarbus leptocheilus, Paralaubuca typus, Pangasianodon hypophthalmus* and *Pangasius larnaudii*, were the species with the highest relative abundance detected in the basin. In the wet season (orange bars; Figure 6.8), *Anabas testudineus, Esomus metallicu*s and *Scaphognathops bandanensis* were the species exhibiting the highest relative abundance. Of note, in the wet season, the relative abundance for all detected species was lower compared to the relative abundance for all detected species in the dry season. On the other hand, non-statistically significant differences were detected in the site occupancy analysis for both seasons (χ^2=27.48; df=30; *p*=0.58).

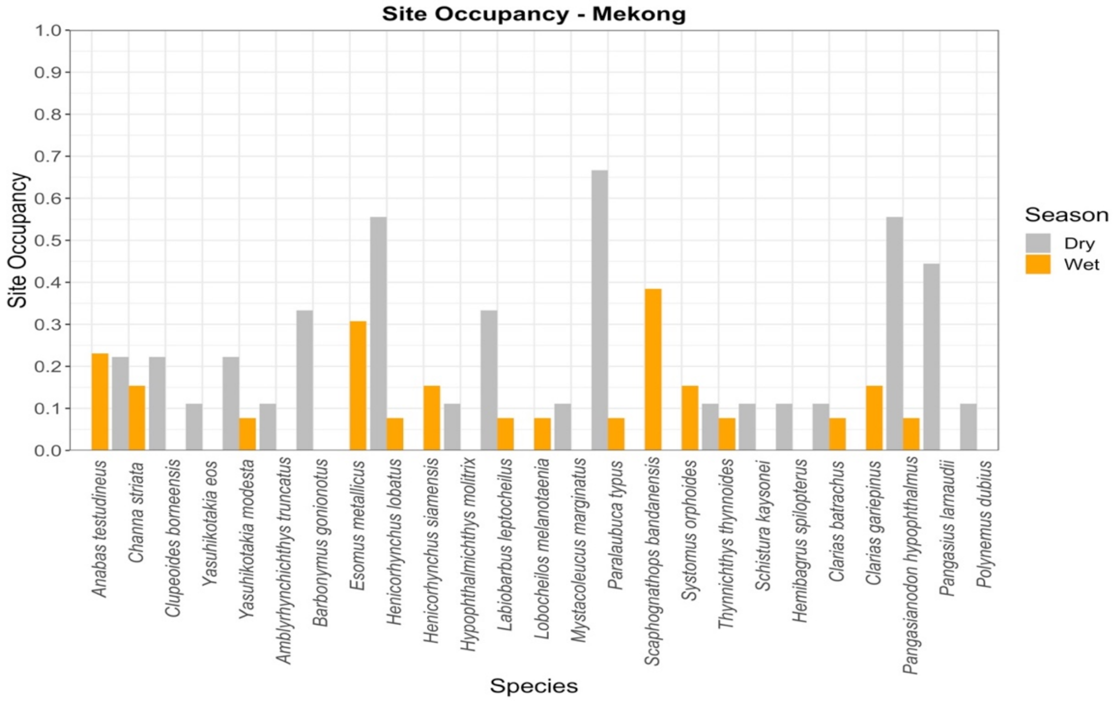


FIGURE 6.8 - SITE OCCUPANCY ANALYSIS FOR THE RELATIVE ABUNDANCE OF INDIVIDUAL SPECIES (I.E. PROPORTION OF READ COUNTS) IN THE BASIN, ACCORDING TO SEASONS.

Figure 6.9 shows important seasonality differences in the fish species detected in individual sites along the Mekong basin. Of note, a higher number of species were found in the dry season (average of 7 species per site) compared to the wet season (average of 3 species per site), following the seasonality differences identified in Figure 6.8 for the relative species abundance. Also, and for the dry season, the diversity on species composition was higher in lateral Cambodian sites (i.e. the 3S system and TSGL system – Tonle Lake Site 1 and 2 and Tonle River), with Kratie and Phnom Penh mainstream river, also exhibiting high diversity on the individual species composition detected (Figure 6.9). In the wet season, apart from the Tonle River, a balanced individual species composition (i.e. maximum of 3 species) was detected throughout the basin (Figure 6.9).

In Laotian sites (i.e. Luang Prabang, Nam Ou and Pakse), and therefore only in the wet season, *Anabas testudineus, Esomus metallicus* and *Scaphognathops bandanensis* were the only species detected, with *Anabas testudineus* comprising over half of the read counts for Nam Ou and Pakse sites. In the 3S system and for the wet season, *Esomus metallicus* was the only species detected in Se Prok and comprised over half of the read counts detected for Se San, with *Systomus orphoides* and *Scaphognathops bandanensis* being detected in Se Kong and Se San. These results detail the findings from Figure 6.8 for the highest relative abundance identified for *Anabas testudineus, Esomus metallicus* and

Scaphognathops bandanensis, in the wet season. In the dry season, *Yasuhikotakia eos* and *Barbonymus goniatus* were mostly detected in Se Prok and Se San, respectively, with these species being only detected in the dry season. In the Tonle Lake, the individual species composition detected between and within sites, regarding the two seasons, were considerably different, apart from *Pagasianodon hypophthalmus*, which was detected in both sites, although in different seasons. Interestingly, *Channa striata*, *Henicorhynchus lobatus*, *Labiobarbus leptocheilus, Paralaubuca typus*, *Pangasius larnaudii* and *Pagasianodon hypophthalmus*, although with differences in the individual species composition in some sites, had the highest identified individual species composition in Kratie, TSGL system and Phnom Penh, in the dry season. The Chi-square analysis showed statistically significant differences in the species composition detected between seasons (χ^2=150080; df=11; p=2.2x10^{-16}).

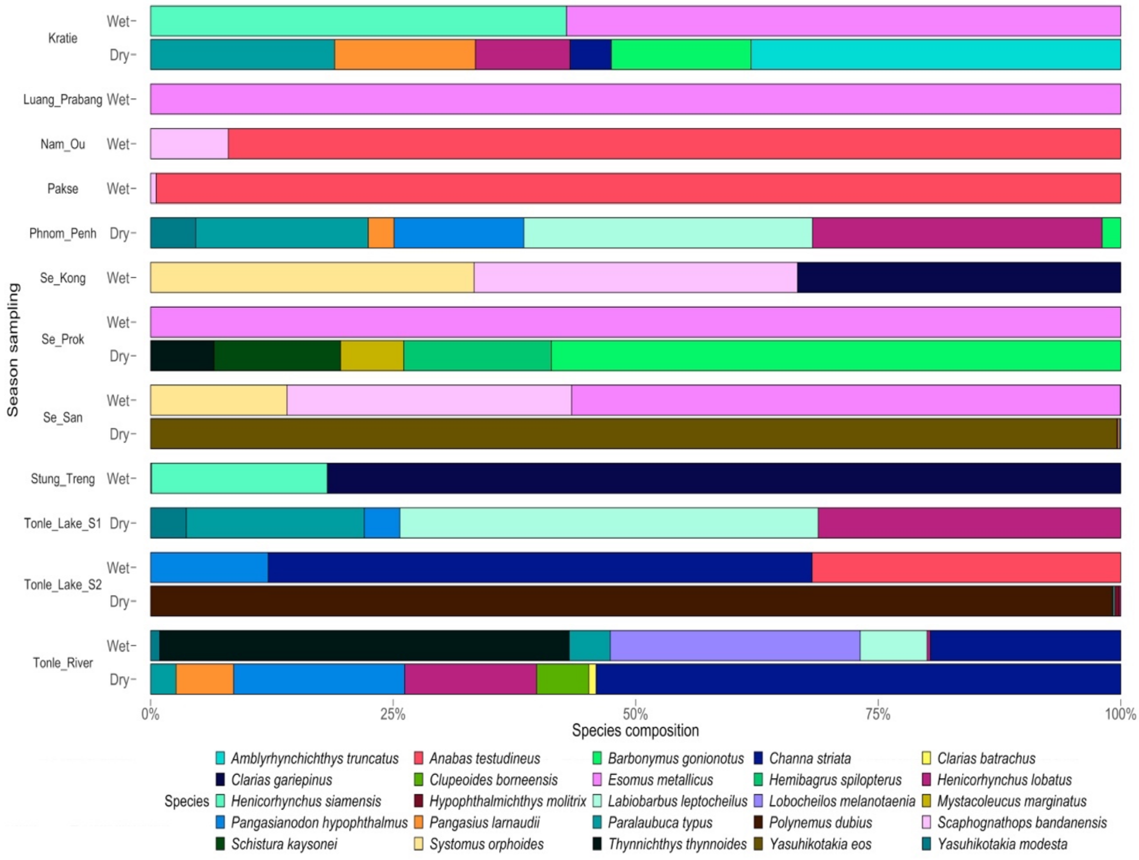


FIGURE 6.9 - SPECIES COMPOSITION (I.E. FOR THE INDIVIDUAL SPECIES' READ COUNTS) DETECTED IN INDIVIDUAL SITES, ACCORDING TO SEASONS.

6.3.6 PATTERNS OF SPATIAL SPECIES DISTRIBUTION AND GUILD ANALYSIS: SEASONAL MIGRATORY BEHAVIOUR AND HABITATS EXPLORED

The identified differences in the seasonal species composition detected in individual sites and the presence of individual species in more than one site (Figure 6.9), show potential patterns of species spatial distribution. Figure 6.10 and Table 6.2 for the wet season and Figure 6.10 and Table 6.3 for the dry season, assess the potential seasonal pattern of spatial distribution for the individual species detected, to characterise the migratory and non-migratory behaviour (i.e. guild analysis) along sites, for the habitats explored.

In the Laotian sites, *Esomus metallicus*, *Anabas testudineus* and *Scaphognathops bandanensis* which are resident, exhibit no migratory behaviour and exhibit medium distance migratory behaviour, respectively (guilds 5, 6 and 4, respectively; Figure 6.10) were the only species detected in both mainstream river and tributary. Furthermore, *Esomus metallicus* was further found in Kratie, Se San and Se Prok in the wet season, in the mainstream and 3S system tributaries, respectively. *Scaphognathops bandanensis*, a greyfish (guild 4; Figure 6.10) was further found in the 3S system (i.e. Se Kong and Se San) and connected Stung Treng's mainstream river in the wet season, potentially demonstrating a spatial migratory distribution from the mainstream river to the floodplain areas of the 3S system. In the wet season, and for the TSGL system, *Channa striata* (guild 6) a no migratory blackfish, *Thynnichthys thynnoides* (guild 5) a resident species and *Henycorhynchus lobatus* and *Pagasianodon hypophthalmus* (guild 3) exhibiting long distance migratory behaviour, showed the highest species composition, with the lake and tributary habitats supporting, therefore, important migratory and no migratory species (Figure 6.10). Therefore, from the guild composition detected in individual sites (Figure 6.10) and the resulting guild analysis of Table 6.2, it is possible to see that in the wet season, 66.4% of the detected species in the basin exhibited no migratory behaviour (i.e. guilds 5 and 6), while 33.6% of the detected species exhibited medium to long distance migratory behaviour (i.e. guilds 4 and 3, respectively).

Regarding the spatial distribution and consequent migratory behaviour of the detected species in the dry season, from Figure 6.10, and as previously mentioned, it is possible to see that *Channa striata*, *Henicorhynchus lobatus*, *Labiobarbus leptocheilus, Paralaubuca typus*, *Pangasius larnaudii* and *Pagasianodon hypophthalmus*, although with differences in the species composition detected in individual sites, were the most detected species in Kratie, TSGL system and Phnom Penh. Of note, apart from *Channa striata*, these species are white and greyfishes (guilds 3 and 4, respectively), exhibiting medium to long distance migratory behaviour. Consequently, the distribution of this species in both mainstream river (Kratie and Phnom Penh) and lateral habitats (TSGL system), potentially indicate the seasonal migratory movements of these species from the lateral habitats to the mainstream river. Thus, the analysis of Table 6.3 show that 81.2% of the detected species in the dry season comprised medium to long distance migratory species (i.e. guilds 4 and 3, respectively), while blackfish (with the mainly contribution of *Channa striata* for the highest individual read counts; Table 6.3) comprising nearly 16%. Importantly, for both wet and dry seasons, the lateral habitats (i.e. lake and tributaries) had the highest detected species composition, exhibiting different migratory behaviour, while the mainstream river, although supporting species exhibiting no migratory behaviour (e.g. in the mainstream river of the Laotian sites), showed the presence of medium to long distance migratory species detected in other sites, reflecting a spatial distribution pattern. Statistically significant differences (χ^2=115600; df=6; *p*=2.2x10^{-16}) were detected regarding the guild composition between seasons.

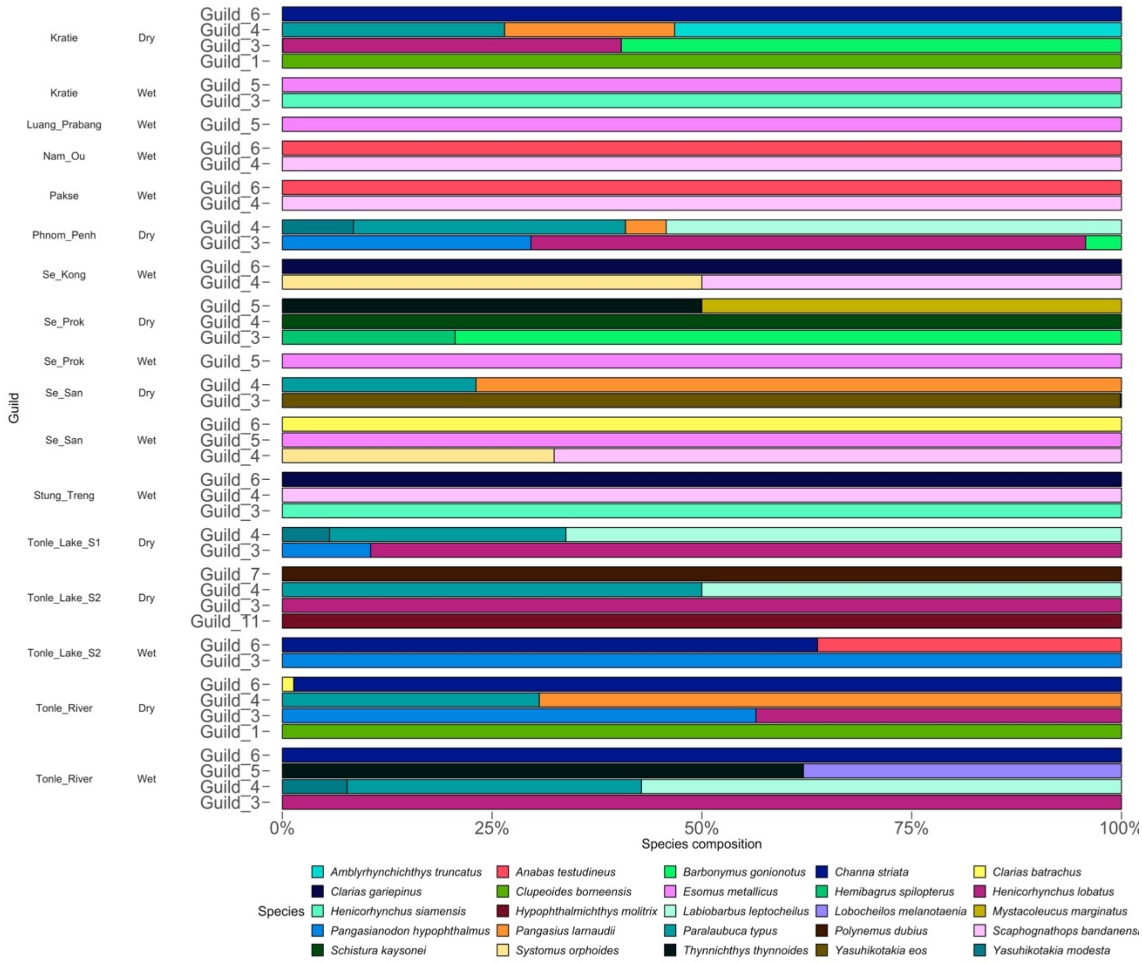


FIGURE 6.10 - SPECIES COMPOSITION, REGARDING THE GUILDS BELONGED, IDENTIFIED IN INDIVIDUAL SITES, ACCORDING TO SEASONS.

TABLE 6.2 - PERCENTAGE (%) OF INDIVIDUAL GUILDS FOUND IN THE WET SEASON.

WET SEASON			
GUILD	**Species**	**Total Read Count**	**Percentage (%)**
3	*Henicorhynchus siamensis*	667	3.22
	Pangasianodon hypophthalmus	8	
	Henicorhynchus lobatus	8	
4	*Scaphognathops bandanensis*	4168	30.37
	Systomus orphoides	1993	
	Yasuhikotakia modesta	22	
	Labiobarbus leptocheilus	163	
	Paralaubuca typus	100	
5	*Esomus metallicus*	8041	45.45
	Lobocheilos melanotaenia	609	
	Thynnichthys thynnoides	998	
6	*Anabas testudineus*	943	20.97
	Clarias gariepinus	3001	
	Clarias batrachus	6	
	Channa striata	501	
TOTAL		**21,228**	

TABLE 6.3 - PERCENTAGE (%) OF INDIVIDUAL GUILDS FOUND IN THE DRY SEASON.

DRY SEASON			
GUILD	**Species**	**Total Read Count**	**Percentage (%)**
1	*Clupeoides borneensis*	3572	1.52
3	*Barbonymus gonionotus*	5906	40.77
	Hemibagrus spilopterus	7	
	Yasuhikotakia eos	9568	
	Pangasianodon hypophthalmus	29491	
	Henicorhynchus lobatus	50970	
4	*Schistura kaysonei*	6	40.43
	Paralaubuca typus	29764	
	Pangasius larnaudii	10842	
	Amblyrhynchichthys truncatus	8799	
	Yasuhikotakia modesta	6171	
	Labiobarbus leptocheilus	39564	
5	*Mystacoleucus marginatus*	3	0.003
	Thynnichthys thynnoides	3	
6	*Channa striata*	36873	15.88
	Clarias batrachus	493	
7	*Polynemus dubius*	3291	1.40
11	*Hypophthalmichthys molitrix*	15	0.006
TOTAL		**235,338**	

6.4 DISCUSSION

This study produced a range of interesting results and highlighted the potential of eDNA to explore distribution and abundance of fish in large tropical river basins. However, the technique was hampered by a number of limitations, which are believed to have directly influenced the low species detection. The identified limitations will be referred herein, whilst discussing the results, for the potential improvements that can be made and further modifications in the applied methodology that can be employed in the future.

6.4.1 DNA DEGRADATION AND THE NEEDS TO OBTAIN A MEANINGFUL AND REPRESENTATIVE SAMPLE IN THIS COMPLEX ENVIRONMENT

The DNA present in environmental samples is generally highly degraded (less than 150 bp; Dejean *et al.,* 2011; Herder *et al.,* 2014; Taberlet *et al*., 2018) and influenced by biotic and abiotic factors (see Chapter 2; Taberlet *et al.,* 2012;2018). However, the DNA concentration obtained in this study was considerably lower (see the analysis in sub-section 6.3.2) compared to other fish metabarcoding studies (e.g. Spens *et al.,* 2016 and Sellers *et al.,* 2018). This indicates that DNA degradation has happened after collection. The DNA captured and preservation methodology applied in this study followed Li *et al.,* (2018) study for the use of 0.45 μm paper filters and Spens *et al.,* (2016) for the preservation of the filtered samples in RNAlater buffer solution, with the study reporting the benefits of using RNAlater buffer solution for reducing the DNAse activity and thus reducing DNA degradation. Accordingly, the DNA captured in filter papers and preserved in the buffer solution, can be detected for nearly one-month storage at 4ºC, without significant decrease in DNA concentration. Yet, in the current study, the filter papers preserved in RNAlater buffer solution were firstly stored in 4ºC during the fieldwork and then for 4 months at the same temperature, after travelling back from overseas to the cold store facility at the University. Moreover, during the travelling period (over 24 hours), the samples were stored without refrigeration due to logistic constrains. Consequently, it is likely that DNA degradation occurred as a direct consequence of the storage conditions, both during overseas transportation, but particularly at the University facility, for the storage temperature and duration, which are expected to have resulted in the observed low DNA concentration obtained and consequent low species detection.

In a complex environment as the Mekong basin, it is expected that the annual high temperature (i.e. annual average of 27 ºC; see Chapter 3), pronounced seasonal variations in the flood regime, large catchment size and diversity of fish species (Rainboth *et al.,* 1996; Campbell *et al.,* 2006;2009; Lamberts, 2006;2008), with unknown amount of DNA released, affect the DNA persistence and dispersion in the water and consequent species detection. Bellemain *et al.,* (2016) on their first and only available eDNA study in the Mekong river, for detecting the distribution of the endangered Mekong giant catfish (*Pangasianodon gigas*), argued the likely role of the river's seasonal flood regime, high temperature and sediment content in influencing the low detectability of the target species and low persistence of DNA in the waters. Likewise, studies have discussed the importance of performing replications of the collected water samples within the surveyed sites and increase the area surveyed (e.g. by performing transects), to obtain a meaningful and representative sample, capable of effectively detect the fish species community or target species inhabiting a given area (Taberlet *et al.,* 2012;2018; Evans *et al.,* 2016; Hänfling *et al.,* 2016; Bista *et al.,* 2017; Deiner *et al.,* 2017; Handley *et al*., 2018). On the other hand, the higher sediment content along the basin (Lu and Siew, 2006), constrains on the available equipment (e.g. access to a single pump-filtration equipment) and lack of access to a laboratory facility close to the field site, need to be taken into account for the potential increase in the filtration time, if higher number of samples are collected (i.e. by performing replications of the collected water and increase the surveyed area). These factors could potentiate DNA degradation, affecting species detection (Bellemain *et al.,* 2016; Yamanaka *et al.,* 2016b; Li *et al.,* 2018).

Therefore, in the present study, the lack of performing replications of the collected water samples to obtain a meaningful and representative sample of the Mekong river's complex environment and DNA degradation associated to the transportation conditions (i.e. for the lack of cold conditions and potential breakdown of the DNA molecules by the movement of the falcon tubes with the preserved DNA material, since no proper storage containers were used due to logistic constrains; Dejean *et al.,* 2011; Herder *et al.,* 2014; Yamanaka *et al.,* 2016b; Taberlet *et al.,* 2018) and the longer storage period in 4ºC than recommended by the eDNA literature (e.g. Deiner *et al.,* 2015; Eichmiller *et al.,* 2016b; Spens *et al.,* 2016; Yamanaka *et al.,* 2016b), provide important understanding for the reasons behind the low DNA concentration obtained in both pre-treatment analysis and further DNA extractions, and consequent downstream low species detection (see later sections). Additionally, the higher sediment content present in the filter papers could indicate a reduced capacity of the DNA extraction methodology applied in effectively isolating the DNA molecules from the sediments. Further improvements in the study design should then perform replications of water samples within sites (although recognising the potential limitations regarding fieldwork conditions), perform transects for collecting water (i.e. decrease the distance between sites), effectively store and transport the samples (i.e. filtered samples should be kept in ice or 4 ºC during transportation and properly stored to avoid the movement of the tubes and consequent breakdown of DNA molecules; Herder *et al.,* 2014) and filter samples should be stored immediately in -20 ºC (if DNA extraction cannot be performed promptly), instead of stored at 4ºC for 4 months.

6.4.2 CROSS-CONTAMINATION AND RELIABILITY OF DOWNSTREAM INFERENCES

The number of processes involved during the eDNA workflow (i.e. from sampling to bioinformatic analyses; see Chapter 2; Taberlet *et al.,* 2012;2018; Herder *et al.,* 2014; Takahara *et al.,* 2015; Hoffmann *et al.,* 2016), although with rigorous quality procedures for fieldwork sampling and laboratory requirements in place (e.g. use and exchange of gloves between sites, bleach and UV sterilization of laboratory, equipment and materials), to avoid potential sources of cross-contamination between samples, make it unlikely to 100% accomplish that. Therefore, for assessing the levels of cross-contamination, methodologic approaches on eDNA studies involve the application of controls in the early stages of the eDNA workflow (Taberlet *et al.,* 2012;2018; Herder *et al.,* 2014; Hänfling *et al.,* 2016; Li *et al.,* 2018;2019). The detection of target species' DNA in the controls can be associated to errors made during the sampling procedure (e.g. DNA present in gloves, failure to effectively sterilise the bottles and equipment used to collect water, cross-contamination by placing the bottles containing purified water next to bottles containing the sampled water, spilling, among other factors). Also, during DNA capture, extraction and PCR amplification, failure to exchange gloves, spill of small drops to the gloves when dropping the water in the filtration equipment, DNA transfer to the materials used to collect the filter papers after filtration, failure to sterilise the filtration equipment and laboratory bench, pipetting mistakes and cross-contamination during the cycling conditions, are all sources of potential cross-contamination (Taberlet *et al.,* 2012;2018; Herder *et al.,* 2014; Ficetola *et al.,* 2015; McKee *et al.,* 2015; Roussel *et al.,* 2015; Cristescu and Hebert, 2018).

In this study, the target species DNA was detected in each of the controls applied (Figure 6.5 and Figure 6.6). Yet, when compared the total read counts (Figure 6.5) and distribution of the read counts (Figure 6.6) of the target species DNA detected in the controls, to the target species DNA in the target samples, the differences on total read counts are remarkably low in the controls compared to the same detected in the target samples. In Hänfling *et al.,* (2016) study, the same was observed, with the authors stating that the levels of contamination detected did not influenced the downstream analyses on the species detected. Accordingly, the same principle was applied in this study. Nevertheless, it is important to understand the potential sources of cross-contamination and apply further modifications in the study design to minimise its presence in future studies. Therefore, in challenging field conditions as encountered in the Mekong river, where the access to a laboratory facility was a major constrain when conducting fieldwork, which most likely compromised the effectiveness of the sterilisation process, and may have contributed to the cross-contamination detected, it is vital to reduce the level of handling and sterilisation steps performed. Consequently, a modification in the applied methodology would consist in using Sterivex encapsulate filters, following the DNA capture and extraction procedure described in Spens *et al.,* (2016). Sterivex filters allow the capture and DNA extraction inside the capsule, reducing the needs of handling the filter and exposing it to open air, whilst reducing the number of sterilisation procedures to the filtration equipment and materials required. Moreover, both Spens *et al.,* (2016) and Li *et al.,* (2018) have assessed and compared the DNA capture yields obtained in this filter compared to other filter papers (including the one used in this study), arguing the ability of Sterivex in capturing high DNA concentrations. Also, Sterivex filters have been demonstrated to be an ideal option when filtering on-site and in challenging environments (Li *et al.,* 2018). As so, Sterivex filters are an effective approach to increase the DNA yields, can be preserved in

RNAlater buffer solution and offer the advantage of minimising cross-contamination, reducing potential sources of bias on species detection.

6.4.3 PATTERNS OF EDNA SPECIES DIVERSITY AND SPATIAL DISTRIBUTION: MIGRATORY BEHAVIOUR, HABITATS AND ROLE OF THE FLOOD REGIME

After the bioinformatic analyses, 25 Mekong fish species were identified (Table 6.1). Although representing less than 4% of the total described Mekong fish richness, the diversity of families found and body-size, reveal the outstanding diversity of species inhabiting this large tropical transboundary river system.

As extensively covered throughout this thesis and revised in the Introduction section of this chapter, the Mekong river is under the influence of the long-term predictable Asian monsoon climate, delimiting a wet and a dry season (Adamson *et al.,* 2009; Delgado *et al.,* 2012). The monsoon climate drives the seasonal variations in the flood regime, affecting the available and connected habitats that are argued to influence the life cycles and migratory behaviour of the fish diversity inhabiting the basin (see Chapter 3; Adamson *et al.,* 2009; Valbo-Jorgensen *et al.,* 2009; Baran, 2010; Delgado *et al.,* 2012). Thus, the identified seasonal differences in the clustering of sites where species were detected (Figure 6.7), relative species abundance (Figure 6.8) and in the individual species composition in individual sites (Figure 6.9), are likely to reflect the influence of the seasonal variations in the river's flood regime in driving the patterns of species diversity detected along the basin, (Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009; Halls, 2010). Whilst it is reported, in the available literature, that in the wet season over 50% of the Mekong fish diversity are found in the mainstream river and tributaries, performing medium to long distance (guilds 4 and 3) migrations to floodplain areas in tributaries and lake habitats, the diversity of species detected (i.e. for the number of species), species composition in individual sites, species detected in more than one site and the guilds belonged (Figure 6.9, Figure 6.10 and Table 6.2), showed a different pattern than the reported in the literature (Rainboth, 1996; Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009; Halls, 2010).

The diversity and abundance (Figure 6.8) of species detected in the wet season were comparable low (average of 3 species per site) than in the dry season (average of 7 species per site), although a higher number of sampled sites were performed in the wet season (n=13) compared to the dry season (n=9). These results might reflect the higher dilution and dispersion of the species eDNA in the wet season, driven by the increase in the flow conditions and flood levels along the basin (Adamson *et al.,* 2009; Bellemain *et al.,* 2016). Noteworthy, the results show the importance of performing replications of water samples and employ a transect approach to collect water in closer areas, which in the wet season would potentially have been able to capture the higher eDNA dispersion and dilution, driven by the hydrologic conditions (Herder *et al.,* 2014; Bellemain *et al.,* 2016; Taberlet *et al.,* 2018). Also, the observed DNA degradation was discussed to have influenced the low species detection (i.e. individual species' read counts present in individual sites; see sub-section 6.4.1), which potentially reflected the lower detection of species exhibiting migratory behaviour in the wet season.

Important differences were present in the species detected in upstream sites (i.e. the Laotian sites), compared to downstream sites (i.e. Cambodian sites). In upstream sites, and therefore only in the wet season, *Anabas testudineus, Esomus metallicus* and *Scaphognathops bandanensis* were the only species detected (Figure 6.9), showing the highest species relative abundance in the wet season for the entire basin (Figure 6.8) and exhibiting no migratory behaviour (guild 6), resident species (guild 5) and medium distance migratory behaviour (guild 4), respectively (Figure 6.10). Importantly, the fact that these species were both found in the mainstream river and tributaries, and attending to the migratory behaviour, shows key adaptive strategies for these species to the higher flood levels, increase in habitat inundation and consequent recycling of nutrients seen in the wet season (Junk *et al.,* 1989; Lamberts, 2006;2008), with the eco-hydrologic interactions occurring between the riparian floodplain habitats and the river, likely to trigger these species to occupy the recently flooded habitats, for the higher food resources available, spawning grounds and shelter option (Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009). Yet, and attending to the low migratory behaviour (i.e. guilds 5, 6 and 4), the fact that these species were observed in more than one site, are likely to reflect different populations of the same species, rather than a spatial migratory distribution.

Further downstream, *Scaphognathops bandanensis* (guild 4) was detected in the 3S system and the connected Stung Treng's mainstream river, which given the medium distance migratory behaviour described for this species (Poulsen *et al.,* 2002;2004; Halls, 2010), it is likely that the higher flood level and consequent eco-hydrologic interactions established in the lateral floodplain habitats of the 3S system, triggered this species to migrate from the mainstream river to the lateral tributaries (Baran, 2006,2010; Halls, 2010). Consequently, the spatial distribution of this medium distance migratory species demonstrates the key role of the system's seasonal flood regime in triggering the migratory behaviour, linked to the consequent eco-hydrologic interactions established in the lateral flooded habitats that fishes rely on for spawning, feeding and sheltering purpose (Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009; Halls, 2010). Overall, and from the results achieved in Figure 6.10 and Table 6.2 for the wet season, the individual species composition was higher in tributaries and lake habitats, with the mainstream river, being an important migratory pathway. Importantly, *Esomus metallicus* and *Thynnichthys thynnoides* (resident species-guild 5), *Scaphognathops bandanensis* and *Systomus orphoides* (greyfish species-guild 4) and *Anabas testudineus* and *Clarias gariepinus* (blackfish species-guild 6) were the species with higher total read counts and contributing the most to the guilds belonged. Additionally, the fact that these species, although with differences between sites, were detected throughout the basin, and attending to the guilds belonged for the migratory behaviour, show that different populations of the same species were detected from upstream Laotian sites to downstream Cambodian sites, rather than a spatial distribution (i.e. migratory dynamics) of the individual species throughout the basin. Table 6.2 further demonstrates the higher detection of species exhibiting no migratory behaviour (guilds 5 and 6) in the wet season, for the nearly 67% of residents and no migratory species composition detected, compared to the 33% of detected species exhibiting medium to long distance migratory behaviour. Hence, the low eDNA detection of migratory species in the wet season, regarding literature knowledge, should be careful interpreted for false negative, rather than a pattern of higher incidence of residents and species exhibiting no migratory behaviour in the wet season (Halls, 2010; Herder *et al.,* 2014; Ficetola *et al.,* 2016).

In the dry season, key differences were identified in the species composition compared to the wet season (Figure 6.9). Interestingly, *Pangasius larnaudii, Paralaubuca typus, Henicorhynchus lobatus, Pangadianodon hypophthalmus, Yasuhikotakia eos* and *Channa striata,* found in more than one site and with the highest detected relative species abundance (Figure 6.8) for all species apart from *Yasuhikotakia eos* and *Channa striata*, are species known to perform medium to long distance migrations (guilds 4 and 3, respectively; Figure 6.10) along the mainstream river, tributaries and lake (apart from *Channa striata*, which is a blackfish; Rainboth, 1996; Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009; Halls, 2010). Likewise, these species were detected in geographically closed sites, with Kratie being the mainstream habitat upstream the TSGL system and Phnom Penh the mainstream habitat downstream the system (Figure 6.1). Thus, a potential spatial distribution, related to a migratory behaviour, can be inferred for these individual species, where in the dry season, these species leave the Tonle Sap Lake, where they stayed during the wet season to feed, spawn and find shelter, and migrate upstream to Kratie mainstream river, through the connected Tonle River or migrate downstream to Phnom Penh mainstream river. Of note, this migratory behaviour out of the TSGL system in the dry season, are likely to be related to the decrease in the flood level, decrease in available inundated habitats and reduced food sources, with species exhibiting no migratory behaviour (i.e. *Channa striata*) showing key adaptive strategies to the reduced flood levels and habitat availability, mainly occupying the permanent lake and river tributary (Figure 6.10; Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Campbell *et al.,* 2006;2009; Valbo-Jorgensen *et al.,* 2009). Also, the decrease in the flood level and habitat flood dynamics in the dry season, have been argued to trigger the distribution of medium to long distance migratory species (guilds 4 and 3, respectively) to areas of higher flood level (e.g. deep pools), in the mainstream river (Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009), with the eDNA detection of these species in Kratie and Phnom Penh mainstream river corroborating this view for a spatial distribution, reflecting a migratory behaviour. Importantly, although a spatial distribution linked to a migratory behaviour can be discussed for these species, it is also possible that the species detected in both Kratie and Phnom Penh mainstream river could reflect different populations of the same species, which were present in the 3S system tributaries during the wet season and in Phnom Penh year-round, and perform migrations to the deep pools located in Kratie (Poulsen and Valbo-Jorgensen, 2000; Poulsen *et al.,* 2002;2004; Baran and Ratner, 2007; Baran, 2010).

Therefore, although eDNA provides important information regarding the potential migratory route for these Mekong fish species, it is vital to understand the ecology of the species detected and the factors influencing the spatial eDNA dispersion and interactions with the river system to effectively describe patterns of species distribution (Schmidt *et al.,* 2013; Ficetola *et al.,* 2015; Civade *et al.,* 2016; Schmelzle and Kinzinger, 2016; Taberlet *et al.,* 2018). Although the spatial dynamics of eDNA and the factors affecting the dispersion and interaction with the Mekong river remain largely unassessed (Bellemain *et al.,* 2016), the locations where these species were detected are in accordance to the reported in the available literature regarding the seasonal migratory distribution of these species, driven by the flood regime and recently flooded habitats (Rainboth, 1996; Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009; Halls, 2010). Hence, the eDNA metabarcoding analyses corroborated the available literature for the identification of patterns of spatial distribution in the fish communities of the Mekong river, helping to characterise the migratory behaviour of the detected species in the dry season, linked to the flood regime conditions for the available habitats. These species (apart from *Channa striata*) contributed to the 81% of guilds 3 and 4 composition present in the dry season (Table 6.3), with tributaries and lake habitats exhibiting higher species composition and the mainstream river being an important pathway for the spatial fish distribution.

In Chapter 4, the historical significance of the predictable seasonal variations in the flood pulse of the TSGL system in influencing the species composition of the *dai* fishery that develop in the dry season (Lamberts, 2006;2008; Campbel *et al.,* 2009; Kummu *et al.,* 2014), revealed that large body species like the eDNA detected *Pangasius larnaudii* and *Pangadianodon hypophthalmus* are significantly influenced by variations in the timing, duration and extent of floodplain flooding, performing migrations out of the TSGL system in the onset of the dry season and being caught in the *dai* nets placed in the Tonle River. In the wet season, these species seek the recently inundated floodplains to feed, spawn and find shelter (Poulsen *et al.,* 2002;2004; Baran, 2006:2010; Halls, 2010). A similar pattern was identified in Chapter 4 for the eDNA detected medium to long distance migratory small body-size species (i.e. *Paralaubuca typus, Yasuhikotakia modesta*, *Labiobarbus leptocheilus* and *Henicorhynchus* sp. - *H. siamensis* and *H. lobatus*; Table 6.3), with these species inhabiting the inundated floodplains during the wet season and leaving the lake in the onset of the dry season, being caught in the *dai* fishery. These dry season eDNA detected medium to long distance migratory species contributed in nearly 40% to the *dai* fishery catch and to the highest fish protein consumption rate in the region and entire Southeast Asia (see Chapter 4; Hortle, 2007;2009; So, 2010; Halls *et al.,* 2013a). Accordingly, the eDNA metabarcoding approach evidenced the reliability of the tool in detecting fishery resources, key to over 1.2 million people for fish protein intake (Hortle, 2007;2009). Additionally, it evidenced the importance of preserving the historical flood pulse dynamics of the TSGL system for the migratory fish species supported, by detecting the seasonal distribution in lateral flooded habitats, showing the reliability of the tool for monitoring the Mekong river's fish communities, while identifying the dispersion dynamics.

6.4.4 THE THREATS TO THE eDNA DETECTED FISH SPECIES BY ENVIRONMENTAL CHANGE PROJECTIONS IN THE MEKONG BASIN

The findings in Chapter 5 evidenced that the combined action of hydropower, irrigation and climate change scenarios will profoundly disrupt the flood pulse dynamics and floodplain inundation profile, resulting in a projected 17% decrease in the *dai* fishery catch, 11% decrease in medium to long distance migratory fish species contribution to the catch and an alarming 17% decrease in fish protein provision to the future populations. Of note, disruptions on the migratory triggers by the modifications in the timing, duration and extent of the floodplain flood dynamics and flood level in the TSGL system, were argued to significantly impact upon the fish abundance and migratory behaviour. Also, the barrier effect from hydropower development and the historical timing of the *dai* fishery were related to the projected losses in catch, species contribution and fish protein availability (see Chapter 5). Accordingly, the five eDNA species detected in the TSGL system in the dry season, exhibited medium to long distance migratory behaviour and are known to contribute nearly 40% to the *dai* fishery, with these species being also detected along the mainstream river and tributaries. The eDNA metabarcoding analyses, by detecting in near real-time the dry season spatial distribution of these species along the basin and the habitats explored, connected to the planned location of the Lower Mekong mainstream dams (i.e. Xayaboury and Don Sahong in Lao PDR and Sambor in Cambodia upstream the TSGL system; see Figure 7.1 of Chapter 7; Baran and Ratner, 2007; Baird, 2011; Baran *et al.,* 2011; Sithirith, 2016; Hecht *et al.,* 2019) evidenced that severe migratory barrier is expected to happen, threatening the abundance and migratory dynamics of these species. These disruptions will likely magnify the decrease in the contributing role of the eDNA detected medium to long distance migratory species to the *dai* fishery in the TSGL system, magnifying food security issues in a region marked by severe malnourishment and where people rely heavily on the fishery resources for the highest fish protein consumption (see Chapter 4; Hortle 2009; So, 2010). Hence, the eDNA metabarcoding analyses evidenced the needs to prioritise environmental conservation actions in the basin, in light of the escalating development of water infrastructures under changing climate, for maintaining the mainstream river migratory pathway and the seasonality of the flood dynamics, for the influence on the lateral habitats flooding dynamics, key to the identified eDNA species abundance and spatial distribution, then reflected in the inland fishery and for the contribution role to the fish protein provision to people for food security (Hortle, 2009; So, 2010; Halls *et al.,* 2013a; DHI, 2015).

6.5 CONCLUSIONS

This study, despite the identified limitations in low DNA concentration obtained and presence of cross-contamination, demonstrated the potential of applying eDNA metabarcoding to detect the Mekong river fish communities. Moreover, the analyses made identified critical differences in the patterns of seasonal spatial distribution for the habitats explored of the eDNA detected species, demonstrating the importance of preserving the flood regime for the eco-hydrologic interactions established that drive species' migratory behaviour. Hence, the patterns of eDNA identified species diversity and seasonal spatial distribution detected higher species diversity inhabiting downstream sites (i.e. Cambodian sites) and higher relative abundance in the dry season. The species composition in the wet season comprised nearly 67% of resident species (guild 5) and species exhibiting no migratory behaviour (guild 6), with nearly 34% of the identified species performing medium to long distance migrations (guild 4 and 3). In the dry season, 81% of the species detected, mainly present in tributaries and lake habitats, exhibited medium to long distance migratory behaviour (guilds 4 and 3, respectively). Additionally, and from the site occupancy analysis, *Pangasius larnaudii, Paralaubuca typus, Henicorhynchus lobatus, Labiobarbus leptocheilus* and *Pangadianodon hypophthalmus* exhibited the highest species abundance in the dry season, with a potential migratory behaviour identified in Kratie, TSGL system and Phnom Penh and linked to the seasonal flood regime.

Of note, these species contribute nearly 40% to the *dai* fishery in Cambodia, with eDNA opening a window of opportunity for detecting fishery resources, vital for food security to over 1.2 million people inhabiting the TSGL system. Finally, the eDNA metabarcoding analyses, by identifying the potential patterns of species composition and spatial distribution along the basin, connected to the projected decreases in *dai* fishery resources by the combined action of the multiple drivers of environmental change in the Mekong river, demonstrated the alarming future disruptions on the eDNA identified species migratory triggers, pathway and suitable habitats, with alarming consequences to the fishery yields and food security to the increasing populations.

The limitations detected opened an opportunity for further studies that should modify the methodological approach here described to improve species detection and reduce the observed contamination levels. Nevertheless, the potential of the tool for monitoring the Mekong fish communities, identify patterns of seasonal distribution and provide evidences of the needs to preserve the flood regime and habitats, should be acknowledged for the potential implementation of conservation measures, particularly in light of the future environmental threats in the basin (Hortle, 2007;2009; Baran, 2010; So, 2010).

CHAPTER 7

Synthesis and Conclusions

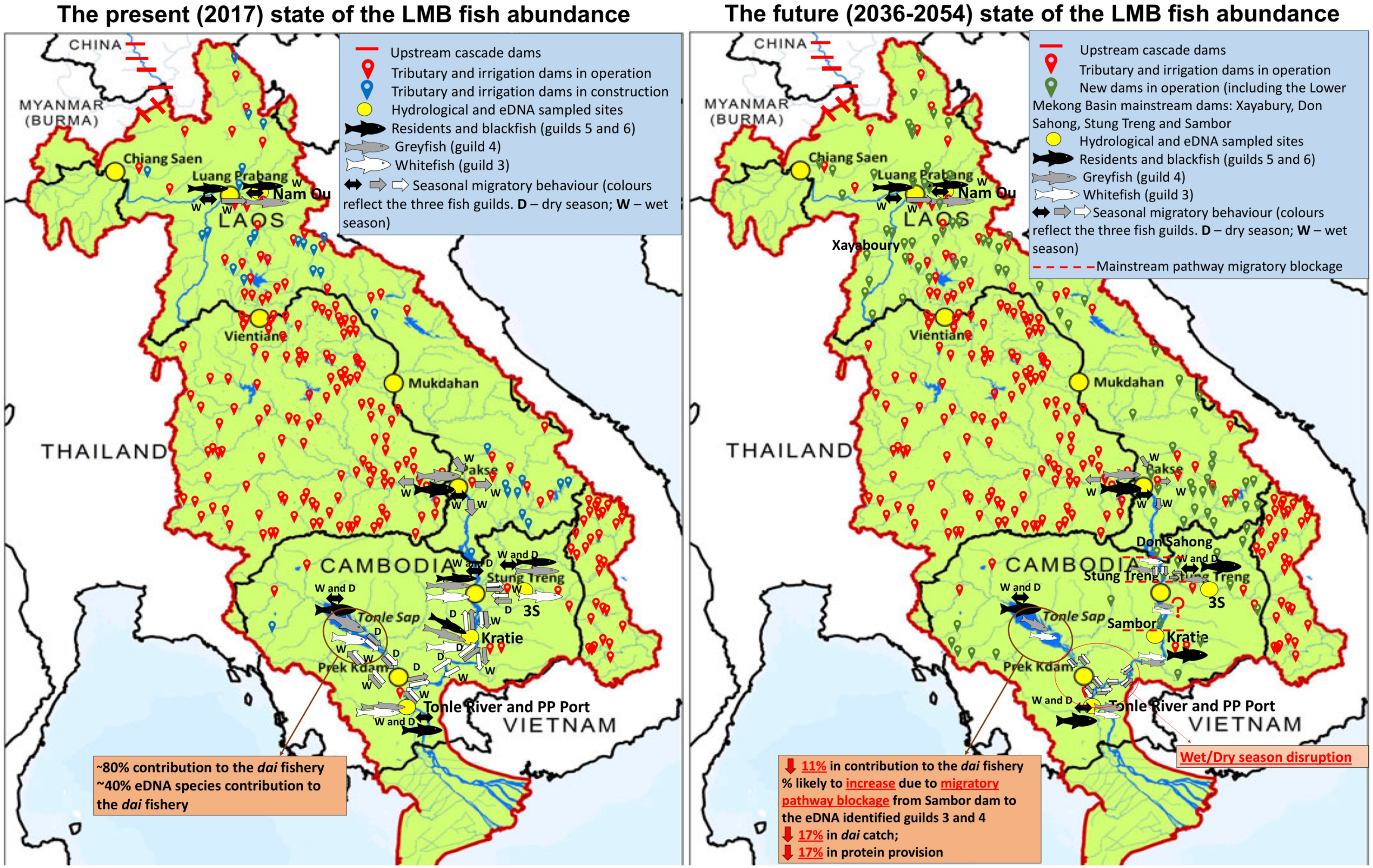
The work presented throughout this thesis joins key interdisciplinary approaches to demonstrate the importance of the Mekong river's seasonal variable hydrologic regime in sustaining and driving the outstanding abundance and diversity of migratory fish species along with habitat adaptive strategies. The habitats are key to the inland fisheries developed along the basin and underpin the food security to over 70 million people (Chapter 3). The TSGL system's historical flood pulse dynamics and connected floodplain inundation, driven by the Mekong river's hydrologic regime, were assessed to understand the importance to the only long-term monitored *dai* fishery and migratory guild species composition of the catch (Chapter 4). Importantly, it provided the baseline knowledge of the needs to preserve the flood pulse eco-hydrologic dynamics, in light of the projected hydropower, irrigation and climate change scenarios in the basin, allowing the quantification, for the first time, of the future of the *dai* fishery resources and food security to increasing populations (Chapter 5). Lastly, eDNA metabarcoding tools detected some of the Mekong's fishes and their seasonal migratory dynamics (Chapter 6). This chapter brings the results from this broad range of methods and techniques together in a holistic manner (section 7.1). These are reflected back in section 7.2 to answer the four research questions established in Chapter 1, along with a discussion of the critical limitations encountered for the implications in the findings detailed. Consequently, section 7.3 builds upon the limitations found and remaining gaps in knowledge to present an open window for further studies. Also, the wider implications of this research are addressed in section 7.4, with the take-home messages and final remarks detailed in section 7.5.

7.1 SYNTHESIS OF THE KEY FINDINGS

The findings of this thesis evidenced two scenarios (Figure 7.1) for the state of the Mekong river's fish abundance, driven by the hydrologic regime and key eco-hydrologic interactions in floodplains that influence the *dai* fishery. Notably, it shows the needs to prioritise environmental conservation actions in the basin to preserve the annual variability in the hydrologic regime, maintaining annual inundated habitats and connected migratory free pathways:

- In the **present scenario (2017)**, the long-term predictable seasonal hydrologic regime has driven the timing, duration and inundation dynamics in floodplain habitats and connected longitudinal and lateral fish migratory corridors, although upstream and tributary dams have been developed. These key eco-hydrologic interactions and free migratory corridors have triggered the migratory behaviour of guilds 3 and 4, contributing ~80% to the *dai* fishery of the TSGL system, particularly in the dry season as detected by the eDNA analyses, with the eDNA identified species of these guilds contributing ~40% to the *dai* fishery. Notably, these guilds are expected to contribute entirely to the highest fish protein consumption in the region and Southeast Asia, being over the triple of the global average;

- In the **future scenario (2036-2064)**, hydropower in the basin will disrupt the most the long-term seasonal variability of the hydrologic regime, resulting in 20% increase in the dry season flood level of the TSGL system, 6% decrease in the wet season flood level, one month earlier (i.e. September) peak flood, and 2 months increase in floodplains flood duration, which combined with climate change and irrigation scenarios resulted in 17% decrease in the *dai* catch and 11% decrease in guilds 3 and 4 contribution to the catch. Notably, these losses in the *dai* fishery, particularly in guilds 3 and 4, are likely to be magnified by the blockage of key migratory corridors from the future LMB mainstream dams, for the locations where these dams are planned and the seasonal habitats explored and migratory behaviour of the eDNA-based identified guilds 3 and 4 fish species. These losses in fishery resources are projected to underpin food security issues in the region.



The present (2017) state of the LMB fish abundance

The future (2036-2054) state of the LMB fish abundance

~80% contribution to the *dai* fishery
~40% eDNA species contribution to the *dai* fishery

⬇ 11% in contribution to the *dai* fishery
% likely to increase due to migratory pathway blockage from Sambor dam to the eDNA identified guilds 3 and 4
⬇ 17% in *dai* catch;
⬇ 17% in protein provision

Wet/Dry season disruption

FIGURE 7.1 - THE PRESENT (2017) AND FUTURE (2036-2064) STATE OF THE MEKONG FISH ABUNDANCE FROM THE FINDINGS OF CHAPTERS 3-6. THE PRESENT AND FUTURE DAM DEVELOPMENT SCENARIOS IN THE BASIN ARE PRESENTED FOR THE IMPACTS UPON THE SEASONAL LONGITUDINAL AND LATERAL MIGRATORY BEHAVIOUR OF GUILDS 3 AND 4, AND CONSEQUENCES TO THE DAI FISHERY AND FISH PROTEIN PROVISION TO FUTURE POPULATIONS.

7.2 ANSWERS TO THE RESEARCH QUESTIONS AND LIMITATIONS FOUND

The main aim of this research was to quantify the individual and combined impacts of the Mekong river's future scenarios of water infrastructure development and climate change on the drivers of fish dynamics and abundance, and predict the consequences to the only long-term monitored *dai* fishery and species composition of the TSGL system (see Chapter 1). This overall aim was addressed through the establishment of four interconnected research questions, which will be addressed, detailing the findings achieved through Chapters 3-6.

1) *How important is the Mekong river's hydrologic regime and sediment flux to the habitats, fish diversity and ecosystem services supported?*

The Mekong river's historical hydrologic regime (i.e. flood level and flow) is seasonally and regionally (i.e. for the different effect in upstream and downstream sections of the basin; see section 3.2 of Chapter 3) driven by the AMCS, the Tibetan Plateau and other periodic weather events (e.g. tropical cyclones and ENSO; Räsänen and Kummu, 2013; Darby *et al.,* 2013;2016), with Adamson *et al.,* (2009) and Delgado *et al.,* (2012) stating that the long-term relatively unchangeable climatic conditions across the basin are key in driving the hydrologic dynamics and the overall high levels of biodiversity. The long-term (1960-2017) mean annual analyses of the Mekong river's flood level and flow conditions along upstream to downstream gauging stations of the basin (see Figure 3.1 of Chapter 3), showed that the present level of upstream cascade dams and lateral tributary dams have not significantly modified the mean annual hydrologic conditions along the basin, particularly at the downstream sections, corroborating the same findings from other studies (Adamson *et al.,* 2006;2009; Piman *et al.,* 2013;2016). Furthermore, seasonal (i.e. for the wet and dry seasons) modifications in the historical flood levels and flow conditions (i.e. increase in both variables in the dry season and decrease in wet season, with variations for the values reported and percentage of change between stations; see section 3.2 of Chapter 3) were quantified by Lu and Siew (2006) and Räsänen *et al.,* (2012;2017), particularly at the upstream and middle sections of the basin, which the authors have related to the upstream cascade dams and to the increased lateral tributary development, particularly in Thailand. However, these were quantified to be marginally felt (i.e. half influence on the 41-68% of the flows reaching Kratie during the dry season; Räsänen *et al.,* 2017) in the downstream sections of the basin, after the Khone Falls region. Consequently, the relatively stable long-term mean annual hydrologic conditions and the predictable seasonal variations (i.e. the timing of wet/dry seasons and duration; Adamson *et al.,* 2009) observed were argued to influence habitats availability, connectivity and inundation patterns, with critical influence for the fish diversity supported and the overall ecosystem services provided (see sections 3.4 and 3.6 of Chapter 3; Valbo-Jorgensen *et al.,* 2009; Baran, 2010; DHI, 2015). Chapter 4 built upon this long-term predictability and seasonality of the hydrologic regime of the basin to assess the influence on floodplain habitats driven by the seasonal flood pulse dynamics of the TSGL system, for the role in supporting over 50% of the Mekong river's migratory fish species (Campbell *et al.,* 2009; Halls *et al.,* 2013a). Moreover, in Chapter 4 it was demonstrated the contributing role of the 19-year ENSO events in magnifying periods of high (La Niña) and low (El Niño) flood levels, following upon section 3.2 of Chapter 3 (Kummu and Räsänen, 2013), which can be shown to affect the annual *dai* fishery yields. However, the available ENSO data constrained further quantifications on the percentage contribution of El Niño/La Niña events to the observed mean annual flood level and the influence on the 19-year period assessed catch yields (see sub-section 4.4.5 of Chapter 4). The extension of the analysis previously made by Kummu *et al.,* (2014), for the complex flow dynamics at the lake (i.e. for the bi-directional unique flow reversals; see sub-section 4.4.4 of Chapter 4), showed that 54% of the annual variations in the flood conditions of the lake were driven by the Mekong river's long-term stable flow dynamics. These stable flow dynamics resulted in a 19-year (1996-2014) non-significant variations in the mean annual flood level of the lake (see sub-section 4.4.1 in Chapter 4) and non-significant variations in the mean monthly flood levels for the same period, showing that the peak flood in the lake (i.e. October) remained equal for the entire period.

This predictability and seasonality of the hydrologic regime were then reflected in the observed variations in the timing, duration and flood extent (i.e. the flood pulse variables; see Table 4.5 in Chapter 4; Junk *et al.,* 1989) in the floodplain habitats and seasonal inundation patterns for the 19-year period assessed, which were quantified to significantly influence in 31% the *dai* catch and support nearly 80% of small and large body-size medium to long distance migratory fish species that composed the long-term catch (i.e. guilds 4 and 3, respectively; see sub-section 4.4.3 and discussion of Chapter 4). Notably, these migratory species were argued to contribute entirely to the highest fish protein consumed in the region and Southeast Asia (70 kg capita^{-1} year^{-1}), being over the triple the global average (i.e. 20.2 kg capita^{-1} year^{-1} to 3.2 billion people, estimated in 2016, with fish contributing 20%; see section 3.5 in Chapter 3; FAO, 2018). This highlights the importance that fish protein place not only to the TSGL system populations, but also for the Mekong basin (i.e. over 70 million people; see Table 3.4 in Chapter 3) more generally, where nearly 48 kg capita^{-1} year^{-1} of fish (i.e. double the global value), estimated in 2007, are consumed along the basin, with migratory fishes contributing 47-80% to this value (Hortle, 2009; Baran, 2010; So, 2010).

The interlinked findings from Chapters 3 and 4 answer this first research question by showing that the long-term predictability and seasonality of the Mekong river's hydrologic regime, although upstream and tributary dams have been developed, have influenced the long-term low level of variation in the timing, duration and inundation dynamics of the key floodplains of the TSGL system, directly triggering and supporting the medium to long distance (i.e. guilds 4 and 3, respectively) migratory fish species of the Mekong river, which contributed nearly 80% to the 19-year assessed *dai* fishery and fish protein consumption in the region. Accordingly, these findings connected to the eDNA identified species from Chapter 6 (see detailed analysis in research question 4), demonstrate the current state of the Mekong fish abundance (see Figure 7.1) and the critical needs to preserve the historical hydrologic regime in the basin, for the seasonal predictable eco-hydrologic interactions established in floodplains and for sustaining key longitudinal and lateral migratory corridors that fish rely on for completing their life cycles, which result in high contribution to the *dai* fishery and fish protein provision. These findings are crucial to predict and understand the future impacts upon the Mekong fish stocks, *dai* fishery and protein provision, driven by individual and combined scenarios of hydropower, irrigation and climate change in the basin, assessed in Chapter 5 (see details in research question 3).

The analysis of the importance of sediment flux on habitats' productivity, fish abundance and ecosystem services supported was constrained by the paucity of the long-term data, particularly for assessing the nutrient-sediment bounded flux along the basin and seasonal recycling dynamics in floodplains, which were argued in Chapter 2 to boost food sources, key to fish production (Davies *et al.,* 2008). Moreover in sections 3.2 and 3.3 of Chapter 3 it was discussed the needs to explore the consequences to fish abundance and migratory dynamics of the observed decrease in sediment and nutrient fluxes reaching the downstream sections of the basin, driven by anthropogenic-driven actions along the basin (i.e. sand mining, irrigation, hydropower and climate change). Therefore, the constraints in the available sediments and nutrients data limited the quantified assessment of the role to the Mekong fish diversity and habitats' productivity, constraining the answer to the research question. However, the recent analysis of Whitehead *et al.,* (2019) for the simulations of the nutrient flux dynamics along the Mekong basin and consequences of water infrastructure and social developments, might open an opportunity for further studies (see section 7.3) to answer this gap in knowledge regarding the importance to fishes.

2) *Can remote sensing tools detect historical variations in the flood pulse dynamics of the Tonle Sap Great Lake (TSGL) system, with influences on the dai fishery and species composition of the catch?*

The analyses of Chapter 4 showed the importance of applying remote sensing tools (i.e. Landsat-TM images) and geographic information systems (i.e. ArcGIS) with hydrologic available time-series data for the TSGL system, to capture the long-term (1996-2014) seasonal variations in the flood pulse dynamics of the lake. In the dry season, 77% of the observed variations in the digitised Landsat-TM lake area were significantly explained by the dry season flood levels of the lake ($R^2=0.77$; $p<0.05$). In the wet season, 89% of the observed variations in the digitised Landsat-TM areas were significantly explained by the wet season flood levels of the lake ($R^2=0.89$; $p<0.05$; see sub-section 4.4.1 of Chapter 4).

The 40 years freely available daily satellite imagery from the Landsat-TM programme, is able to capture, with high accuracy and in real-time, the modifications occurring in different systems, including the TSGL system, driven by hydrologic changes, vegetation cover, land-use, among other factors (McAlister and Mahaxay, 2009; Fujii *et al.,* 2010; Fragal *et al.,* 2016; Dang *et al.,* 2018). Fragal *et al.,* (2016) by applying a LandTrendr algorithm, characterised the anthropogenic or natural influence in the observed changes in the vegetation cover of the Lower Amazon area, using time-series of 37 Landsat-TM images between 1984-2009. Furthermore, in the TSGL system, Dang *et al.,* (2018) recently applied Landsat-TM images to quantify spatio-temporal suspended sediment dynamics in the Mekong floodplains and reaching the Mekong delta, showing significant correlations and high explanatory percentage (R^2 from 0.66 to 0.92) for the sediments rating curves driven by the Landsat-TM analysis. The authors concluded that remote sensing are effective tools to understand temporal and spatial sediment dynamics in large floodplains driven by hydrologic variations. Noteworthy, a key limitation found in Chapter 4 for applying Landsat-TM images to systems under predictable moonsoon climates as the TSGL system, was the high cloud coverage in the wet season, which limited the monthly measurements of the lake area (see sub-section 4.3.2 of Chapter 4). Nevertheless, even measuring the lake area once every two months, the Landsat-TM digitised areas, combined with the hydrologic time-series data available for the lake, were capable of capturing with high accuracy (i.e. for the explanatory variable: R^2) the long-term seasonal flood pulse dynamics, and the resulting low level of variations in the floodplain inundation dynamics, which significantly influenced the *dai* fishery ($R^2=0.31$; $p<0.05$) and the migratory dynamics of guilds 3 and 4 that together contributed nearly 80% to the species composition of the catch, during the 19-year period assessed, and entirely to the highest fish protein comsuption in the region, as previsously discussed when answering the research question 1. Hence, the application of remote sensing tools were capable of capturing with high accuracy the seasonal complex flood pulse dynamics of the TSGL system, compared with the analysis made by Kummu *et al.,* (2014), for the application of a DBM (i.e. Digital Bathymetric Model) of the lake, with this model not differentiating the wet and dry season complex flood dynamics' influence on the lake's flood area, which, as answered in research question 1, had key implications to floodplain flood dynamics, migratory fish behaviour and *dai* fishery yields (see sub-section 4.5.1 of Chapter 4).

3) *How will the flood pulse and floodplain flood dynamics of the TSGL system be impacted by future individual and combined action of the multiple drivers of environmental change scenarios of the Mekong basin? What are the consequences to the dai fishery, species composition and food security?*

Building upon the findings from Chapter 3 for the long-term (1960-2017) relatively stable hydrologic regime in the lower catchment of the basin (where the TSGL system is located; see Figure 3.1 of Chapter 3) and the baseline analyses made in Chapter 4 for the importance of the seasonal variations in the flood pulse dynamics of the lake in driving the predictable and low level of change, during the 19-year period, of the timing, duration and inundation dynamics in floodplains, which significantly influenced the *dai* fishery and migratory fish species behaviour and contribution to the catch; in Chapter 5, and for the first time to the TSGL system, the future (2036-2064) impacts to the flood pulse dynamics, floodplain inundation profile and *dai* fishery resources, driven by individual and combined hydropower, irrigation and climate change scenarios of the Mekong basin were projected. These analyses were based on Hoang *et al.,* (2016;2019) simulated scenarios of the daily flow conditions at Kratie (2036-2064), under the individual and combined hydropower, irrigation and climate change scenarios, to extrapolate future daily water levels at the lake, by applying a baseline (1996-2014) rise and fall metrics assessing the one-day delay daily flow conditions at Kratie and the water level conditions at Kampong Luong (i.e. the gauging station inside the lake; see sub-section 5.2.3 of Chapter 5). Importantly, when extrapolating the future daily water level conditions at the lake under the individual and combined scenarios of water infrastructure development and climate change, an averaged 3-meter water level gap in the rise-fall metrics was found for the future period assessed, with the further attempts made to the rise and fall metrics to reduce this water level gap (see sub-section 5.2.3 of Chapter 5) not being effective. Consequently, the 3-meter water level gap was discussed in sub-section 5.4.1 of Chapter 5, to potentially have influenced the wet season estimations of flood levels, permanent lake area and inundation dynamics.

The hydropower development scenarios in the Mekong basin were quantified to profoundly disrupt most of the historical flood level of the lake (i.e. 20% increase in the dry season and 6% decrease in the wet season), the permanent lake area (i.e. 26% increase in the dry season and 5% decrease in the wet season), the flood peak (i.e. nearly 1 month earlier compared to the baseline October scenario) and floodplain habitats inundation duration and timing (i.e. 2 months increase (8 months total floodplain flooded) – middle August to early May, compared to the 6 months baseline floodplain flooded: late July – early January). Moreover, the combined impacts of hydropower and climate change scenarios resulted in a significant reduction in floodplain habitats inundation extent (i.e. 7% decrease). Importantly, the quantified disruptions on the flood pulse and floodplain inundation dynamics, resulted in an alarming projection of 17% decrease in the *dai* fishery catch, 11% decrease in guilds 3 and 4 contribution to the catch and 17% decrease in the fish protein availability to future populations (see sub-sections 5.3.3 and 5.4.4 of Chapter 5), with these projections reflecting the combined impacts of hydropower, irrigation and climate change scenarios on the flood pulse and floodplain inundation profile, and hydropower scenarios impacting the most as previously described.

This decrease in fish protein come at a time when the region and entire Mekong basin are projected to increase in population to 2 million and 100-145 million people in 2050, respectively (Varis *et al.,* 2012), raising some alarming future food security issues. On top of this, and although not assessed in this thesis, the disruption on the flood pulse dynamics of the TSGL system is likely to impact rice farming activities, which will not be able to replace the fish protein loss, increasing food security threats and urging processed informed conservation measures to be implemented by decision makers, to secure the vital fisheries to people, while addressing the basin's development plans (see further details in section 7.4).

The findings from Chapter 5 show unprecedent scenarios of migratory fish species (i.e. guilds 3 and 4) decrease in contribution to the *dai* catch, with resulting alarming losses in the fishery yields and fish protein to people. These losses reflect the consequences of disruptions on the migratory triggers, for the quantified changes in the timing of the historical seasonal flood level variations. Also, disruptions on the timing, duration and flooded habitats inundation extent and connectivity, and consequent effect to the *dai* fishery historical operation activity (i.e. for the time that fish remain in flooded floodplains and the March-October operation season; see sub-section 5.4.3 of Chapter 5), and migratory pathway blockage from the planned locations of the dams and the seasonal habitats occupied by these medium to long distance migratory species, as evidenced by the species detected with eDNA in Chapter 6 (see below), are explanatory factors for the quantified decrease in the medium to long distance migratory fish species of the *dai* fishery and an alarming fish protein decrease. These findings demonstrate the needs to preserve the historical hydrologic regime, habitat inundation dynamics and migratory corridors, to sustain migratory fish stocks, *dai* fishery and fish protein provision to future populations (see below).

4) *Can eDNA metabarcoding analyses detect the Mekong river's fish communities and identify patterns of seasonal dispersion, demonstrating the needs to improve the biomonitoring and environmental conservation actions in the basin, in light of the projected environmental threats?*

Knowledge concerning tropical freshwater fish diversity and ecology, the migratory behaviour, habitats and role of the hydrologic regime and sediment flux in driving the distribution patterns, has been hampered by the identified limitations in the traditional surveys (see section 1.3 of Chapter 1; Fischer, 2013). Few applications of novel molecular-based survey tools in tropical freshwater systems have been applied (see section 2.4 of Chapter 2; Bellemain *et al.,* 2016; Robson *et al.,* 2016). In section 3.4 of Chapter 3 it was argued that over 50% of the Mekong fish species perform seasonal medium to long distance (i.e. guilds 4 and 3, respectively) longitudinal and lateral migrations, in the Lower Mekong Basin, driven by the predictable seasonal variations in the hydrologic regime and sediments carried by the flow (see section 3.2 of Chapter 3; Adamson *et al.,* 2009; Darby *et al.,* 2016), promoting key eco-hydrologic interactions in floodplains (i.e. extensive inundation providing spawning, feeding and sheltering grounds, and recycling of nutrients from the terrestrial land and nutrients carried by the flow; Poulsen *et al.,* 2002;2004; Baran 2006;2010; Lamberts, 2008; Valbo-Jorgensen *et al.,* 2009; Arias *et al.,* 2012;2013;2014; Baran *et al.,* 2015).

The lack of mainstream dams and reduced tributary dam development in the lower catchment (see Figure 7.1), have resulted in connected longitudinal and lateral free migratory corridors, key for migratory fishes to reach spawning, feeding and sheltering habitats. This was further demonstrated in Chapter 4, for the role of the long-term hydrologic regime of the basin in driving the flood pulse dynamics in the TSGL system and floodplain habitats inundation timing, duration and extent, crucial for guilds 3 and 4 and demonstrated by their high contribution to the *dai* fishery and fish protein provision to over 1.2 million people (see the discussion in research question 1). Furthermore, the alarming projections made in Chapter 5 for the losses in *dai* catch, fish protein availability to future populations and decrease in guilds 3 and 4 contribution to the catch, demonstrated the needs to prioritise environmental conservation actions in the basin for preserving the long-term hydrologic regime, habitat inundation dynamics and migratory corridors connectivity, key to medium to long distance migratory fishes (see the discussion in research question 3). Consequently, Chapter 6 gathered the findings from the previous chapters to explore a preliminary assessment of the potential for applying eDNA metabarcoding tools in the Mekong river to detect in near real-time (since eDNA in water has a persistence time of nearly 1 month; see section 2.4 of Chapter 2; Herder *et al.,* Taberlet *et al.,* 2018) how many fish are there along the basin? Where and when? and how future anthropogenic-driven environmental changes in the basin will impact the fish diversity and abundance detected, and vital fisheries to people?

Although key limitations were found regarding the low number of eDNA-based Mekong fish species detected (i.e. 25 species representing <4% of the 801 total described fish species for the Mekong basin; Baran 2006;2010; Hortle, 2009; Valbo-Jorgensen *et al.,* 2009; Halls, 2010), which were related to the lack of obtaining a meaningful and representative sample in this complex environment (i.e. lack of collecting replicated water samples within sites) and DNA degradation of the filter-samples from the overseas transportation conditions and storage temperature and duration within the University of Hull facilities (see sub-section 6.4.1 of Chapter 6); the eDNA identified species allowed a preliminary understanding of the diversity of families (see Table 6.1 of Chapter 6) of the fishes inhabiting the Mekong river, while assessing the potential seasonal migratory behaviour along the basin and habitats explored. Of note, the identified limitations evidenced the needs to apply further studies, with modifications in the applied methodology, to increase the number of species detected (Dejean *et al.,* 2011; Deiner *et al.,* 2015; Spens *et al.,* 2016; Yamanaka *et al.,* 2016b; Li *et al.,* 2018; Taberlet *et al.,* 2018), allowing a further understanding of the diversity of species, relative abundance and distribution along the basin, which will result in a better understanding of the threats by dam development in the basin to the species and consequences to the inland fisheries and fish protein provision to people (see section 7.3). In the wet season, 46% and 21% of the identified species (i.e. for the proportion of individual species' total read counts and guilds belonged) were residents and blackfish species, exhibiting no migratory behaviour (i.e. guilds 5 and 6, respectively), with the remaining 33% of the identified species belonging to guilds 4 and 3, respectively (see Table 6.2 of Chapter 6), and these species being mostly found in the mainstream river and tributaries habitats.

These findings contrast with the available literature (Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009; Halls, 2010), reporting the higher presence of guilds 3 and 4 in the mainstream and lateral habitats, linked to the higher flood levels' trigger for these species to perform longitudinal and lateral migrations along the basin (see section 3.4 of Chapter 3). The low DNA yields (see sub-section 6.3.2 of Chapter 6) and consequent low species detection can explain this result. These are associated with the potential reduced capacity of the DNA extraction methodology, to effectively separate the DNA from the high sediment content present in the filter papers. Also, the lack of performing replications in water collections and transect, to approach the higher dilution, dispersion and degradation of the eDNA material in the wet season, linked to the higher flood level, flow and turbidity, and DNA degradation from the filter-samples' storage conditions, were argued to have influenced the findings (see sub-section 6.4.1 of Chapter 6; Bellemain *et al.,* 2016). In the dry season, 81% of the species detected belonged to guilds 3 and 4 (see Table 6.3 of Chapter 6), mainly found in the mainstream river, tributaries and lake habitats. The reduced flood level and decrease in flooded habitats, particularly in the TSGL system where a higher number of species (i.e. higher individual species' read counts) were found (see sub-section 6.3.6 of Chapter 6), corroborated the previous discussion in section 3.4 of Chapter 3 and the answer to the first research question, for the role of the seasonal hydrologic regime in driving the migratory behaviour of medium to long distance migratory species (Poulsen *et al.,* 2002;2004; Baran, 2006;2010).

The most abundant eDNA identified guilds 3 and 4 species in the dry season (i.e. *Henicorhynchus lobatus, Labiobarbus leptocheilus, Pangasianodon hypophthalmus, Pangasius larnaudii* and *Paralaubuca typus*; see sub-section 6.4.4 of Chapter 6) contributed ~40% to the *dai* fishery species composition of Chapter 4. Thus, linking the findings from Chapter 5 (i.e. 11% decrease in guilds 3 and 4 contribution to the *dai* catch) with the locations where future mainstream dams are planned (Figure 7.1) and the likely migratory behaviour for the habitats explored of the eDNA identified guilds 3 and 4, it is likely that future projections of a decrease in *dai* catch, guilds 3 and 4 contribution to the catch and fish protein provision to future populations, will be magnified, driven by the blockage of the previously connected free longitudinal and lateral migratory corridors, resulting in fish stocks' decrease (Figure 7.1).

Hence, the eDNA metabarcoding analyses, by detecting in near-real time the seasonal species composition along the basin, habitats explored and potential migratory routes, evidenced the reliability of the tool to monitor the Mekong fish communities and the needs to apply conservation actions of the long-term hydrologic regime, habitat inundation dynamics and longitudinal and lateral migratory corridors, for the sustainability of the fish stocks, the *dai* fishery and fish protein to future populations (Hortle, 2007;2009; Baran, 2010; So, 2010).

7.3 OUTLOOK FOR FURTHER STUDIES

In section 7.2 the identified limitations opened opportunities to develop further studies aiming to understand the complexity of the eco-hydrologic interactions established in the Mekong river (i.e. hydrologic regime – sediment flux – nutrient recycling – habitats productivity) and the role on the fish species supported, inland fisheries and food security to people (Hortle, 2007;2009; Baran, 2010).

The importance of the long-term spatio-temporal dynamics of the hydrologic regime and the sediment loads transported along the Mekong river in influencing the fish diversity supported and habitats' productivity, was constrained by the paucity of the available sediments data. Particularly, the sediment-bounded nutrient flux dynamics along the basin, the seasonal recycling from floodplain habitats and consequent importance to different fish species production, reflected in the inland fisheries, was not quantified in this thesis linked to the identified gaps in the long-term available nutrients data (see section 3.3 of Chapter 3). On the other hand, Baran *et al.,* (2015) stated the key role of the nutrient recycling in floodplain habitats for fish production, with Poulsen *et al.,* (2002;2004), Baran (2006) and Valbo-Jorgensen *et al.,* (2009) arguing that the higher food sources in lateral habitats during the wet season, driven by the nutrient recycling from the nutrients transported in the flow and the recently inundated areas, trigger the migratory movement of medium to long distance migratory fish species, resulting in optimal conditions for larvae and juvenile to growth and adults to build fat layers (see section 3.4 in Chapter 3). However, the lack of a quantified baseline assessment on the role of nutrients (e.g. phosphates and nitrates) in influencing different migratory fish production (e.g. building upon the analyses in Chapter 4), limited further predictions on how changes in nutrient flux, by hydropower trapping and/or disruption in the historical floodplain inundation dynamics, would magnify the quantified scenarios of guilds 3 and 4 decrease in the percentage contribution to the *dai* fishery, catch and fish protein loss (Chapter 5).

The recent study by Whitehead *et al.,* (2019) using the INCA Model, provide a new option to quantify the importance of nutrient fluxes (focusing on nitrates and phosphates) to the migratory fish species and project the consequences to guilds 3 and 4 production and to the *dai* catches of a decrease in nutrient fluxes from water infrastructure development and climate change scenarios. The authors showed the reliability of the model in estimating scenarios of nutrient flux change reaching the Mekong delta, driven by water infrastructure and social developments, for the role on agriculture productivity (see sub-section 3.6.1 of Chapter 3 for detailed values). Thus, and although recognising that the complex bi-directional flow reversals at the TSGL system (Kummu and Sarkkula, 2008; Kummu *et al.,* 2014) would potentially require a different parameterisation and operation module of the INCA Model to capture those complex hydrodynamics and correctly estimate the nutrients flowing in and out of the system, while quantifying the nutrients stored and recycled in the seasonally inundated floodplains; the assessment made by Whitehead *et al.,* (2019) evidenced a potential tool to be further investigated to answer how sediment flux (particularly the nutrient-bounded sediments) dynamics in the Mekong river influence the fish species and habitats' productivity?

In Chapter 5, the extrapolations of the future water levels at the lake, under the individual and combined scenarios of hydropower, irrigation and climate change projections, were driven by the discharge scenarios of Hoang *et al.,* (2016;2019) VMod Model approach and the establishment of a baseline rise and fall metrics for the discharge conditions in Kratie and influence on the lake's water levels. These baseline metrics resulted in an averaged 3-meter water level gap, which was argued to influence the wet season predictions of the flood levels, permanent lake area and floodplain inundation dynamics. Thus, and recognising that the VMod is a hydrologic model and that future daily water level simulations for the lake are absent from other studies, future studies should aim to simulate the complex hydrologic dynamics in the TSGL system to directly obtain future scenarios of daily flood levels at the lake, under individual and combined scenarios of hydropower, irrigation and climate change of the basin. One potential option would be to use the flow dynamic equation (Equation 4.1) applied in Chapter 4, from Kummu *et al.,* (2014) analysis, for capturing the bi-directional flow reversals at the Tonle Sap River (i.e. the tributary of the lake), apply the equation to simulate the future daily flows using a modified version of the VMod's operation module to account for the inflows and outflows, and evaluate the contribution of the mainstream flow (e.g. using the flow conditions at Kratie, since it is the closest station) for the resulting simulations, following Kummu *et al.,* (2014) analysis that the mainstream flow contribute nearly 54% to the flood pulse of the TSGL system. Then, and since the flow equation is based on the daily flood levels at the lake, the daily simulated flows from the VMod Model would allow the extrapolations of the future daily flood levels at the lake.

The eDNA analyses in Chapter 6 were limited by the low number of species detected along the different sites and seasons (i.e. average of 3 species per site in the wet season and 7 species per site in the dry season; see sub-section 6.3.5 of Chapter 6). This was particularly observed in the wet season, regarding the individual species composition and potential migratory behaviour for the habitats explored (see sub-sections 6.3.5 and 6.3.6 of Chapter 6), for the lower detection of individual medium to long distance (i.e. guilds 4 and 3, respectively) migratory species' total read counts in the mainstream and lateral habitats (i.e. in tributaries and lake), since literature states that the wet season conditions (i.e. higher flood levels, inundated habitats and connectivity) trigger the longitudinal and lateral migratory pattern of guilds 3 and 4 (Poulsen *et al.,* 2002;2004; Baran, 2006;2010; Valbo-Jorgensen *et al.,* 2009; Halls, 2010). The lack of replication of the collected water samples in each site, distance between sampled sites and DNA degradation after DNA capture of the collected samples, linked to the transportation conditions to the UK (i.e. no cold conditions in place and lack of appropriate conditioning of filter-samples to avoid the movement of the falcon tubes) and the duration and temperature storage of the captured DNA material (i.e. 4 months in 4ºC), were argued as the reasons for the low species detection along sites on both seasons (see sub-section 6.4.1 of Chapter 6). Also, and knowing that eDNA is highly degraded (i.e. less than 150 bp; Taberlet *et al.,* 2018) and affected by biotic and abiotic factors (i.e. temperature, UV-radiation, turbidity, flow conditions, species abundance and DNA release and bacterial activity; see section 2.4 of Chapter 2), for the persistence and degradation in the environment, and with Bellemain *et al.,* (2016) arguing that these factors are likely to be exacerbated in the Mekong river, for its tropical monsoonal climate influence on the seasonal variations in the flood regime along the basin (see section 3.2 of Chapter 3), the choice of capture and preservation methods should aim to minimize the degraded eDNA material, while reducing the handling and exposure to open air of the captured DNA, to maximise species detection and reduce the cross-contamination issues detected (see sub-section 6.4.2 of Chapter 6; Deiner *et al.,* 2015; Spens *et al.,* 2016).

Consequently, for increasing the number of eDNA species detected along sites in both seasons and improve the understanding on the seasonal migratory behaviour and linkage with the habitats explored (particularly in the wet season), further studies should improve the methodology applied in Chapter 6. This means, perform replications of the collected water samples in each site and apply a transect approach to increase the chances of high species detection (see sub-section 6.4.1 of Chapter 6). Yet this can be a challenge when performing fieldwork in complex environments like the Mekong, where high turbidity would increase the filtration time and the lack of access to a suitable laboratory facility would increase the cross-contamination among samples, this way potentially compromising the reliability of the species detected and findings reported by the study. Thus, and following the findings from Spens *et al.,* (2016) and Li *et al.,* (2018), the application of Sterivex filters would be suitable to apply in eDNA metabarcoding studies in the Mekong river, for reducing the sterilization procedures, filter handling and exposure of filters to open-air, this way reducing potential sources of cross-contamination, while being able to capture and preserve the low eDNA fragments present in the water, with these studies reporting high species detection from Sterivex filters. Furthermore, to reduce DNA degradation, the falcon tubes containing the filter-samples should be transported in sterilized storage containers that would avoid the movement of the tubes, reducing the breakdown of the DNA molecules, and throughout the transportation period, samples should be kept in ice or at 4 ºC temperature. Likewise, when at the laboratory facility of the University of Hull (UK), the captured DNA material should be immediately stored in -20 ºC in a dedicated DNA-free freezer, instead of stored for 4 months in a cold-room at 4ºC (see sub-section 6.4.1 of Chapter 6). Additionally, effective DNA extraction methodologies should be tested to assess the potential influence of high sediment content present in the filter papers to the low DNA recovery and downstream low species detection.

To sum up, by applying these changes in the methodological approach, further studies would likely be able to detect higher abundance of species along the basin and further understand the eDNA dynamics. This would improve the knowledge on the seasonal migratory dynamics and habitats explored, which combined to the findings on the role of the long-term hydrologic regime on the migratory movements (Chapters 3-4) and future disruptions on the flood pulse, habitats inundation dynamics and blockage of key migratory pathways (Chapter 5), would allow an increased understanding of the future of the Mekong fish abundance (Figure 7.1) and the consequences to the *dai* fishery and fish protein provision to future populations.

7.4 THE WIDER IMPLICATIONS OF THIS RESEARCH

The findings presented throughout this thesis help to disentangle fundamental knowledge gaps identified in Chapters 2 and 3, with scientific, political and social implications. The methodological approach in Chapter 4, based on the long-term analysis of the hydrologic regime of the Mekong basin (see section 3.2 of Chapter 3), showed the importance of preserving the historical flood pulse dynamics (i.e. for the timing, duration and flood magnitude) established in a vital tropical wetland system and connected floodplains, for the role in driving the migratory fish guilds behaviour, which contributed nearly 80% to the *dai* fishery and to the highest fish protein consumption in the region. Of note, future projections of hydropower development, combined to irrigation and climate change scenarios in the basin, showed profound disruptions in the flood pulse dynamics and resulting floodplain inundation pattern, with alarming losses in the *dai* fishery, species composition and food security in the region (Chapter 5). These losses are likely to be magnified by the blockage of key longitudinal to lateral previously connected migratory routes and habitats for guilds 3 and 4, driven by the mainstream location of the planned dams, as identified in Chapter 6, with five dry season eDNA identified guilds 3 and 4 species contributing nearly 40% to the *dai* catch.
The complexity of the seasonal eco-hydrologic interactions established in floodplains, driven by the predictable variability in the flood pulse, have characterised the fish communities present and their adaptive strategies, particularly regarding migratory fish species that have contributed highly to the *dai* fishery (Chapter 4). Additionally, the projected future losses in catch and migratory species composition and contribution to the catch (Chapter 5), evidenced that medium to long distance migratory species are less capable of adapting to the new environmental conditions in the system, particularly regarding changes in the timing of the flood pulse and migratory pathway

blockage. This is likely to result in disruptions in the spawning cycle with expected reductions in fish stocks (Baran, 2010; DHI, 2015).

The perturbations in the long-term environmental conditions may result in fish assemblage changes, where non-migratory blackfish species (e.g. Channidae family) are expected in the future to increase the composition and contributing role to the *dai* fishery, compared to medium and long distance grey and whitefishes. This is because blackfishes are more capable of adapting to changes in the timing, duration and magnitude of high and low floods, and habitat flood dynamics, as well as to modifications in water quality parameters (e.g. low dissolved oxygen and high turbidity; see Chapter 2; Lucas and Baras, Val *et al.,* 2006; Baran, 2010). Of note, the potential change in fish assemblage and role on the *dai* fishery needs to be addressed to assess potential impacts on trophic chains and for food security in the region, regarding the capacity of blackfish to replace the fish protein loss by grey and whitefish, which are vital to people. Thus, the eDNA approach presented offers an opportunity to address this issue, by detecting the spatio-temporal fish species diversity present throughout the basin, particularly in the TSGL system where the *dai* fishery develops. This would help to increase the knowledge on the influence of environmental perturbations to fish assemblage and changes in the community level, by comparing the fish guilds relative abundance identified throughout the sampled sites.

The projected decrease in fish protein provision to the expected 100-145 million people (Varis *et al.,* 2012; see Chapter 5), bring alarming food security challenges in a region marked by high poverty and malnourishment. Likewise, it is expected that future populations would need to intensify agriculture production to face the fish protein loss, yet and as demonstrated in sub-section 5.4.4 of Chapter 5 by Orr *et al.,* (2012) and in section 3.6 of Chapter 3 by Whitehead *et al.,* (2019), the agriculture intensification would result in increased water use, land change and threats to water quality (i.e. for the higher pesticides in water; Whitehead *et al.,* 2019). These, combined to disruptions in the hydrologic regime and decrease in sediment and nutrient load, would likely exacerbate the threats to fish abundance and amplify the losses in fish protein, while disrupting the productivity of soils for agriculture, with the agriculture production likely to not be enough to compensate for the fish protein loss. Therefore, the increasing trend in aquaculture production discussed in section 3.6 of Chapter 3 might compensate the inland fishery protein loss (DHI, 2015). Yet aquaculture has been growing mainly in the Vietnamese delta, which is under high threat from land change for irrigation purpose and increased salinity intrusion from sea level rise (Van Manh *et al.,* 2015; Hoang *et al.,* 2018; Kondolf *et al.,* 2018; Whitehead *et al.,* 2019), with unknown consequences to the aquaculture production and fish protein provision to people. Furthermore, even considering that aquaculture production is enough to compensate the inland fisheries protein loss, the low economic power in Cambodia (see section 3.5 of Chapter 3; DHI, 2015), where the highest annual fish protein consumption is observed (i.e. 70 kg person^{-1} year^{-1}, So, 2010), make it unreliable that people would import aquaculture products from Vietnam. Accordingly, the results in this thesis provide the scientific comprehensive knowledge of the needs to implement environmental priority conservation areas in strategic development plans, seeking the balanced basin's economic development, while sustaining the natural resources.

According to Winemiller *et al.,* (2016), the Amazon, Mekong and Congo basins are expected to possess the highest hydropower development, while sustaining 75% of the entire freshwater fish diversity (see section 2.3 of Chapter 2). Although the Amazon basin has been under high scrutiny for the importance of the flood pulse dynamics to the fishery resources (Castello *et al.,* 2015;2017; Isaac *et al.,* 2016; Lima *et al.,* 2017), gaps in knowledge on the fish abundance and distribution dynamics, constrains the baseline knowledge and future projections, under the expected hydropower, irrigation and climate change scenarios at the basin (Castello and Macedo, 2016; Latrubesse *et al.,* 2017). Thus, the eDNA metabarcoding analyses in Chapter 6 could provide answers to these identified gaps. Moreover, the Amazon and Congo basins sustain high population densities relying on fishery resources for food security and livelihood, with losses in the fish protein provision likely to significantly hamper food security issues in the regions (Winemiller *et al.,* 2016). Hence, remote sensing tools (Chapter 4), numerical modelling (Chapter 5) and eDNA metabarcoding (Chapter 6), could help to predict the future of the fish resources supported by those basins and the consequences to food security to people.

7.5 TAKE HOME MESSAGES AND FINAL REMARKS

The analyses from Chapters 3 and 4 demonstrated the importance of the long-term seasonal hydrologic regime of the Mekong basin in driving the timing, duration and inundation extent of floodplain habitats of the TSGL system. These habitats were key to the migratory behaviour and abundance of guilds 3 and 4, contributing nearly 80% to the 19-year assessed *dai* fishery, and to the fish protein provision to people, critical for food security in the region. Moreover, the eDNA metabarcoding analyses in Chapter 6, corroborated the importance of the long-term seasonal hydrologic regime, for the habitats explored by the eDNA-based detected fish species, linked to the connected longitudinal and lateral free migratory corridors, with the five eDNA guild 3 and 4 species detected in the dry season contributing nearly 40% to the *dai* fishery. Thus, these analyses showed that the present state (Figure 7.1) of high fish abundance and migratory species contribution to the *dai* fishery, and protein provision to people, result from the long-term predictable seasonal hydrologic conditions and the influence on habitats inundation dynamics, and the free migratory connectivity from the low mainstream and tributary dam impoundment on the migratory movements.

However, the findings from Chapter 5 show that future hydropower development in the basin, connected to climate change and irrigation scenarios, are projected to profoundly disrupt the long-term hydrologic regime, impacting upon the flood pulse and floodplain inundation dynamics in the TSGL system, causing an alarming projected 17% decrease in the *dai* catch and fish protein provision to future populations, and 11% decrease in the contribution of guilds 3 and 4 to the *dai* fishery. Also, blockage of the previously connected free migratory corridors, linked to the mainstream locations of the planned dams, are expected to severely disrupt the access to key spawning, feeding and sheltering grounds for the eDNA identified species, affecting fish stocks and the *dai* fishery, magnifying future food security issues in the region (Figure 7.1).

Hence, this thesis evidenced the needs to preserve the historical hydrologic regime of basin, habitat inundation dynamics and the migratory corridors, for securing fish stocks, fishery yields and protein availability to future populations, where the fish protein consumption is over triple the global average.

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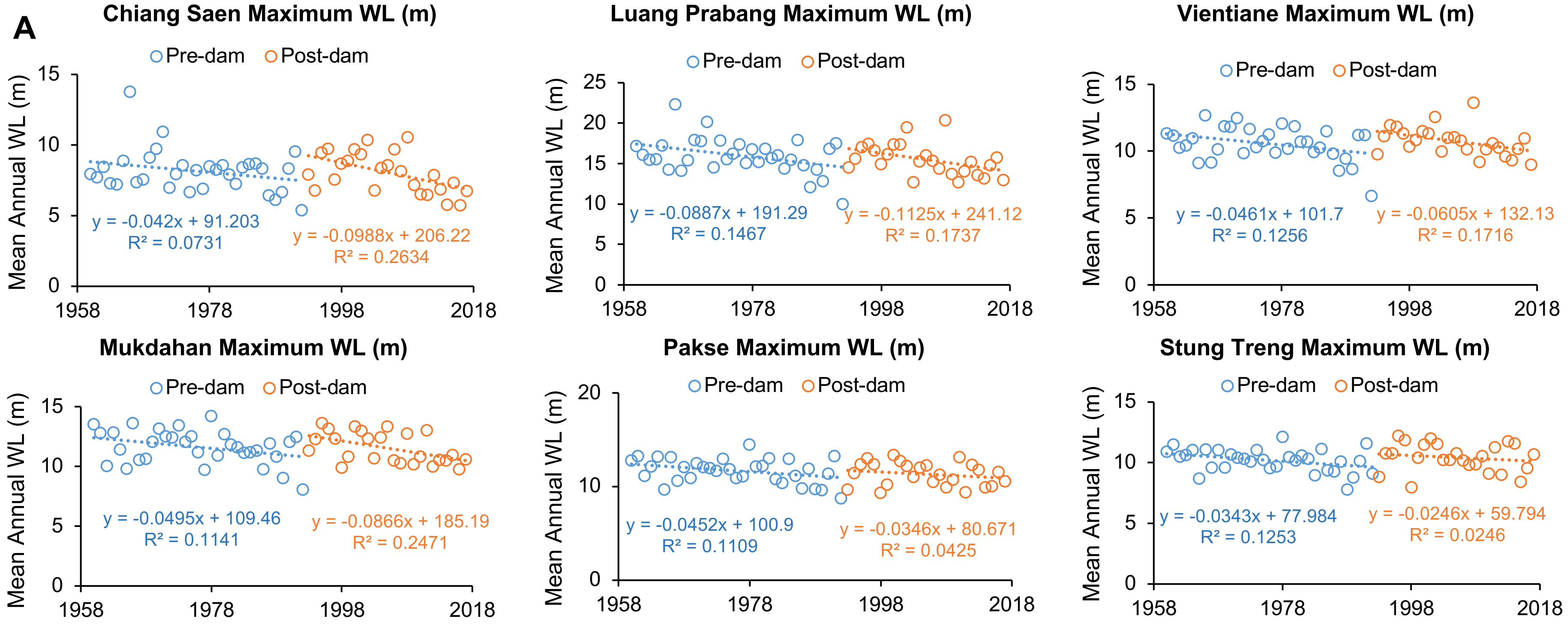
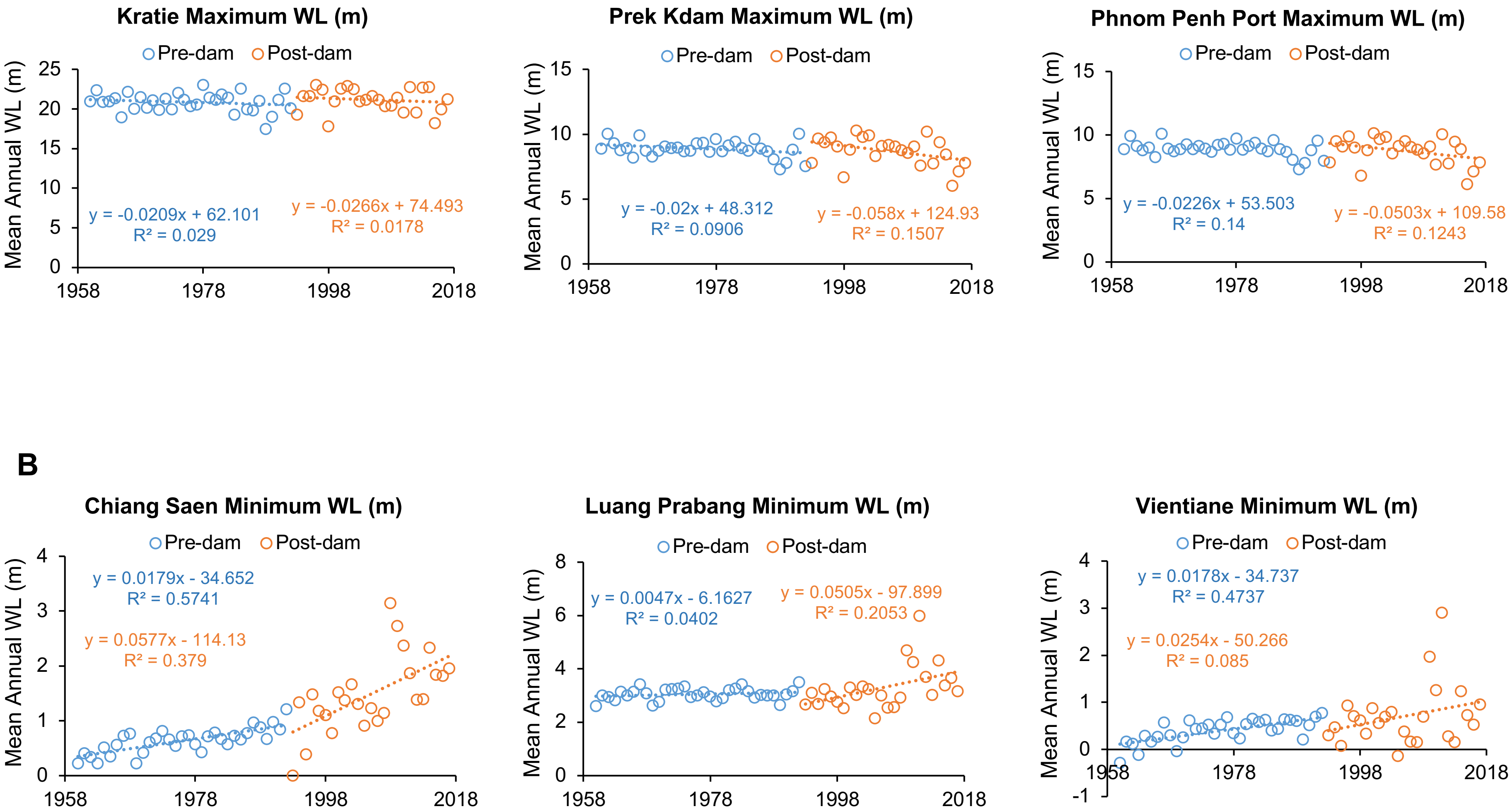
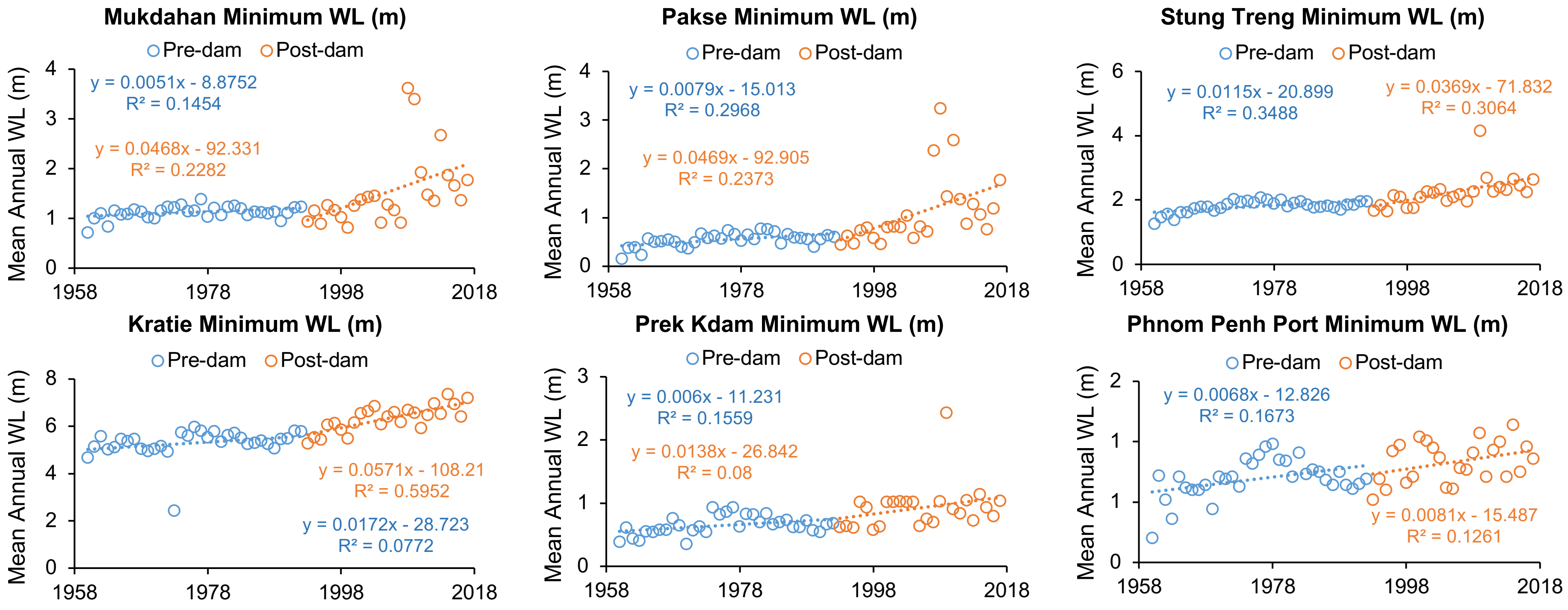
APPENDIX 1

Time-series (1960-2017) analysis of the mean annual **(A)** maximum and **(B)** minimum water level (m) variations, for the pre and post-dam development (i.e. based on the 1993 Manwan dam operation start), along the Mekong basin's gauging stations.

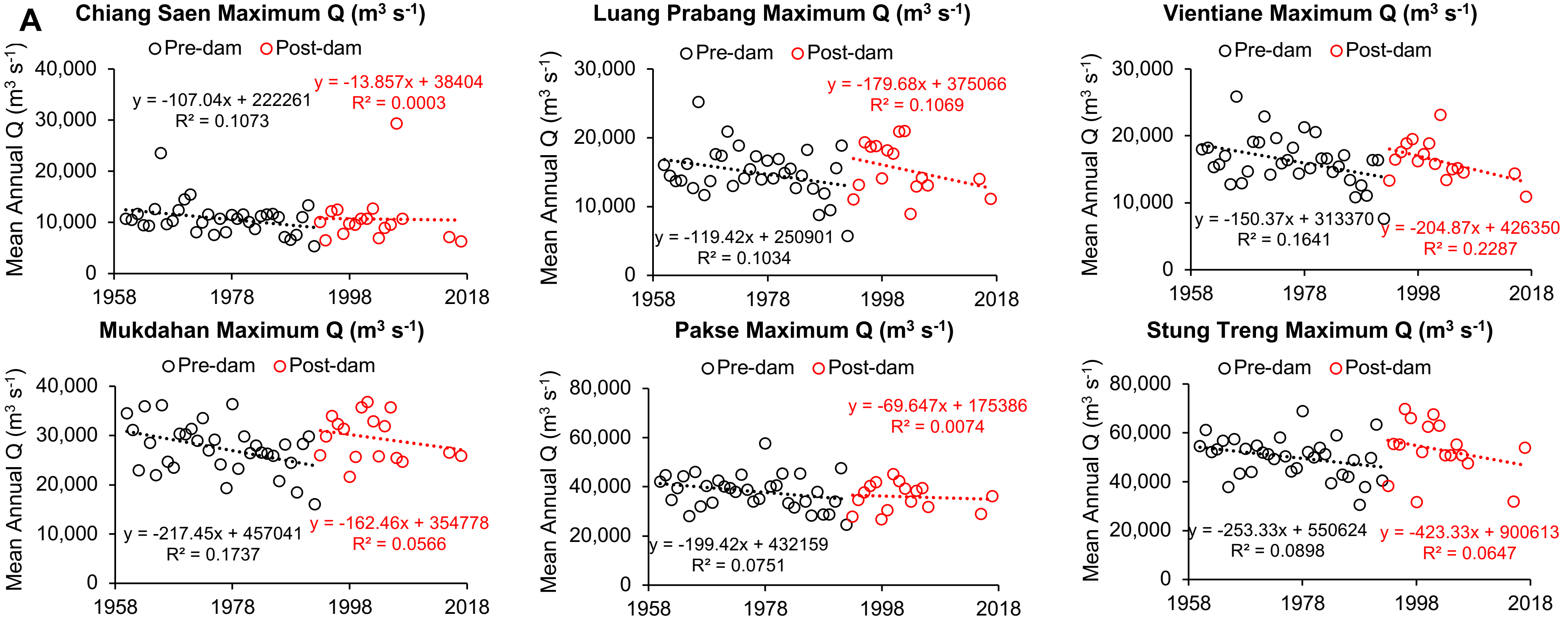


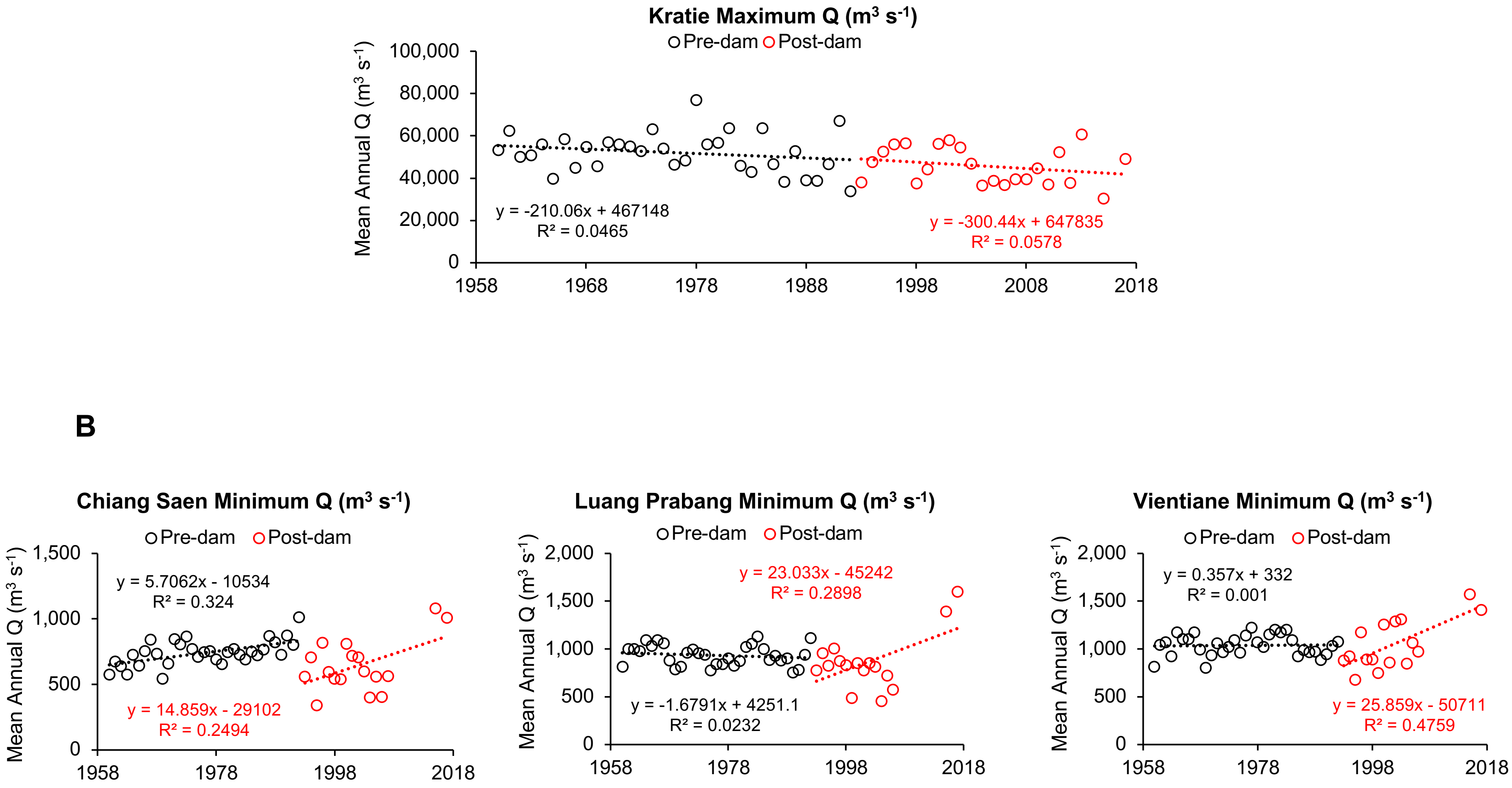

Kratie Maximum WL (m)
Pre-dam
Post-dam
Mean Annual WL (m)
y = -0.0209x + 62.101
R² = 0.029
y = -0.0266x + 74.493
R² = 0.0178
0
5
10
15
20
25
1958
1978
1998
2018
Prek Kdam Maximum WL (m)
Pre-dam
Post-dam
Mean Annual WL (m)
y = -0.02x + 48.312
R² = 0.0906
y = -0.058x + 124.93
R² = 0.1507
0
5
10
15
1958
1978
1998
2018
Phnom Penh Port Maximum WL (m)
Pre-dam
Post-dam
Mean Annual WL (m)
y = -0.0226x + 53.503
R² = 0.14
y = -0.0503x + 109.58
R² = 0.1243
0
5
10
15
1958
1978
1998
2018
B
Chiang Saen Minimum WL (m)
Pre-dam
Post-dam
Mean Annual WL (m)
y = 0.0179x - 34.652
R² = 0.5741
y = 0.0577x - 114.13
R² = 0.379
0
1
2
3
4
1958
1978
1998
2018
Luang Prabang Minimum WL (m)
Pre-dam
Post-dam
Mean Annual WL (m)
y = 0.0047x - 6.1627
R² = 0.0402
y = 0.0505x - 97.899
R² = 0.2053
0
2
4
6
8
1958
1978
1998
2018
Vientiane Minimum WL (m)
Pre-dam
Post-dam
Mean Annual WL (m)
y = 0.0178x - 34.737
R² = 0.4737
y = 0.0254x - 50.266
R² = 0.085
-1
0
1
2
3
4
1958
1978
1998
2018

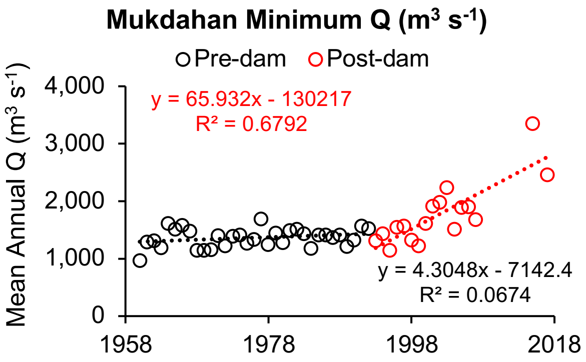

Mukdahan Minimum WL (m)
○Pre-dam ○Post-dam
y = 0.0051x - 8.8752
R² = 0.1454
y = 0.0468x - 92.331
R² = 0.2282
Mean Annual WL (m)
0
1
2
3
4
1958
1978
1998
2018
Pakse Minimum WL (m)
○Pre-dam ○Post-dam
y = 0.0079x - 15.013
R² = 0.2968
y = 0.0469x - 92.905
R² = 0.2373
Mean Annual WL (m)
0
1
2
3
4
1958
1978
1998
2018
Stung Treng Minimum WL (m)
○Pre-dam ○Post-dam
y = 0.0115x - 20.899
R² = 0.3488
y = 0.0369x - 71.832
R² = 0.3064
Mean Annual WL (m)
0
2
4
6
1958
1978
1998
2018
Kratie Minimum WL (m)
○Pre-dam ○Post-dam
y = 0.0571x - 108.21
R² = 0.5952
y = 0.0172x - 28.723
R² = 0.0772
Mean Annual WL (m)
0
2
4
6
8
1958
1978
1998
2018
Prek Kdam Minimum WL (m)
○Pre-dam ○Post-dam
y = 0.006x - 11.231
R² = 0.1559
y = 0.0138x - 26.842
R² = 0.08
Mean Annual WL (m)
0
1
2
3
1958
1978
1998
2018
Phnom Penh Port Minimum WL (m)
○Pre-dam ○Post-dam
y = 0.0068x - 12.826
R² = 0.1673
y = 0.0081x - 15.487
R² = 0.1261
Mean Annual WL (m)
0
1
1
2
1958
1978
1998
2018

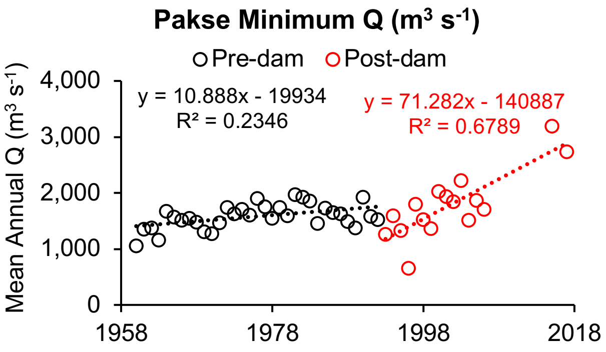
APPENDIX 2

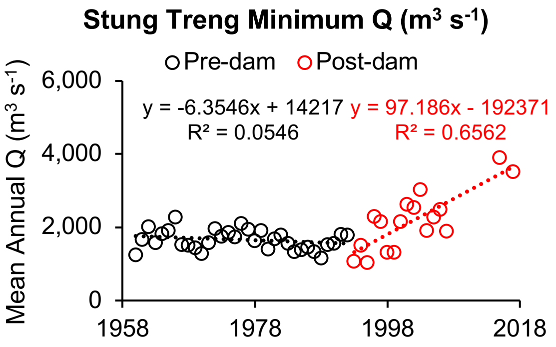
Time-series (1960-2017) analysis of the mean annual **(A)** maximum and **(B)** minimum discharge (m^3 s^{-1}) variations, for the pre and post-dam development (i.e. based on the 1993 Manwan dam operation start), along the Mekong basin's gauging stations.

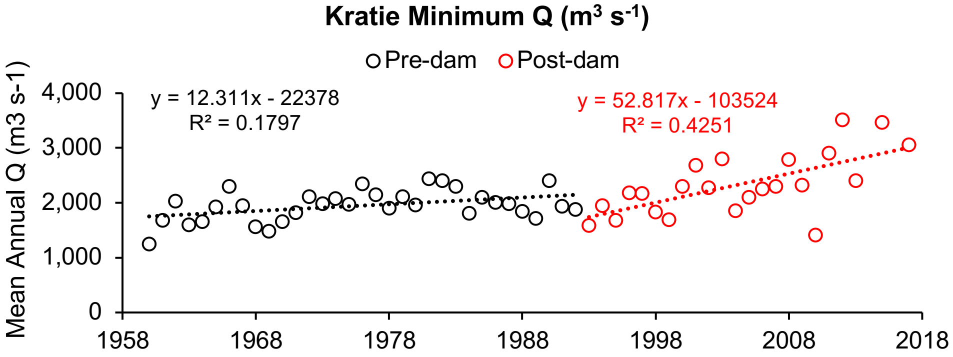



Kratie Maximum Q (m^3 s^{-1})
○Pre-dam ○Post-dam
Mean Annual Q (m^3 s^{-1})
100,000
80,000
60,000
40,000
20,000
0
1958
1968
1978
1988
1998
2008
2018
y = -210.06x + 467148
R² = 0.0465
y = -300.44x + 647835
R² = 0.0578
B
Chiang Saen Minimum Q (m^3 s^{-1})
○Pre-dam ○Post-dam
Mean Annual Q (m^3 s^{-1})
1,500
1,000
500
0
1958
1978
1998
2018
y = 5.7062x - 10534
R² = 0.324
y = 14.859x - 29102
R² = 0.2494
Luang Prabang Minimum Q (m^3 s^{-1})
○Pre-dam ○Post-dam
Mean Annual Q (m^3 s^{-1})
2,000
1,500
1,000
500
0
1958
1978
1998
2018
y = 23.033x - 45242
R² = 0.2898
y = -1.6791x + 4251.1
R² = 0.0232
Vientiane Minimum Q (m^3 s^{-1})
○Pre-dam ○Post-dam
Mean Annual Q (m^3 s^{-1})
2,000
1,500
1,000
500
0
1958
1978
1998
2018
y = 0.357x + 332
R² = 0.001
y = 25.859x - 50711
R² = 0.4759


Mukdahan Minimum Q (m³ s⁻¹)
○Pre-dam ○Post-dam
Mean Annual Q (m³ s⁻¹)
4,000
3,000
2,000
1,000
0
1958
1978
1998
2018
y = 65.932x - 130217
R² = 0.6792
y = 4.3048x - 7142.4
R² = 0.0674


Pakse Minimum Q (m³ s⁻¹)
○Pre-dam ○Post-dam
Mean Annual Q (m³ s⁻¹)
4,000
3,000
2,000
1,000
0
1958
1978
1998
2018
y = 10.888x - 19934
R² = 0.2346
y = 71.282x - 140887
R² = 0.6789


Stung Treng Minimum Q (m³ s⁻¹)
○Pre-dam ○Post-dam
Mean Annual Q (m³ s⁻¹)
6,000
4,000
2,000
0
1958
1978
1998
2018
y = -6.3546x + 14217
R² = 0.0546
y = 97.186x - 192371
R² = 0.6562


Kratie Minimum Q (m³ s⁻¹)
○Pre-dam ○Post-dam
Mean Annual Q (m3 s-1)
4,000
3,000
2,000
1,000
0
1958
1968
1978
1988
1998
2008
2018
y = 12.311x - 22378
R² = 0.1797
y = 52.817x - 103524
R² = 0.4251

APPENDIX 3

Commissioned dams in the Mekong River basin (installed capacity more than 20 MW) with information of the country, location, year of approval, installed capacity (MW) and height (m; adapted from: MRC, 2009; *International Rivers, 2013).

Dam name	Country	Location	Year	Installed capacity (MW)	Height (m)
Manwan*	China	Mekong	2007	1,550	126
Dachaoshan*	China	Mekong	2003	1,350	118
Gongguoqiao*	China	Mekong	2012	900	130
Jinghong*	China	Mekong	2009	1,750	118
Xiaowan*	China	Mekong	2010	4,200	292
Nuozhadu*	China	Mekong	2012	5,850	262
Nam Ngum 1	Lao PDR	Nam Ngum	1971	148.7	75
Nam Ngum 2	Lao PDR	Nam Ngum	2011	615	181
Theun-Hinboun	Lao PDR	Theun/Hinboun	1998	220	38
Xeset 1	Lao PDR	Xeset	1994	45	16
Xeset 2	Lao PDR	Xeset	2009	76	16
Nam Mang 3	Lao PDR	Nam Mang	2004	40	28
Houay Ho	Lao PDR	Houayho/Xekong	1999	150	76.5
Nam Leuk	Lao PDR	Leuk/Nam Ngum	2000	60	45
Nam Lik 2	Lao PDR	Lik	2010	103	328
Nam Theun 2	Lao PDR	Theun/Xe Bang Fai	2010	1,075	48
Ubol Ratana	Thailand	Nam Pong	1966	25.2	35.1
Sirindhorn	Thailand	Lam Dom Noi	1971	36	42
Chulabhom	Thailand	Nam Phrom	1972	40	70
Pak Mun	Thailand	Mun	1994	136	17
Lam Ta Khong	Thailand	Lam Ta Khong	2002	500	40.3
Yali Falls	Vietnam	Se San	2001	720	65
Sesan 3	Vietnam	Se San	2006	79	79
Sesan 3A	Vietnam	Se San	2007	96	35
Plei Krong	Vietnam	Se San/Kroong Po Ko	2008	100	65
Buon Kuop	Vietnam	Se Prok	2009	280	34
Buon Tua Sra	Vietnam	Se San/Kroong Po Ko	2009	86	83
Sesan 4	Vietnam	Se San	2009	360	60
Sre Pok 3	Vietnam	Se Prok	2009	220	52.5
Total				**10,611**	

Dams proposed for the LMB mainstream (adapted from: ICEM, 2010).

Dam name	Country	Installed capacity (MW)	Height (m)
Pak Beng	Lao PDR	1,230	76
Luang Prabang	Lao PDR	1,410	68
Xayaburi	Lao PDR	1,260	32
Pak Lay	Lao PDR	1,320	35
Sanakham	Lao PDR	700	38
Pak Chom	Lao PDR	1,079	1,200
Ban Koum	Lao PDR	1,872	53
Lat Sua	Lao PDR	686	27
Don Sahong	Lao PDR	240	10.6
Stung Treng	Cambodia	980	22
Sambor	Cambodia	2,600	56
Total		**13,377**	

Power demands (MW) in 2005, 2010, 2015 and 2020 in the LMB (adapted from: MRC State of Basin Report, 2010).

Country	2005	2010	2015	2020
Cambodia	302	407	699	-------
Lao PDR	291	648	1,216	1,487
Thailand	20,538	25,612	33,897	44,695
Vietnam	9,255	20,000	31,495	50,000
Total	**30,386**	**46,667**	**67,307**	**96,182**

APPENDIX 4

Information on the Landsat-TM image acquisition year, month (01 – January; 02 – February; 03 – March; 04 – April; 05 – May; 06 – June; 07 – July; 08 – August; 09 – September; 10 – October; 11 – November and 12 – December), Landsat Programme and band resolution (meters).

Year	Months	Landsat Programme	Resolution (m)
1996	02; 04; 05; 08; 10	Landsat 5	30
1997	01; 03; 05; 08; 12	Landsat 5	30
1998	02; 04; 05; 07; 11	Landsat 5	30
1999	01; 03; 05; 07; 12	Landsat 5	30
2000	02; 04; 06; 08; 11	Landsat 5	30
2001	01; 04; 06; 09; 11	Landsat 5	30
2002	01; 03; 05; 07; 11	Landsat 7	30
2003	01; 03; 05; 08; 12	Landsat 7	30
2004	02; 04; 07; 10; 12	Landsat 5	30
2005	02; 04; 07; 09; 11	Landsat 5	30
2006	01; 03; 05; 08; 12	Landsat 5	30
2007	02; 04; 06; 07; 12	Landsat 5	30
2008	02; 04; 06; 08; 12	Landsat 5	30
2009	02; 04; 07; 09; 12	Landsat 5	30
2010	02; 04; 07; 10; 12	Landsat 5	30
2011	02; 07; 10; 11	Landsat 7	30
2012	01; 05; 07; 11	Landsat 7	30
2013	01; 03; 05; 07; 12	Landsat 7 and 8	30
2014	02; 04; 07; 11	Landsat 8	30

*Information on the day of each Landsat-TM image collection was recorded, but for the sake of simplicity in stating it in this table, only months are reported here.

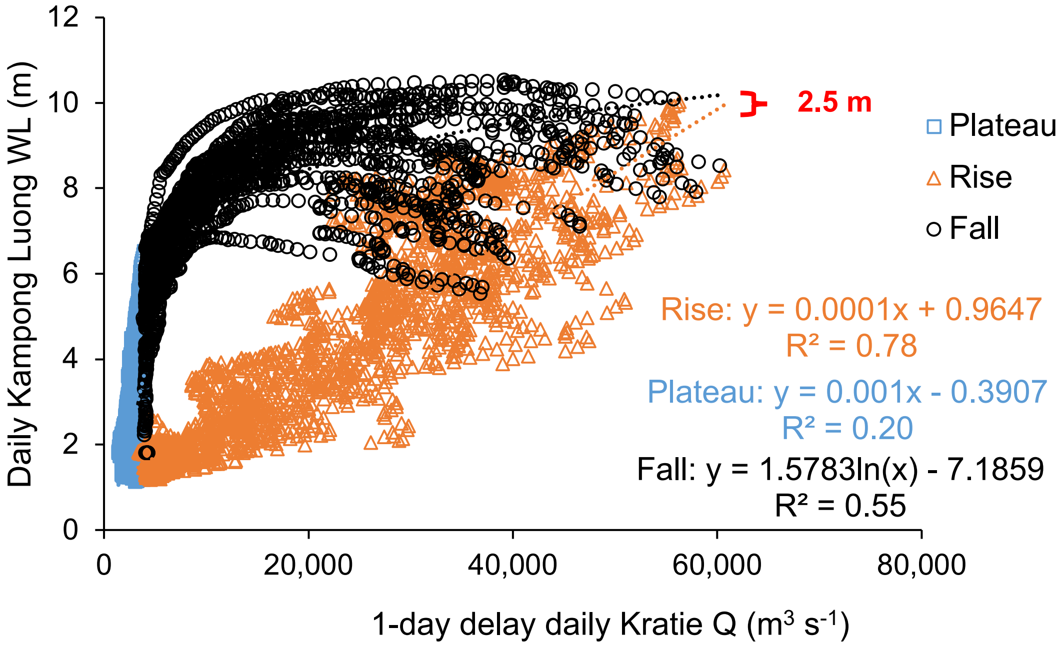
APPENDIX 5

Oceanic Niño Index (ONI) by years and three months measurements, showing years with extreme El Niño events (red) and years with extreme La Niña events (blue) (source: NOAA website).

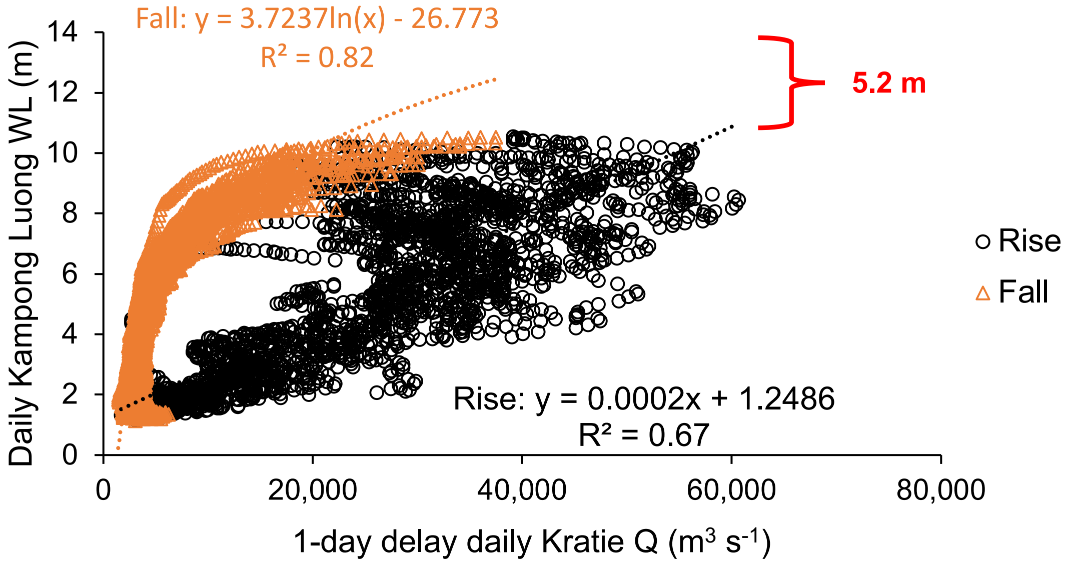
Year	DJF	JFM	FMA	MAM	AMJ	MJJ	JJA	JAS	ASO	SON	OND	NDJ
1996	-0.9	-0.8	-0.6	-0.4	-0.3	-0.3	-0.3	-0.3	-0.4	-0.4	-0.4	-0.5
1997	-0.5	-0.4	-0.1	0.3	0.8	1.2	1.6	1.9	2.1	2.3	2.4	2.4
1998	2.2	1.9	1.4	1	0.5	-0.1	-0.8	-1.1	-1.3	-1.4	-1.5	-1.6
1999	-1.5	-1.3	-1.1	-1	-1	-1	-1.1	-1.1	-1.2	-1.3	-1.5	-1.7
2000	-1.7	-1.4	-1.1	-0.8	-0.7	-0.6	-0.6	-0.5	-0.5	-0.6	-0.7	-0.7
2001	-0.7	-0.5	-0.4	-0.3	-0.3	-0.1	-0.1	-0.1	-0.2	-0.3	-0.3	-0.3
2002	-0.1	0	0.1	0.2	0.4	0.7	0.8	0.9	1	1.2	1.3	1.1
2003	0.9	0.6	0.4	0	-0.3	-0.2	0.1	0.2	0.3	0.3	0.4	0.4
2004	0.4	0.3	0.2	0.2	0.2	0.3	0.5	0.6	0.7	0.7	0.7	0.7
2005	0.6	0.6	0.4	0.4	0.3	0.1	-0.1	-0.1	-0.1	-0.3	-0.6	-0.8
2006	-0.8	-0.7	-0.5	-0.3	0	0	0.1	0.3	0.5	0.7	0.9	0.9
2007	0.7	0.3	0	-0.2	-0.3	-0.4	-0.5	-0.8	-1.1	-1.4	-1.5	-1.6
2008	-1.6	-1.4	-1.2	-0.9	-0.8	-0.5	-0.4	-0.3	-0.3	-0.4	-0.6	-0.7
2009	-0.8	-0.7	-0.5	-0.2	0.1	0.4	0.5	0.5	0.7	1	1.3	1.6
2010	1.5	1.3	0.9	0.4	-0.1	-0.6	-1	-1.4	-1.6	-1.7	-1.7	-1.6
2011	-1.4	-1.1	-0.8	-0.6	-0.5	-0.4	-0.5	-0.7	-0.9	-1.1	-1.1	-1
2012	-0.8	-0.6	-0.5	-0.4	-0.2	0.1	0.3	0.3	0.3	0.2	0	-0.2
2013	-0.4	-0.3	-0.2	-0.2	-0.3	-0.3	-0.4	-0.4	-0.3	-0.2	-0.2	-0.3
2014	-0.4	-0.4	-0.2	0.1	0.3	0.2	0.1	0	0.2	0.4	0.6	0.7

APPENDIX 6

- Baseline (1996-2014) observed Kratie's one-day delay daily discharge relation to the observed daily Kampong Luong water level, with a plateau stage:



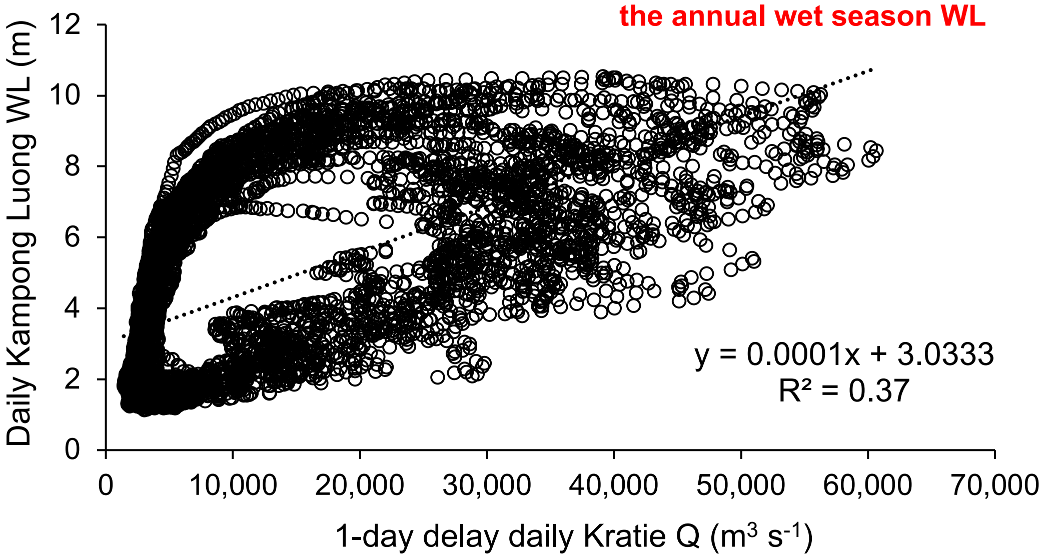
- Baseline (1996-2014) observed Kratie's one-day delay daily discharge relation to the observed daily Kampong Luong water level, following a water level peak flood:



- Baseline (1996-2014) observed Kratie's one-day delay daily discharge relation to the observed daily Kampong Luong water level, applying a single annual linear and non-linear regression fit:

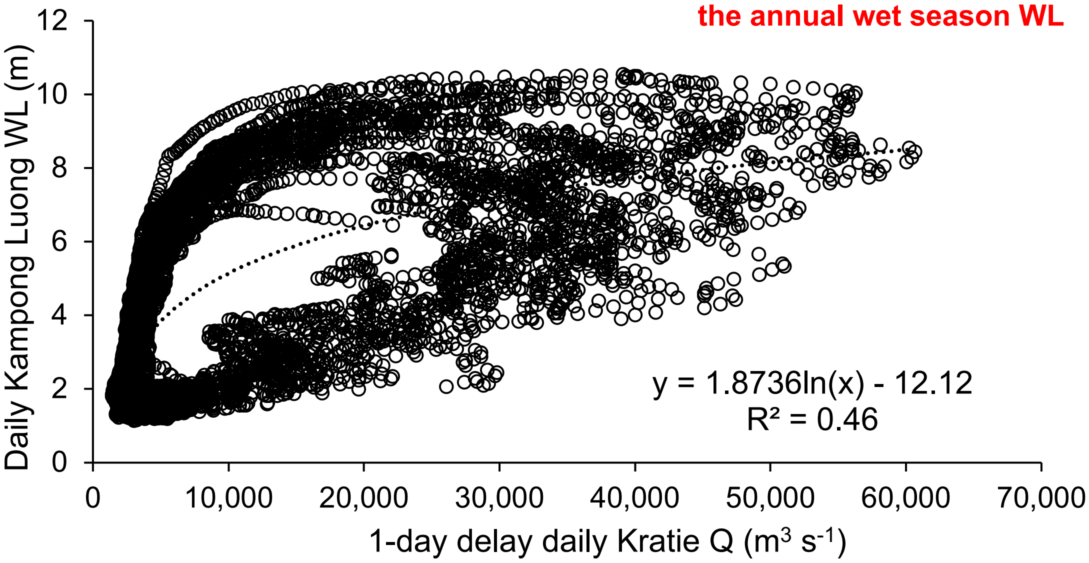
  - Linear Regression fit:

3.3 m under estimation of the annual wet season WL

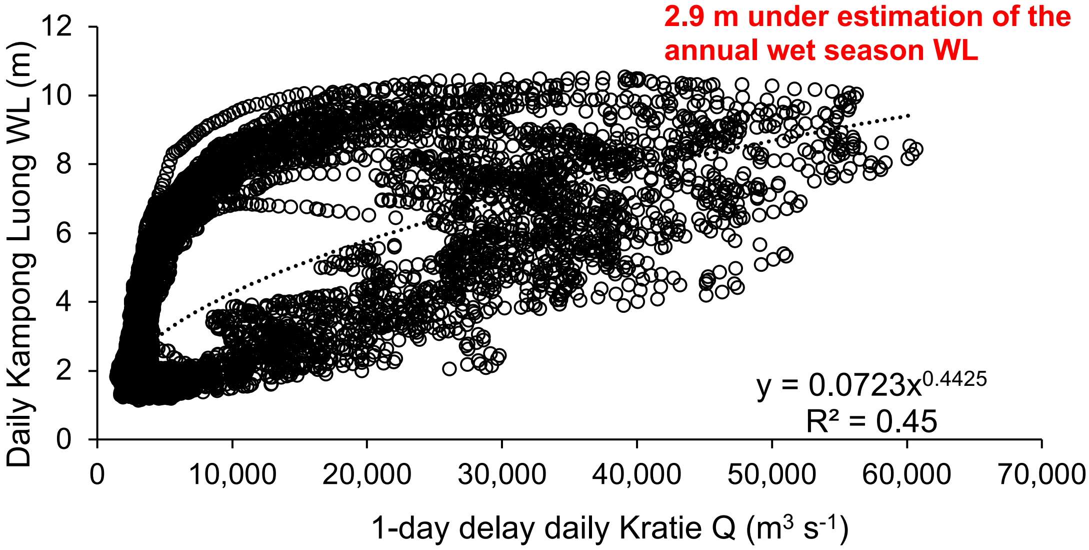


  - Logarithmic Regression fit:

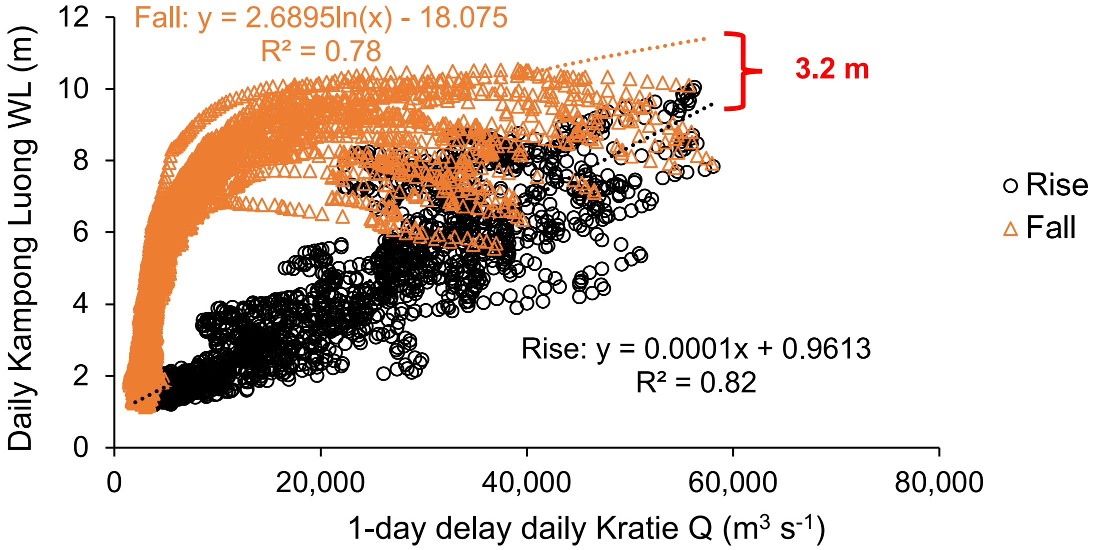
2.7 m under estimation of the annual wet season WL



  - Power Regression fit:



- ➢ Baseline (1996-2014) observed Kratie's one-day delay daily discharge relation to the observed daily Kampong Luong water level, following the hydrologic year approach:



APPENDIX 7

Wet season – September 2016			
Sample ID	**Location**	**GPS coordinates**	**Depth Profile**
Vientine	Mekong River	17°57'57.50"N 102°34'2.26"E	Surface: 10-20 cm Bottom: 8 m
Nam Ou	Tributary	20°7'59.33"N 102°18'32.66"E	Surface: 10-20 cm Bottom: 4 m
Luang Prabang	Mekong River	19°55'59.86"N 102°10'1.24"E	Surface: 10-20 cm Bottom: 10 m
Pakse	Mekong River	15°8'30.98"N 105°44'50.23"E	Surface: 10-20 cm Bottom: 12 m
Stung Treng	Mekong River	13°34’17.05’’N 105°58’57.00’’E	Surface: 10-20 cm Bottom: 15 m
Se Kong	Tributary	13°33’53.27’’N 106°3’35.32’’E	Surface: 10-20 cm Bottom: 9 m
Se San	Tributary	13°36’3.75’’N 106°19’48.40’’E	Surface: 10-20 cm
Se Prok	Tributary	13°30’26.93’’N 106°20’19.83’’E	Surface: 10-20 cm Bottom: 7 m
Kratie	Mekong River	12°46'46.74"N 105°56'47.95"E	Surface: 10-20 cm Bottom: 17 m
Phnom Penh	Mekong River	11°38’55.44’’N 104°57’15.24’’E	Surface: 10-20 cm Bottom: 14 m
Tonle Sap River	Tributary	11°40´12.39’’N 104°51’33.62’’E	Surface: 10-20 cm Bottom: 10 m
Tonle Sap Lake Site 1	Lake	13°10’45.93’’N 103°56’40.13’’E	Surface: 10-20 cm Bottom: 8 m
Tonle Sap Lake Site 2	Lake	13°3’34.15’’N 103°54’.31.39’’E	Surface: 10-20 cm Bottom: 12 m

Dry Season – January 2017			
Sample ID	**Location**	**GPS coordinates**	**Depth Profile**
Stung Treng	Mekong River	13°34’17.05’’N 105°58’57.00’’E	Surface: 10-20 cm Bottom: 8 m
Se Kong	Tributary	13°33’53.27’’N 106°3’35.32’’E	Surface: 10-20 cm Bottom: 6 m
Se San	Tributary	13°36’3.75’’N 106°19’48.40’’E	Surface: 10-20 cm Bottom: 6 m
Se Prok	Tributary	13°30’26.93’’N 106°20’19.83’’E	Surface: 10-20 cm Bottom: 5 m
Kratie	Mekong River	12°46'46.74"N 105°56'47.95"E	Surface: 10-20 cm Bottom: 9 m
Phnom Penh	Mekong River	11°38’55.44’’N 104°57’15.24’’E	Surface: 10-20 cm Bottom: 10 m
Tonle Sap River	Tributary	11°40´12.39’’N 104°51’33.62’’E	Surface: 10-20 cm Bottom: 7 m
Tonle Sap Lake Site 1	Lake	13°10’45.93’’N 103°56’40.13’’E	Surface: 10-20 cm Bottom: 4 m
Tonle Sap Lake Site 2	Lake	13°3’34.15’’N 103°54’.31.39’’E	Surface: 10-20 cm Bottom: 5 m

APPENDIX 8

eDNA metabarcoding species composition (percentage of read counts) between sites, according to depth profile (i.e. surface and deep) and season (i.e. wet and dry season).