Laterality, reproduction and parental care: an investigation in fish

This thesis is submitted for the degree of Doctor of
Philosophy, Biological and Marine Sciences,
University of Hull

by

Stephanie Eilidh McLean
BSc (Hons), University of Edinburgh
MRes, University of Glasgow

August 2021

For my mum,

I hope you are proud of all that I have achieved.

Keep smiling down on me!

Declaration of authorship

I declare that the work herein is intellectually my own. Each data chapter received contributions from my supervisor (Lesley Morrell, LM), collaborators within the parental cluster at the University of Hull (James Gilbert, JG; Isabella Capellini, IC; Andrew Furness, AF) and the animal care technicians (Alan Smith, AS; Vic Sweetez, VS) as follows:

Chapter 2

I led the experiment and performed all data collection and subsequent analyses. LM helped co-design the experiment and VS aided with experimental apparatus construction. I wrote the first draft of each chapter and performed subsequent editing, following advice from my supervisor (LM) and collaborators (IC; AF).

Chapter 3

I led the experiment and performed all data collection and subsequent analyses. LM helped co-design the experiment and VS aided with experimental apparatus construction. I wrote the first draft of each chapter and performed subsequent editing, following advice from my supervisor (LM).

Chapter 4

I led the experiment and performed all data collection and subsequent analyses. LM helped co-design the experiment and VS aided with experimental apparatus construction. I wrote the first draft of each chapter and performed subsequent editing, following advice from my supervisor (LM).

Chapter 5

I led the experiment, which was co-designed with LM. I performed data collection, aided by AS who provided assistance and expertise with fish breeding, and subsequent data analyses. I wrote the first draft of each chapter and performed subsequent editing, following advice from my supervisor (LM).

Chapter 6

I led the experiment, which was co-designed with LM. I performed data collection, aided by AS who provided assistance and expertise with fish breeding, and subsequent data analyses. I wrote the first draft of each chapter and performed subsequent editing, following advice from my supervisor (LM).

Additionally, the work contained in this thesis has been published.

Chapter 2 published as: McLean, S., & Morrell, L. J. (2020). Consistency in the strength of laterality in male, but not female, guppies across different behavioural contexts. *Biology letters*, *16*(5), 20190870.

Chapter 3 published as: McLean S & Morrell L. J. (2021) Sex Differences in Laterality Are Associated with Reproduction in Threespine Stickleback. *The American Naturalist*, *197*(6).

Please note that chapter 2 differs from the published manuscript as it contains an additional aim (confirming the value of the 'detour test' as a method to examine laterality experimentally) that was not relevant to the research questions proposed in the published manuscript but that is relevant for the thesis as a whole. Additionally, the colour scheme of the graphs in chapters 2 and 3 have been altered from the published version to align with the rest of the thesis presentation.

I further declare that no part of this work has been submitted as part of any other degree. This copy has been supplied on the understanding that it is copyright material and that no quotation from the thesis may be published without proper acknowledgment.

Stephanie McLean, 2021

Acknowledgements

The research within this thesis was made possible by University of Hull funding. The guidance and support of my supervisor, Lesley Morrell, really contributed to making the research within this thesis what it was and for that I am extremely grateful. Working with you was like working with a friend: direction and assistance were there when needed but not without freedom, support and inspiration to make the thesis my own. With a few laughs along the way of course - I really couldn’t have asked for more!

I am also extremely grateful to my secondary supervisors James Gilbert and Isabella Capellini and the rest of the Parental Care Cluster at Hull, Andrew Furness and Yannis Dimopoulos. Your support, advice and thought provoking conversations were greatly appreciated.

I would like to extend my thanks to the technical staff, Alan, Vic, Sonia and Rose, who ensured the aquariums were well looked after and that the fish had all that they needed. Additionally, the knowledge and help Alan provided with fish behaviour and breeding and Vic’s handyman skills were invaluable.

To my friends at Hull, Emma Peasland, Andrew Furness, Yannis Dimopoulous, Stuart Nattrass, Bethany Muller, Branden Kusanto, Nawel Zaibi, Graham Sellers, Mike Winter, Marco Benuucci, Lynsey Harper and Rob Donnelly you provided support that I didn’t know I needed to get through this journey. Thank you for your friendship, encouragement and laughter. A special thanks to Emma, Andrew, Stuart and Yannis for being great office companions.

To Cristina Di Muri and Nathan Griffiths, I would not have been able to do this without you – you were my life and soul in Hull. I am so incredibly grateful to have met both of you and am so lucky that with you both I know I have made friends for life.

I would also like to share my appreciation for my best friends from home, Rebecca McEwan, Alyssa Jones, and Kelsey Wilson who have supported me on my academic journey, especially during this PhD from a distance with so much love and laughter. You are all wonderful humans!

A special thanks is needed for Stacey Lorimer, without whose friendship I probably would have never considered undertaking a PhD. Since we met during our BSc we have been inseparable and although you may not know it, you inspired me and really helped me explore my love for science. Not only that, you have been an endless lifeline of support. You truly are one in a million.

I am very fortunate to have an amazing other half, Max Evans, who has been on this emotional roller coaster with me and not once complained. For that I owe you everything. Max, you have provided me (and Chia) with a home, looked after me and kept me alive, encouraged me to ensure that I strive to do my best and provided an unconditional amount of love and support, especially on tougher days. I am also fortunate to have my fur baby Chia, who is an endless source of happiness who has provided unlimited snuggles and walkies, which were a welcomed distraction.

Most importantly I would like to thank my family because without them I would not have completed this PhD. To my sister Natalie I thank you for teaching me to aim high, believe in myself and never give up. You have been by my side through thick and thin and have always been there when I've needed you. You are the reason I have made it to where I am today. To my dad, Iain, you have provided invaluable support both emotionally and financially when needed. You have always ensured I stayed on the right path in life and have been by my side for every achievement or hurdle that I have faced. You have also read every thesis I have submitted to date and pretended like you understand everything in it and for that you deserve a medal!

Last, but by no means least I would like to acknowledge my late mum, Joyce, and gran, GG. You have both always been my biggest supporters and have always let me know how proud you are of me. Gran, I loved having you by my side for every step of this journey from watching me graduate and asking if you needed to wear a hat to the ceremony to reading every paper I've published to see what I've been working on and wanting to share it with your friends. Our weekly phone calls were a lifeline and source of solace for me and something I have missed significantly in the recent months.

Mum, my life could have taken a very different path when I lost you, but I am grateful that our relationship made me strong and want a better life for myself. I really hope you are proud of all I have achieved because this one is for you!

Abstract

Cerebral lateralisation or 'laterality', the partitioning of different cognitive functions in specific brain hemispheres, is a selectively advantageous trait that can enhance cognition. The selective advantages of exhibiting laterality are hypothesised to be the primary selective force driving its widespread evolution in both vertebrate and invertebrate taxa. However, substantial variation persists within this trait, particularly between the sexes. The underlying drivers of this variation are poorly understood, with social behaviours, especially those tightly associated with fitness, having received little consideration in this regard. In this thesis, I explored the relationship between cerebral lateralisation at the behavioural level, and reproduction and reproductive social behaviours, specifically parental care.

In the first section of this thesis (chapters 2 - 4), I investigated whether variation in laterality, particularly between the sexes, is associated with reproduction and the performance of reproductive behaviours. In chapter 2, I provide evidence that in a live bearing species (guppies, *Poecilia reticulata)* there is variation in the pattern of laterality exhibited between the sexes, whereby individual males are consistent in the expression of laterality across three different behavioural contexts, while females are not, and instead exhibit substantial within-individual variation.

In chapter 3, I showed that in a uniparental species (threespine stickleback, *Gasterosteus aculeatus*), variation in laterality both between the sexes and within a single sex was attributed to reproduction and variation in individuals experience of performing reproductive behaviours respectively. Males, the caring sex, were more strongly lateralised than the non-caring sex (females) during reproduction, and laterality was reduced outside of the breeding season in males. Additionally, males with experience of mating and performing parental care behaviours were more strongly lateralised than males absent in this experience.

In chapter 4, I explored whether laterality and its associated costs and benefits vary in different social contexts, while reproductive and performing parental care and in a non-reproductive non-social state, in a biparental substrate brooding cichlid fish, *Telmatochromis temporalis*. While I demonstrate no variation in laterality in a reproductive and non-reproductive state, I identified a cost of exhibiting laterality, poorer performance in a task requiring communication and cooperation between the left and right brain hemispheres, that is consistent regardless of sex or social context.

In the latter section of this thesis (chapters 5 and 6), I assessed laterality in a fitness related reproductive social behaviour, parental care. Specifically, I explored whether parental care behaviours are lateralised in *T. temporalis* (chapter 5) and whether any biases in such behaviours are flexible when the selective pressures thought to select for laterality are altered (chapter 6). I provide the first evidence that parental care behaviours, specifically brood defence behaviour and visual hemisphere use during care, are lateralised in fish (chapter 5), and report that lateral biases in parental care behaviours may be flexible under differing selective pressures, here social and predation pressures, that result from experimental removal of the female biparental parent (chapter 6).

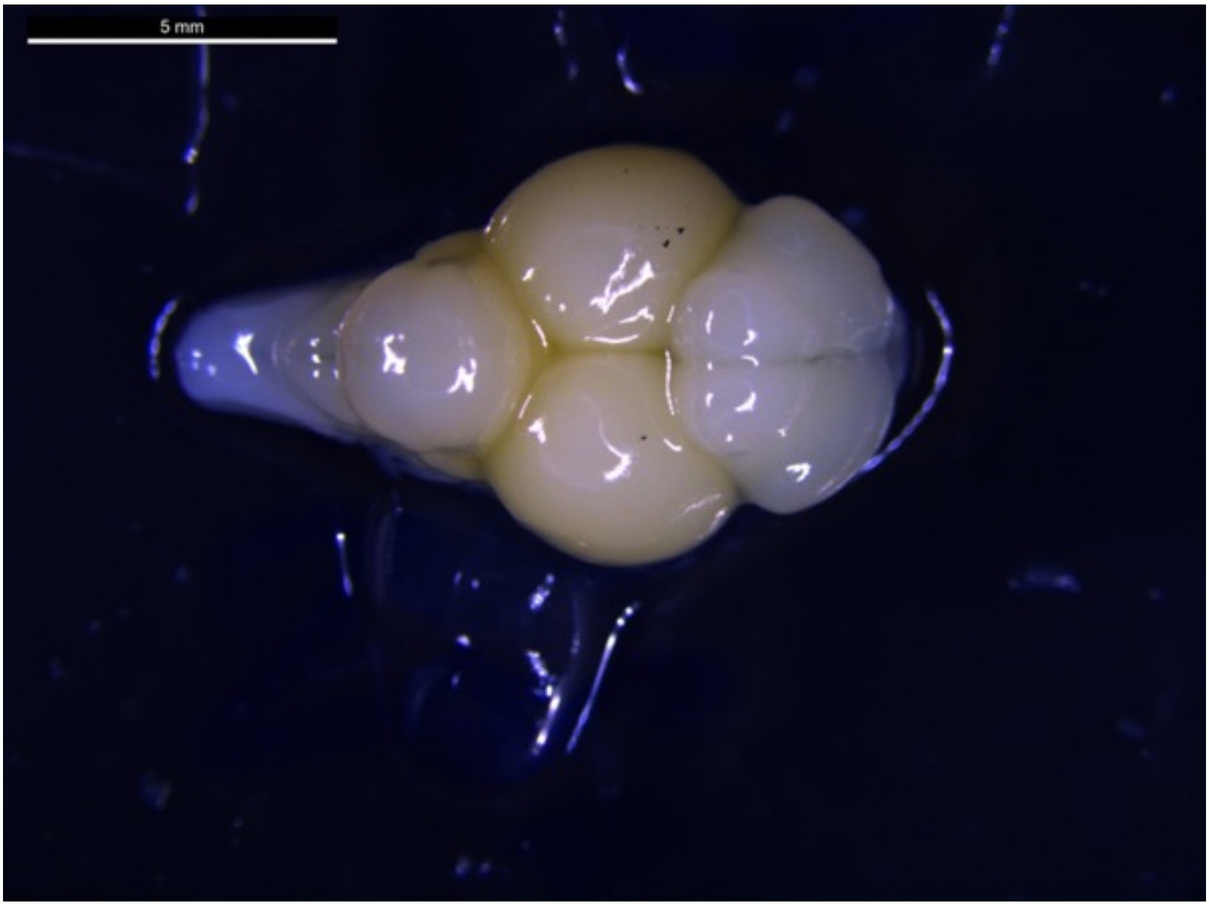
Together, my findings suggest that reproduction and reproductive behaviours, particularly parental care, represent key but previously unidentified drivers of variation in laterality both within a sex and between the sexes. These findings provide new insights that help further our understanding of how variation in cerebral lateralisation evolved and why it may be maintained.

Table of Contents

List of tables

List of figures

Chapter 1: General Introduction



Dorsal view of a 1 year old male *Dimidiochromis compressiceps* brain

© user: Tiffany Armstrong | Personal photo

1.1 What is cerebral lateralisation?

Cerebral lateralisation or 'laterality' is the phenomenon where brain hemispheres are asymmetrical either structurally or functionally (Warren 1980; Walker 1980; Bisazza et al. 1998a). Originally thought to be associated with the evolution of complex cognitive processes such as language and tool use, cerebral lateralisation was thought to be a trait unique to humans (Bisazza et al. 1998a; Harris 2000). However, a century after it was first discovered, functional laterality was demonstrated in a non-human animal, the Chaffinch (*Fringilla coelebs*; Nottebohm 1971). In the past 50 years evidence of cerebral lateralisation across the animal kingdom has grown vastly and has now been widely documented in all major taxonomic groups, including mammals (Bradshaw and Rodgers 1993; Walker 1980), birds (reviewed in Güntürkün 1997), fish, amphibians, reptiles (reviewed in Bisazza et al. 1998a), and invertebrates (reviewed in Frasnelli et al. 2012; Niven and Bell 2018).

There are two main forms of laterality: structural and functional asymmetries. Structural asymmetries are physical characteristics that have a skewed distribution or bilateral characteristics that exhibit increased growth on a particular side (Leary and Allendorf, 1989; Van Valen 1962). Studies investigating structural asymmetries have largely considered morphological characteristics and neural structures. For example, the human skull has impressions resulting from the right brain hemisphere being narrower in the anterior region while the left brain hemisphere is wider in the posterior region (Bradshaw 1989). In animals, males of the four-eyed fish (*Anableps anableps*) exhibit leftward or rightward angling of the gonopodium, the male sex organ, with females also exhibiting asymmetry in the direction of genital opening (Neville 1978). In convict cichlids, *Amatitlania nigrofasciata*, habenular nuclei, highly conserved pathways that connect the forebrain to ventral midbrain (Sutherland 1982) responsible for several functions including learning, memory, and feeding and mating behaviours (Sandyk 1991), are larger in the left than the right brain hemisphere in both sexes (Gutiérrez-Ibáñez et al. 2011).

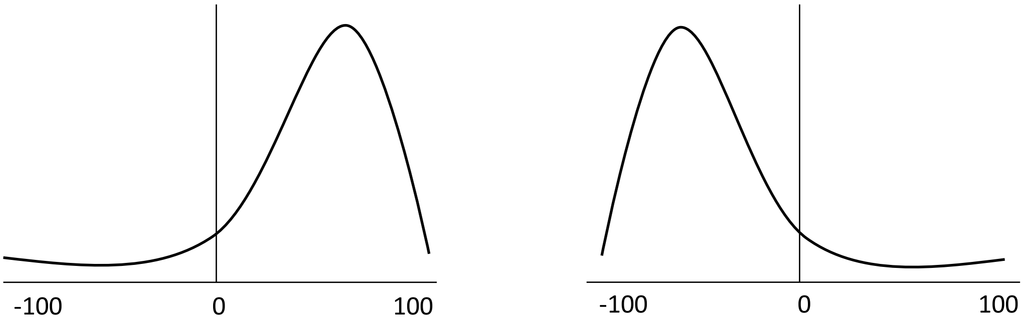
Laterality can also be expressed as functional asymmetries in different sensory modalities that are commonly observed at the behavioural level as side biases in behaviours (Bisazza et al. 2001a), or as use of one side or half of a bilateral characteristic (Koboroff et al. 2008; Brown and Magat 2011a). These behavioural biases stem from cerebral lateralisation and represent underlying asymmetries in cognitive processing and functioning, and the degree of lateralisation of an individual's brain (Vallortigara and Rogers 2005; Bisazza and Brown 2011). In humans, each brain hemisphere controls the opposing side of the body. Speech and language are processed by the left brain hemisphere, while emotion and spatial tasks are processed by the right brain hemisphere (Bisazza et al. 1998a). As a result of left hemisphere control, the right of the mouth moves more than the left during speech, while emotions are more strongly expressed on the left side of the face as a result of right hemisphere control (Wolf and Goodale 1987). In animals, functional asymmetries have been demonstrated in sensory modalities such as olfaction (Westin 1990) and the lateral line organ (de Perera and Braithwaite 2005) but are most predominant in visual lateralisation, which is largely context dependent and variable across animal taxa. In fish, left and right visual hemisphere biases have been observed for social stimuli (Sovrano 2004; Sovrano and Andrew 2006) and predator inspection (Facchin et al. 1999) respectively, while in toads (*Bufo* spp) left and right visual hemisphere biases are exhibited in predator inspection (Lippolis et al. 2002) and foraging contexts (Vallortigara et al. 1998) respectively.

Laterality, whether evident functionally or structurally, is most commonly measured using a relative lateralisation index, L_R = [(right-left)/(right+left) x100] and can occur at two levels: the population and the individual (Lehman 1981; Bisazza et al. 1997; Figure 1.1). Population level laterality occurs when the majority of individuals in a population (>50%) exhibit the same lateral bias i.e. the majority of individuals are lateralised in a specific direction (left or right; Denenberg 1981; Rogers 2002; Figure 1.1A) and is represented by the relative laterality index (L_R), which ranges from -100 to 100 representing a population exhibiting consistent leftward and rightward biases respectively (Bisazza et al. 1997). On the other hand, individual level laterality results when individuals exhibit a consistent lateral bias, irrespective of direction, meaning a population can be composed of both lateral phenotypes (Denenberg 1981; Rogers 2002; Figure 1.1B). Individual level laterality represents the strength of lateral preferences and is measured using the absolute laterality index (L_A), calculated as $|L_R|$, which ranges from 0 (individuals exhibiting an equal left and right preference) to 100 (individuals exhibiting a consistent preference for a specific direction; Bisazza et al. 1997; Figure 1.1B).

1.2 The costs and benefits of cerebral lateralisation

Cerebral lateralisation is a selectively advantageous trait, that enables individuals to better cope with divided attention, the sharing of attention or focus across different tasks or functions, which directly limits an individual's ability to successfully perform tasks or functions (Brown and Bibost 2014). To date, three main selective advantages of cerebral lateralisation have been proposed: an enhanced neural capacity, hemisphere dominance and simultaneous information processing (Vallortigara and Rogers 2005).

A) Directional asymmetry: population level, L_R



B) Antisymmetry: individual level, L_A

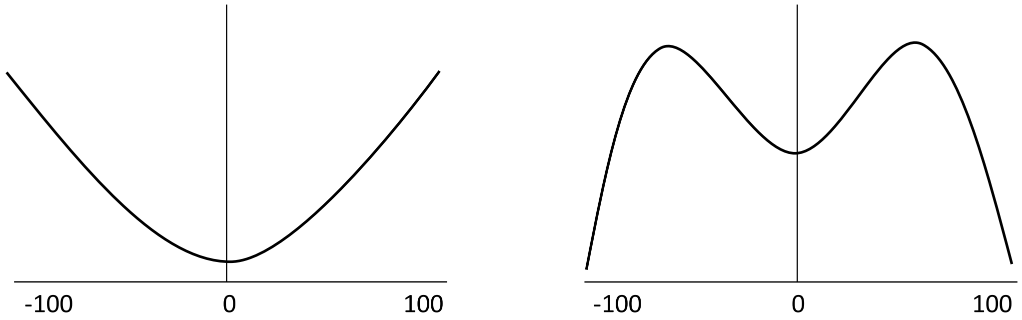


Figure 1. 1: Frequency plots showing examples of A) directional asymmetry at the population level and B) antisymmetry at the individual level. The values on the x-axis represent the lateralisation index [(right-left)/(right+left x 100)], A)L_R and B)L_A. In A), both figures show a population where the majority of individuals are lateralised in the same direction causing an aligned population bias for either the right (-100) or left (100). In B), two forms of antisymmetry are represented in which the majority of individuals in the population are lateralised but there are approximately equal numbers of right and left biased individuals. Figure adapted from Vallortigara and Rogers 2005.

An enhanced neural capacity is thought to result from having one of two possible brain hemispheres available to perform additional functions (Rogers 2002; Vallortigara 2006), thus reducing the need for expensive neural tissue and circuitry associated with repeating the same function in multiple hemispheres (Levy 1977). While there is no experimental evidence to support the mechanism through which an enhanced neural capacity is achieved and it remains theorised, several behaviours across the animal kingdom are processed in a single brain hemisphere, such as prey capture and predator vigilance (Bisazza et al. 1998a).

Lateralised individuals are also said to exhibit hemisphere dominance, which decreases interference between differing functions (Rogers 2002; Vallortigara 2006), reducing the likelihood of conflicting responses from different brain hemispheres to stimuli visualised by organisms with laterally placed eyes (Andrew 1991; Vallortigara 2000). This could speed up neural processing by avoiding slow inter-hemispheric interactions or by allowing more efficient parallel processing (Levy 1969). In pigeons (*Columba livia domestica*), a species with left brain hemisphere dominance for visual object processing (Rogers 1996), the degree of lateralisation was positively related to performance in visual object discrimination in a pebble-grit discrimination task, whereby enhanced performance was related to right eye dominance in the majority of birds. Lateralisation of visual hemisphere use in pigeons is suggested to increase the efficiency of object recognition processes resulting from concentrating them in a single brain hemisphere, thus preventing conflicting responses and search sequences in the opposing hemisphere (Güntürkün et al. 2000).

Additionally, laterality is also suggested to increase the efficiency of information processing since it permits the partitioning of different information types into specific and unique brain hemispheres, allowing separate and parallel processing of information, thus enhancing the brains ability to conduct simultaneous information processing (Rogers 2000; 2002; Vallortigara 2006). There is experimental evidence of simultaneous information processing, colloquially known as ‘multi-tasking’, in goldenbelly top minnows, *Girardinus falcatus*. In a situation where attention was shared between two simultaneous tasks, predator vigilance and prey capture, lateralised individuals were twice as fast at non-lateralised individuals at prey capture in the presence of a predator but no differences were evident when a predator was absent. This difference resulted from lateralised individuals viewing prey and predators in different eyes, thus using separate and parallel brain hemispheres to process information simultaneously (Dadda and Bisazza 2006a). Thought to result from the selective advantages of cerebral lateralisation, individuals who are lateralised have been found to outcompete their non-lateralised counterparts in a number of behaviours including spatial reorientation (Sovrano et al. 2005), numerical discrimination (Dadda et al. 2015), foraging while simultaneously performing predator inspection (Dadda and Bisazza 2006a), termite fishing (McGrew and Marchant 1999), visual discrimination (Gunturkun et al. 2000) and schooling (Bisazza and Dadda 2005).

Cerebral lateralisation, however, is also associated with costs. The physical world is extremely unpredictable in terms of predator-prey interactions, social interactions and foraging opportunities whereby stimuli can present on either the left or right side of a focal individual. One proposed cost of cerebral lateralisation is that it makes individuals’ behaviour more predictable. For example, consistent directional biases likely increase the predictability of an individual from an ecological perspective, which could be especially detrimental in situations where such biases could be learned or exploited (Rogers 2002; Ghirlanda and Vallortigara 2004). This is more likely when lateral biases are strong and in situations where fitness related behaviours are compromised, such as predator-prey interactions (Deckel 1995; Lippolis et al. 2002; Ventolini et al. 2005). For example, in scale eating cichlids, *Perissodus* *microlepis*, individuals with mouth openings of the rarer phenotype had greater hunting success than the common phenotype in a population where both phenotypes were maintained as a result of frequency dependent selection based on prey alertness (Hori 1993).

A further cost of cerebral lateralisation is a reduction in the ability to transfer and integrate information that reaches both brain hemispheres, resulting in a reduced efficiency in neural processing when communication and cooperation in information processing is required, or when different stimuli related to a single decision are viewed in different visual hemispheres, and information processing is partitioned into different brain hemispheres (Rogers 2002; Dadda et al. 2009). In goldbelly topminnows (*Girardinus falcatus*) trained to find the central door in a row of nine, non-lateralised individuals made the correct choice in the majority of trials while lateralised individuals most often chose a door corresponding to their ‘dominant side’, either left or right. Similarly, lateralised topminnows in a shoal choice task, where shoals were seen in differing eyes, choose the better-quality shoal less frequently due to strong inherent side biases, than non lateralised individuals who most often choose the shoal of the highest quality (Dadda et al. 2009).

Despite these costs and benefits, laterality is widely documented throughout the animal kingdom in a number of behaviours, including predator avoidance and escape, foraging, reproduction, exploratory behaviour and social behaviours (see Rogers 1996; Bisazza et al. 1998a). In recent years our understanding of behavioural, morphological, anatomical and physiological asymmetries has grown vastly with demonstrations of laterality in all major taxonomic groups (Rogers et al. 2013), yet there are still several unanswered questions that remain regarding the evolution of laterality. Specifically, when did laterality evolve, why did laterality evolve and why does laterality persist?

1.3 When did cerebral lateralisation evolve?

Among vertebrates and insects several characteristics of axes during development are conserved (Gilbert and Barresi 2016), including myosin 1D, which is involved in left/right axis specification in *Drosophila* (Juan et al. 2018). Nodal gene pathways responsible for the evolution of body plans and left/right differentiation are likely to have been found in the common ancestors of bilaterians, since homologous gene sequences of the Nodal family are present in both vertebrates (Boorman and Shimelf 2002) and Bilateria (Grande and Patel 2009). This suggests that mechanisms of left/right axis specialisation are conserved over long phylogenetic distances, allowing the formation of asymmetries in neural circuits, the basis of cerebral lateralisation (Niven and Fransnelli 2018). A bilateral ancestor of insects and vertebrates, an unbilaterian, has been hypothesised (Niven and Frasnelli 2018). Although its exact form is debated (Northcutt 2012), the vermiform bauplan of this ancestor suggests that it is unlikely to have exhibited any behavioural or cognitive functions equivalent to those of vertebrates or insects, and thus any laterality if present, would likely be restricted to turning biases. Behavioural and neural laterality that underpin such biases, or control specialised appendages most likely evolved independently in vertebrate and invertebrate taxa. Therefore, the evolution of laterality in vertebrate and invertebrate taxa is most likely analogous (Niven and Frasnelli 2018), thus vertebrates and invertebrates represent separate lineages to assess the evolutionary pressures driving laterality.

Fossil records suggest that asymmetries in the head region have dated back to the most primitive chordates (calcichordates; Jefferies 1979) with further evidence that ancestors of chordates received different sensory inputs on the left and right sides of the body resulting from laying on the right side (Jefferies and Lewis 1978). Additionally, visualisation of potential prey allowing termination of inhibition responses may have been a critical step in the evolution of cerebral lateralisation in the ancestors of modern vertebrates (Andrew et al. 2000). Thus, cerebral lateralisation is likely ancient in vertebrates, occurring after duplication of the brain in to two halves or hemispheres.

Differentitation between left and right is a key requirement for laterality to evolve. In insects, laterality likely depends upon early developmental specification of the left/right body axis but the resultant patterns of laterality are likely produced independently (Niven and Fransnelli 2018). Indeed, a common origin of left/right axis specification is proposed in insects, based on studies of the molecular basis of left/right axis specification in *Drosophila melanogaster* (Coutelis et al. 2008), similar to the anterior-posterior and dorsal-ventral axes in insects, which are also proposed to have a common origin (Raff and Kaufman 1991; Kalinka and Tomancak 2012). Laterality in insects is not solely determined by left/right body axes development and likely also involves motor control, learning and memory and sensory perception. However, the pattern of behavioural lateralisation in insects often differs from laterality in other behaviours or from asymmetries in morphological traits, even when the behaviours that show lateralisation share similar morphological structures (Niven and Frasnelli 2018). This suggests that the neural substrates responsible for laterality in insects act independently with regard to susceptibility to selection pressures and the production of laterality.

1.4 Why did cerebral lateralisation evolve?

Two main hypotheses have been proposed to explain why brain lateralisation evolved: the first suggests that laterality results from specialisation in feeding structures and the second suggests that laterality evolved in line with the evolution of two laterally placed eyes (reviewed in Andrew 2002). Evidence for the first hypothesis comes from the lancelet (*Branchiostoma lanceolatum*), an organism that exhibits asymmetry in feeding structures during the larval stage. The mouth is present on the left-hand side of the body until the adult stage when the mouth moves to a central position, however neural processing of feeding behaviours remain controlled by the left hemisphere of the brain (Rogers et al. 2013). This hypothesis proposes that left hemisphere control of feeding behaviours has persisted throughout vertebrate evolution, which has been demonstrated in both toads (*Bufo* spp) and chicks (*Gallus gallus domesticus*). In *B. bufo* and *B. virdis* the majority of tongue strikes directed at prey were performed when the prey entered the right visual hemisphere suggesting left hemisphere control of feeding behaviours (Vallortigara et al. 1998). Similarly, in chicks, glutamate injections in the left, but not the right, visual hyperstriatum (a region of the forebrain) were found to reduce performance in a food-pebble discrimination task further suggesting left hemisphere control of feeding behaviour (Deng and Rogers 1997).

Alternatively, the second hypothesis is based on the idea that the evolution of two eyes allows a greater degree of information about an individual's surrounding environment to be obtained, resulting in a larger number of stimuli being processed at a given time (Wiper 2017). The increased demand to process multiple stimuli could have driven specialisation of processing different cognitive functions into specific brain hemispheres, particularly in species with laterally placed eyes such as fish, where there is little binocular overlap between visual hemifields and complete decussation at the optic chiasma resulting in two eyes that each receive a unique view of the surrounding environment (Brown et al. 2004). In species with a large degree of binocular overlap, the same strategy could be supported for stimuli seen in the extremes of the lateral monocular fields of vision, which are likely to evoke turning responses. Evidence in support of this hypothesis comes from Vallortigara et al's (1998) study on toads (*Bufo* spp) that found individuals exhibited more efficient prey capture when prey were detected in the right, than in the left, visual field suggesting left hemisphere control of feeding behaviour. Additionally, in the stripe-faced dunnart (*Sminthopsis macroura*), individuals responded more often when a model predator was presented in the left than in the right visual hemifield suggesting that the right brain hemisphere is responsible for controlling fear responses (Lippolis et al. 2005), similar to the partitioning of different emotive responses within different brain hemispheres in humans. While both hypotheses have received some empirical support, the available evidence, while supporting one hypothesis, is insufficient to entirely rule out the alternative and thus, it is plausible that these hypotheses proposed to explain the evolution of laterality are not mutually exclusive.

1.5 Why does cerebral lateralisation persist?

As laterality can provide a selective advantage under certain circumstances and heritability of laterality has been demonstrated in fish (Bisazza et al. 1997), rodents (Collins 1993) and primates (Hopkins et al. 2001), we might expect cerebral lateralisation to be driven to fixation in populations where these advantages are experienced (Reddon et al. 2009b; Dadda et al. 2015). However this is not the case, as substantial variation in laterality persists throughout the animal kingdom, which highlights two puzzling issues. Firstly, why does so much variation in laterality persist? Despite the advantages of laterality for individual efficiency being clear, substantial individual variation in laterality remains, whereby populations often contain both lateralised and non-lateralised phenotypes, and not infrequently strongly lateralised individuals are outnumbered by non or weakly lateralised individuals (Bisazza et al. 1997; Takeuchi and Hori 2008). Secondly, why would individuals align their lateral biases within a population? Individuals exhibiting the same consistent directional biases will likely be more predictable, thus representing a clear disadvantage for members of the population with the common lateral phenotype (Hori 1993). Understanding how this variation develops and why it is maintained is critical to furthering our understanding of the evolution of laterality in animals.

1.6 Laterality in fish

Investigations of laterality in fish, reptiles and amphibians began more recently than in mammals and birds, the first non-human entity in which laterality was demonstrated (reviewed in Bisazza et al. 1998a). These investigations have grown vastly in the last 25 years with teleost fish now representing the most commonly used model system to study various aspects of cerebral lateralisation (reviewed in Bisazza et al. 1998a; Bisazza and Brown 2011). Unlike mammalian research, which has often focused on investigating left-right differences in brain functions, studies investigating laterality in fish commonly assess directional differences in behaviours or bilateral characteristics, whereby these biases are assumed to represent underlying asymmetries in brain structure and functioning (Vallortigara and Rogers 2005). As a result, laterality has been considered in a wide range of contexts, with the behavioural consequences of laterality known for a wide range of ray-finned fishes (Bisazza and Brown 2011). Consistent with other vertebrates, studies investigating lateralisation of sensory modalities in fish are dominated by visual lateralisation, with olfactory navigation in the eel (*Anguilla anguilaa;* Westin 1998) and lateral line use and exploration in blind Mexican cave fish (*Astyanax mexicanus*; de perera and Braithwaite 2005) representing the only exceptions. Some studies have assessed motor asymmetries in fish, but these are often confounded by visual lateralisation making it difficult to isolate the motor component of the behaviour. Of the limited studies that have investigated motor asymmetries, only a few can be considered sound including goldbelly topminnow turning biases in the dark (*Girardinus falcatus*; Bisazza et al. 2001a), fin use in gourami fish (*Trichogaster trichopterus;* Bisazza et al. 2001b) and sound production in catfish (*Ictalurus punctatus;* Fine et al. 1996).

Of all the contexts considered, ecologically relevant behaviours, such as foraging and predator avoidance, have received the most attention. This is likely because these behaviours are relatively simple to measure in a standardised context and side biases in these behaviours could influence behavioural performance and ultimately individual fitness. For example, individual biases in foraging behaviour have been found in some fish species with more strongly lateralised individuals exhibiting a higher rate of prey capture (Kurvers et al. 2017). In antipredatory behaviour, fish have been shown to exhibit turning biases during predatory escape behaviour (Cantalupo et al. 1995), which have been linked to enhanced escape reactivity in some species (Dadda et al. 2010). However, despite a strong research bias towards foraging and predator escape behaviours, laterality has also been demonstrated in a number of other behaviours in fish including aggression (Bisazza and Santi. 2003), lateral displays (Arnott et al. 2011) and rotational swimming (Bisazza and Vallortigara 1997).

Lateralisation of social behaviours represents a growing area of research interest since theoretical models predicted that behavioural responses that require interaction and coordination, such that the fitness of an asymmetric individual depends on what other asymmetrical individuals do, could select for aligned directional biases in laterality at the population level (Ghirlanda and Vallortigara 2004). To date, interactions within social species, interactions between individuals living within groups or between individuals performing social interactions have been suggested as selection pressures that may favour the evolution of aligned directional biases in laterality (Frasnelli and Vallortigara 2018). In fish, support for this theory comes from examining lateralisation in a highly social, cooperatively breeding cichlid species, *Neolamprologus pulcher*, where males exhibit a population level right eye/left brain hemisphere bias when viewing a social stimulus (Reddon and Balshine 2010). Additionally, in a study of 16 fish species from 13 families, all of the shoaling species examined exhibited population level laterality in turning preferences when viewing a dummy predator, while only 40% of non-shoaling species exhibited the same biases (Bisazza et al. 1997). Such population biases in social species have also been shown to be beneficial in a social context in yellow and blueback fusiliers, *Caesio teres*. Individuals exhibiting the same directional bias in predatory escape behaviour as the majority of individuals within their school had enhanced escape performance relative to those at odds with the group (Chivers et al. 2016). Despite the growing interest into social behaviours, reproduction and reproductive social behaviours remain relatively unexplored and underrepresented, which is surprising given their association with, and related implications for, reproductive success and individual fitness. To date laterality has been demonstrated in (i) mating behaviours of insects and molluscs, (ii) courtship behaviours in birds and insects and (iii) social interactions and communication between parent and offspring in mammals. In contrast, demonstrations of laterality in this regard in fish are limited to asymmetry of male and female genitalia and resultant mating regimes (see Table 1.1 for an overview of laterality in reproductive behaviours).

Regardless of behavioural context, variation in laterality is common between species, populations, individuals and sexes (reviewed in Bisazza et al. 1998a). Research investigating variation in laterality is abundant, with the majority of studies focusing on identifying the underlying drivers of variation within and between species, populations and individuals. In the last 30 years, several potential drivers have been identified in this regard including genetic mechanisms or genetic pathways, early life experience (specifically the influence of differing selection pressures such as predation, light exposure and environmental enrichment), frequency dependent selection, age, individual personality traits and parental effects that allow parents to alter the development trajectories of their offspring producing individuals with phenotypes best suited to the environment they will experience (see Table 1.2 and references within).

Sex is an increasingly recognised but understudied source of variation in cerebral lateralisation (Bianki and Filippova 2003) despite several vertebrate taxa, including mammals (Ward et al. 1990; Alonso et al. 1991), birds (Andrew and Brennan 1984) and fish (Reddon and Hurd 2008; Irving and Brown 2013) all reporting sex specific variation in motor, neural and behavioural asymmetries (Table 1.2). The true extent of this variation is not known and likely underestimated due to a vast quantity of the available research considering only a single sex or animal models where the sexes are unidentifiable non-invasively (Reddon and Hurd 2008).

Table 1.1: Overview of lateralisation in reproductive/parental behaviours in vertebrates and invertebrates.

Species	Sex	Reproductive or parental behaviour	Biases	Reference(s)
VERTEBRATES				
Zebra finch, *Taeniopygiua guttata*	Male	1. Lateralised visual stimulation of courtship. 2. Visual lateralisation of mate choice	1. Right eye bias 2. Right eye bias	Templeton et al. 2014
Onesided livebearer fish of the genus Jenynsia, *Cyprinodontiformes, Anablepidae*	Male & female	Lateralised genitalia	Left and right biased	Torress-Dowdall et al. 2020
Harbor Porpoise, *Phocoena phocoena*	Male	Laterality in sexual approach	Males position their ventral side on the females left side	Keener et al. 2018
Black-winged stilts, *Himantopus himantopus*	Male & female	1.Courtship displays 2.Copulatory attempts	1. Left eye bias 2. Left eye bias	Ventolini et al. 2005
Humpback whale, *Megaptera novaeangliae*	Female	Nursing (suckling) behaviour	Calf located on the mothers right side	Zoidis & Lomac-MacNair 2017
Siamese fighting fish, *Betta splendens*	Male	Aggression during reproduction	Right eye bias	Forsatkar et al. 2015
Striped plateau lizard, *Sceloporus virgatus*	Female	Aggressive rejection displays during courtship	Left eye bias	Hews et al. 2004
Barnacle geese, *Branta leucopsis*; White fronted geese, *Anser albifrons*	Male & female	Lateralised spatial interactions between long term monogamous pairs	Left eye bias in following partner that leads	Zaynagutdinova et al. 2020
Pacific walrus, *Odobenus rosmarus* Horse, *Equus ferus caballus* Siberian tundra reindeer, *Rangifer tarandus sibiricus*	Female	Mother-infant interactions	Infants keep mother on their left side	Karenina et al. 2017

Table 1.1 continued:

Species	Sex	Reproductive or parental behaviour	Biases	Reference(s)
Saiga antelope, *Saiga tatarica* Domestic sheep, *Ovis aries* Muskox, *Ovibos moschatus* Beluga whale, *Delphinapterus leucas* Orca, *Orcinus orca* Eastern grey kangaroo, *Macropus giganteus* Red kangaroo, *Osphranter rufus*	Female	Mother-infant interactions	Infants keep mother on their left side	Karenina et al. 2017
INVERTEBRATES				
Rice weevil, *Sitophilus oryzae* Confused flour beetle, *Tribolium confusum*	Male	Copulation approach	Left side bias	Benelli et al. 2017a
Earwig, *Labidura riparia*	Male	Reproductive organs	Right dominance	Kamimura 2006
Bedbugs, *Cimex hemipterus; Cimex lectularius*	Male	Reproductive organ of females (spermalege)	Right side bias in males when spremalege duplication in female	Kamimura et al. 2014
Fruit fly, *Drosophila pachea*	Male	Reproductive organs (male genitalia external lobes)	Left lobe longer and thinner	Lang & Orgogozo 2012

Table 1.1 continued:

Species	Sex	Reproductive or parental behaviour	Biases	Reference(s)
Khapra beetle, *Trogoderma granarium*	Male & female	1. Male recognition and mounting 2. Female post-copulation kicks 3. Male foreleg tapping acts	1. Left bias (female) 2. Left bias 3. Right bias	Benelli et al. 2017b
Parasitic wasp, *Leptomastidea abnormis*	Male	Antennal tapping during copulation	Right bias	Romano et al. 2016
Australian cuttlefish, *Sepia apama*	Male	1. Female inspection during mating interactions 2. Approach of female during mating attempt	1. Left eye bias 2. Right biased	Schnell et al. 2019

Identifying the drivers of variation in cerebral lateralisation, especially between the sexes, is important from both an evolutionary and ecological perspective since laterality has been shown to impact fitness related behaviours. Thus, identifying the underlying mechanism(s) responsible for variation in laterality is critical not only to enhance our understanding of how this variation develops and is maintained, but it is also essential to better understand how variation in laterality will influence behavioural performance and ultimately fitness and how this may vary among the sexes.

1.7 Parental care

Parental care is defined as "any parental trait that increases the fitness of offspring, often at a cost to the parents own survival and reproduction, that is likely to have originated for, or is currently maintained, for that function" (Royle et al. 2012). Parental care is a highly diverse trait. Under its broad definition parental care can include, but is not limited to, allocating resources to eggs prior to mating, offspring provisioning before or after hatching, waste-removal, nest tending and guarding (Royle et al. 2012). These behaviours can be performed by either sex (uniparental care), both parents (biparental care) or by non-parents such as siblings (alloparental care; Kokko and Jennions 2008; Royle et al. 2012). There are significant taxonomic patterns of which sex will care for the young in vertebrates (Reynolds et al. 2002). In mammals, female only care is predominant and found in around 90% of families, with a small occurrence of biparental care in around 10% of families and no occurrence of male only care. In contrast, biparental care is the dominant form of care in birds (90%) with a small number of species exhibiting female only care (8%) and even fewer species exhibiting male only care (2%; Gross 2005). In contrast, fish are the vertebrate group with the most diverse and unique patterns of parental care (Gross 2005). While the majority of fish species do not provide parental care (80% of families), male only care is dominant in the remaining species that do perform care (50% of the remaining 20% of species), with occurrences of both female only (30% of caring species) and biparental care (20% caring species). Thus, not only have fish evolved all forms of parental care, they are the only taxa where male care is the dominant form. In fish, parental care may range from burial of eggs, to internal gestation and live bearing but guarding of eggs or a brood is the most commonly observed behaviour (Gross and Sargent 1985; Sargent and Gross 1986).

Within species, cognitive flexibility skills are said to differ between the sexes as a result of differences in ecological roles and associated demands (Brandão et al. 2019). Most commonly these differences are attributed to differences in roles during reproduction of males and females, which affect physiology and brain structure (Gonzalez-Voyer et al. 2009). Parental care is extremely cognitively demanding (Reddon and Hurd 2009a). In a multi-species analysis of African Cichlid fish in Lake Tanganyika, brain size was associated with parental care type, whereby uniparental care was associated with a larger brain than biparental care (Mowrey and Portman 2012; Gonzalez-Voyer et al. 2009). Similarly, in threespine sticklebacks (*Gasterosteus aculeatus)*, a species with uniparental male care, males have larger brains than females, which are associated with the cognitive demands of reproduction and parental behaviour exhibited by the caring sex (Kotrschal et al. 2012; Samuk et al. 2014). Not only is parental care itself cognitively demanding, caring parents must also perform multiple tasks simultaneously, including all aspects of parental care while performing additional behaviours critical to survival including foraging and predator avoidance (Royle et al. 2012).

Table 1.2: Overview of the factors driving variation in laterality.

Source of variation	Variation observed	Species	Reference(s)
Genetic mechanisms or pathways	Strong and weakly lateralised paw preference resulting from a single major gene control response and differential allele expression at the locus driving this variation.	Mice, *Mus musculus*	Biddle et al. 1993
Early life experience - *Predation*	1. Predation: High predation pressures led to right eye dominance in predator viewing but no lateral biases in low predation pressures.	*Brachyrhaphis episcopi*	Brown et al. 2004
	2. Individuals reared with predator cues wmore strongly lateralised.	Guppies, *Poecilia reticulata*	Broder & Angeloni 2014
- *Light exposure*	Offspring of parents reared on high-levels of light were significantly lateralised in visual and motor tests, while those from low levels were not.	Goldbelly topminnows, *Girardinus falcatus*	Dadda & Bisazza 2012
- *Environmental enrichment*	Males were more lateralised in social context when reared in enriched conditions, but females were more lateralised when reared in impoverished conditions.	Rainbowfish, *Melanotaenia dubulayi*	Bibost et al. 2013
Frequency dependent selection	Frequency of left and right mouth-opening phenotypes oscillated around unity. Maintained by frequency dependent selection based on preys alertness.	Scale eating cichlid, *Perissodus microlepis*	Hori 1993
Age	Increased left turning bias in t-maze when shelter present from 3 to 45 days posthatch.	Cuttlefish, *Sepia officinalis*	Jozet-Alves et al. 2012a

Table 1.2 continued:

Source of variation	**Variation observed**	**Species**	**Reference(s)**
Individual personality traits	Bolder individuals (quicker to emerge from a shelter) were more strongly lateralised when exploring a familiar environment.	Convict cichlid, *Archocentrus nigrofasciatus*	Reddon & Hurd 2009c
	In females, bolder individuals were more strongly lateralised.	Guppies, *Poecilia reticulata*	Irving & Brown 2013
Parental effects	Tadpoles exposed to risk as embryos exhibited stronger lateralisation in a rotational task relative to predator-naive controls.	Wood frog tadpoles, *Lithobates sylvaticus*	Lucon-Xiccato et al. 2016a
	Prenatal light exposure to one eye during embryonic development causes the visual Wulst contralateral to the light exposed eye to develop dominance over its equivalent in the other brain hemisphere.	Domestic chicks, *Gallus gallus domesticus*	Deng & Rogers 2002
Sex	Females had lower strength of laterality at the individual level than males but no differences in direction.	Rats, *Rattus norvegicus*	Alonso et al. 1991
	Males show a right eye bias when viewing a visual stimulus but females use either the right or left eye.	Domestic chicks, *Gallus gallus domesticus*	Andrew & Brennan 1984

Table 1.2 continued:

Source of variation	Variation observed	Species	Reference(s)
Sex	Female non-aggressors and male aggressors had a left detour bias when differences in behavioural trait (aggression) and sex were considered.	Convict cichlid, *Archocentrus nigrofasciatus*	Reddon & Hurd 2008
	In females, bolder individuals were more strongly lateralised. A trend not observed in males when individual differences in behaviour (personality) and sex were considered.	Guppies, *Poecilia reticulata*	Irving & Brown 2013

The evolution of diversity in parental care likely reflects the costs and benefits of parental care to male and female parents, which in turn depends on several factors including offspring dependency on care, potential mating opportunities and associated paternity assurance, environmental factors and life history traits (Clutton-brock 1991). The study of parental care behaviour has vastly enhanced our understanding of a wide range of areas of biology including sexual selection and mating systems, sociality, phenotypic plasticity and crucially life history theory since parental care impacts both offspring and parental fitness. Within the field of parental care, much of the research has focused on understanding the different forms of parental care, the duration of care that is provided and the extent to which each sex provides care (Clutton-Brock 1991).

1.8 Linking laterality and parental care

The advantages of cerebral lateralisation (an enhanced neural capacity, hemisphere dominance and simultaneous information processing) suggest that caring parents could obtain a potential fitness benefit from having a lateralised brain. These advantages could drive the widely observed variation in laterality present throughout the animal kingdom, particularly between the sexes (Bianki and Filippova 2003), as a result of differences in sexual selection and the involvement of each sex during parental care (Bateman 1948; Magurran 2005). Additionally, aligned directional biases in laterality at the population level are thought to occur when individuals interact in such a manner, that the fitness of an asymmetric individual is dependent on what other asymmetrical individuals do (Rogers 2000; Ghirlanda and Vallortigara 2004). Thus, biparental and alloparental care represent selective pressures that could drive aligned directional biases in lateralised behaviours at the population level as a result of the requirement for individuals to interact and coordinate behavioural responses during care. In some cases, alignment of lateral biases could result in more efficient or successful performance in behaviours, e.g. if biparental parents both attack potential brood predators utilising their dominant side, and such biases are associated with enhanced performance in brood defence. Indeed, other social reproductive behaviours including courtship and copulation have been shown to exhibit population level asymmetries, which have been associated with enhanced reproductive success (Ventolini et al. 2005; Romano et al. 2016), but considerations of parental care are limited.

Lateralisation of parental care behaviours, whether at the individual or population level, could have two-fold implications for individual fitness and ultimately selection. Firstly, consistent directional biases in parental care behaviour could impact behavioural performance during care, which could impact both the fitness of parents and offspring. Specifically, consistent directional biases in predatory behaviours have been associated with enhanced escape reactivity (Dadda et al. 2010) thus, consistent directional biases in parental care behaviours could improve behavioural performance during care, which could in turn positively impact the quality or success of the care provided and ultimately parental reproductive success and offspring survival. Secondly, consistent directional biases in parental care behaviour could also impact the performance of parents in other behavioural domains that are performed simultaneously during the caring period, including ecologically relevant behaviours such as successfully finding food and avoiding predation, which are tightly associated with individual fitness. For example, caring parents could partition information relating to parental care into a specific brain hemisphere leaving the other hemisphere free and available to process additional functions, such as foraging (Vallortigara and Rogers 2005). As a result parents may also exhibit enhanced performance in additional behaviours performed during care as a result of the enhanced ability to perform simultaneous information processing that is associated with being lateralised (Rogers 2000, 2002) and/or additionally, as a result of exhibiting laterality in these additional behaviours by partitioning the relevant information processing into a specific brain hemisphere. Thus, it is surprising that laterality has rarely been studied with regard to parental care.

Evidence of population level lateralisation in parent-offspring communication is evident for 11 species of terrestrial and marine mammals. Offspring of these species prefer to keep the mother on their left side indicating right brain hemisphere dominance for information processing relating to social stimuli (Karenina et al. 2017), while in fish asymmetries in brain size have been found between male and female threespine stickleback whereby males, the caring sex, have 23% heavier brains than females, the non-caring sex, after controlling for body mass (*Gasterosteus aculeatus*; Kotrschal et al. 2012). This asymmetry has been suggested to result from the cognitive demands associated with the male only parental care system in this species but this hypothesis has not been directly tested. To date the relationship between laterality and parental care is largely unknown.

1.9 Laterality and parental care in fish

There are likely several advantages to studying laterality in fish relative to other model species that have previously been considered including humans, primates and rodents. The best example is visual lateralisation, the most commonly studied sensory modality with regard to laterality. Humans have frontally placed eyes with optic nerve fibres that partially decussate at the optic chiasm meaning that approximately half of the fibres from each eye reach each of the brain hemispheres. Thus, our perception of the external environment is barely altered when one eye is covered, and even if information was to predominantly reach one hemisphere (as might result from other sensory modalities), fast and efficient interhemispheric communication would be enabled by the corpus callosum (Atchison and Smith 2000; Bisazza and Brown 2011). Unlike humans, fish have laterally placed eyes with little crossover at the optic chiasm. Thus each eye largely sees an independent view of the surrounding environment except for a small frontal overlapping portion. In fish, all the nerve fibres from one eye cross to the contralateral brain hemisphere such that a stimulus viewed in the left visual hemifield is primarily processed by the right brain hemisphere and vice versa (Bisazza and Brown 2011; Figure 1.2).

In fish, laterality is easily observable at the behavioural level as side biases in behaviours or as a preference for one side or half of a bilateral characteristic. By covering a single eye or presenting a stimulus in one visual hemifield we can draw inferences about the way brain hemispheres function and how information is processed by measuring differences in the behavioural responses of individuals (Bisazza et al. 2001a; Bisazza and Brown 2011). These differences also suggest that functional left-right preferences in eye-use are more important in the everyday behaviour of individuals with laterally placed eyes, such as fish, relative to those with binocular vision, including birds. For example, domestic chicks (*Gallus gallus domesticus)* can discriminate between familiar and unfamiliar stimuli seen with the left eye or both eyes but not when visualised with the right eye only (Vallortigara and Andrew 1994). Some species (birds and some reptiles) are able to reduce this limitation by scanning the external environment with both eyes in sequence as a result of their mobile necks (Clayton and Krebs 1994) however, this does not extend to fish who would have to re-orientate their entire body (Bisazza and Brown 2011). As a result, laterality is widely studied in a number of fish species across a range of behavioural contexts (reviewed in Bisazza et al. 1998a), and substantial amounts of variation in laterality are found to persist between individuals, species, populations and sexes (Irving and Brown 2013; Bisazza et al. 1997; Brown et al. 2004; Reddon and Hurd 2008).

In addition, fish are the vertebrate group that exhibit the greatest diversity in parental care (both behaviour performed and type of care; Gross and Sargant 1985; Sargant and Gross 1986), with males of many fish species playing a greater role in reproduction relative to other vertebrate species (Amundsen 2003). Fish are also relatively easy to breed in laboratory conditions and parental care behaviours in fish are observable and measurable at the behavioural level, especially brood guarding and defence, the most predominant form of care (Gross and Sargant 1985; Sargant and Gross 1986). Thus fish represent a model system to examine the relationship between laterality and reproduction and parental care.

1.10 Study species

As parental care is so diverse both across and within animal taxa, and there is substantial variation in the parental care behaviours that are performed, the level of care given and who is responsible for providing care (Royle et al. 2012), I used three species of fish, that are representative of some of the variation in parental care, to address the research questions that this thesis proposes. The first, guppies, *Poecilia reticulata*, are a small freshwater live-bearing species, native to Trinidad that can be found in an array of habitats including rivers with high levels of water flow and smaller more stagnant ponds (Magurran and Phillip 2001). Live bearing has evolved from egg laying 21-22 times in all fish species and is thought to have evolved to increase offspring survival (Goodwin et al. 2002). Several advantages to live bearing are known including shielding offspring from extreme environmental conditions and predation using internal development (Clutton-Brock 1991; Wourms and Lombardi 1992), an increased rate of embryonic development resulting from adult body temperature staying above ambient temperature (only true in ectotherms; Fry, 1971) and the production of larger offspring with an enhanced survival rate resulting from internal provisioning of offspring (Amoroso 1968; Wourms 1977; Wourms and Lombardi 1992). However, the extent to which live bearing is a form of parental care is a widely debated topic: some argue there is little parental involvement once the young are birthed and no post birth care of offspring (Plath et al. 2007), while others argue it is an extreme form of parental care with several costs for live bearing parents including vast energetic and physiological changes associated with carrying young and developing placenta like structures and reductions in mobility making parents more vulnerable to predation (Goodwin et al. 2002).

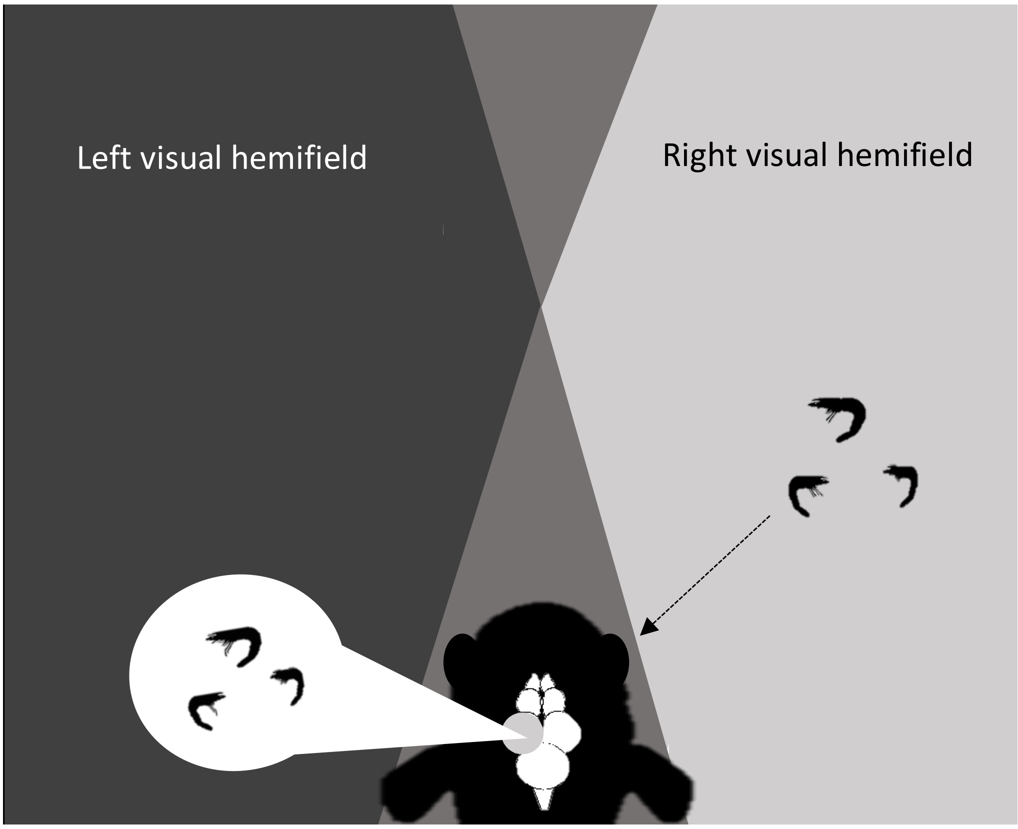


Figure 1.2: Schematic of vision and associated hemispheric processing in fish. Fish have relatively little overlap in visual hemifields (see only small frontal portion) and almost 100% crossover to the contralateral brain hemisphere at the optic chiasma. Thus visual stimuli seen in the right visual hemifield are processed in the left brain hemisphere (shown here with fish visualising prey). Figure adapted from Bisazza and Brown 2011.

Guppies represent a model species for exploring variation in laterality for a number of reasons. Firstly, we have a broad understanding of mating dynamics and of sexual selection in fish as a result of an expanse of research in this species (Amundsen 2003). Secondly, the reproductive cycle is short and there is a clear division of roles in reproduction (Houde 1997). Females perform male mate choice which is largely based on mate colouration, specifically caretonoid (orange) pigmentation (Brooks and Endler 2001), but females are subject to high levels of sexual harassment from males who try to sneak extra copulations likely because female guppies store sperm (Magurran and Seghers 1994a, 1994b; López-Sepulcre et al. 2013). Following internal fertilisation females carry the young until birth and no further parental care is performed by either sex. Additionally, due to extensive research on guppies in the last 30 years investigating various aspects of ecology and evolution, sex differences in behaviours are also well established in this species (Magurran 2005) making them a model system to investigate sex differences in laterality across different behavioural contexts.

The second species, threespine sticklebacks, *Gasterosteus aculeatus*, are a common species found in both marine and freshwaters in the northern hemisphere (von Hippel 2010). The reproductive biology of threespine sticklebacks is extremely well studied and documented, likely due to the series of distinctive stages of reproduction and the male only uniparental care system in this species (Tinbergen 1952). In sticklebacks, male acquire territories in spring, the start of the breeding season, which they defend against other male conspecifics, and start to exhibit nuptial colouration. Males will construct a nest and begin to court gravid females using an elaborate “zig-zag” dance. Once males have eggs laid in their nest, which can be from several different courted females, the males will fertilise the eggs and chase away any female partners. The males will then ventilate the eggs by fanning them with their pectoral fins, increasing in frequency until hatching. Males defend the newly hatched larvae from brood predators until the offspring are free-swimming and independent (Tinbergen 1952). These distinctive stages of reproduction (territory defence, construction of a nest, courting, spawning and parental care) can be experimentally manipulated and observed within a laboratory setting. Additionally, since there is a clear division of roles in reproduction and parental care, whereby only a single sex cares for the young (males) and there is a stark contrast in the cognitive demands associated with reproduction and the performance of reproductive behaviours between the caring and non-caring sex, threespine sticklebacks provide a model system to explore the whether there are sex differences in laterality and whether laterality is linked to the performance of parental care behaviours.

Lastly, I used a biparental substrate brooding cichlid fish, *Telmatochromis temporalis*, endemic to Lake Tanganyika to explore the later questions that this thesis addresses. When not reproductive, *T. temporalis* are a non-social solitary living species. However, during reproduction, individuals form pair bonds and are monogamous biparental breeders (Kuwamura 1997; Mboko 1998). Approximately one week prior to spawning, male and female *T. temporalis* will form a pair bond, during which time the female remains in or close to the nest site, which is often a nest of a small hole or a burrow under a stone (Mboko 1998). The female will deposit eggs inside the nest and the male will ejaculate sperm externally at the nest site. Both parents defend the brood until the offspring reach independence (~16mm; Kuwamura 1997; Mboko 1998): males have territories around the nest site which they guard from other males and predatory conspecifics, while females defend the brood from smaller or more immediate threats (Mboko 1998; Mboko and Kohda 1999). As *T. temporalis* breed relatively easily in the lab and the directional biases in the performance of biparental care can be easily observed at the behavioural level, this species is suited to experiments that assess whether individuals and populations exhibit lateral biases in parental care behaviours. Additionally, both sexes have an equivalent role in care and within the species different levels of cooperation and social interaction are evident in a reproductive and non-reproductive state, thus making *T. temporalis* a model system to explore questions about laterality and its associated costs and benefits in different contexts with varying social pressures.

1.11 Thesis rationale

Laterality has been shown to impact behavioural performance which can in turn have implications for individual fitness and survival. Much of the research on laterality to date has been biased towards ecologically relevant behaviours, such as foraging and predation, with little investigation into social behaviours, especially reproductive social behaviours, that are highly variable between the sexes. This is surprising given social behaviours represent a selective pressure that is thought to drive aligned lateral biases at the population level and the potential two-fold implications for parent and offspring fitness that directional biases in such behaviours could have. Understanding variation in laterality and how it could impact behavioural performance and ultimately fitness is critical to better understand how variation in laterality develops and why it is maintained. This thesis will examine the relationship between laterality, reproduction and parental care, a reproductive social behaviour, using fish as a model system. Specifically, this thesis will address two overarching research questions:

(i) can reproduction and reproductive social behaviours explain variation in laterality, particularly between the sexes?

(ii) are reproductive social behaviours, namely parental care behaviours, lateralised?

Below, I consider the specific aims of each chapter, which contribute to these two key questions.

1.11.1 Chapter 2: Consistency in the strength of laterality in male, but not female, guppies across different behavioural contexts

Chapter 2 (published as McLean & Morrell 2020) had two specific aims:

(i) to confirm the value of the "detour test" (Bisazza et al 1997, 1998a) as a method to examine laterality experimentally. The detour test, commonly used to assess laterality in fish, involves assessing the turning direction of a fish approaching a barrier which partially obscures an object behind it, and indicates which eye the fish preferentially uses to view the object (Bisazza et al. 1997, 1998a, 1998b). Different objects, such as conspecifics, predators, novel objects or food/shelter can be used to represent different ecological contexts (Bisazza et al 1997, 1998a, 1998b).

(ii) to investigate whether there are sex differences in laterality, in a live bearing species *Poecilia reticulata*. Specifically, to establish if there are sex differences in the strength, direction or consistency of laterality exhibited in three different behavioural contexts in a live bearing species where sex differences in behaviour are well established (Magurran 2005).

1.11.2 Chapter 3: Sex differences in laterality are associated with reproduction in Threespine Stickleback

In chapter 3, I extend the work of chapter 2 by investigating whether variation in laterality is driven by reproduction and the performance of reproductive behaviours in a species that performs uniparental male care and has a clear division of labour, threespine stickleback *Gasterosteus aculeatus*. Specifically, the aims of this chapter were to (i) assess whether the caring sex is more strongly lateralised than the non-caring sex and (ii) assess whether laterality is linked to the performance of reproductive behaviours, including nest construction and courting, mating, and parenting.

1.11.3 Chapter 4: The costs and benefits of laterality in Telmatochromis temporalis in different reproductive states

Costs and benefits of exhibiting consistent directional biases in behaviour are known (Vallortigara 2006). However, whether the balance of these costs and benefits and associated expression of laterality is consistent in different social contexts when individuals likely face different selection pressures is completely unknown. In this chapter, I use a biparental substrate brooding cichlid fish, *Telmatochromis temporalis,* to examine laterality in exploratory behaviour, and assess individuals in two tasks previously used to assess the costs and benefits of laterality, efficiency in tasks requiring interhemispheric communication and simultaneous information processing respectively under different social conditions: while reproductive and actively performing biparental care and in a non-reproductive, non-social state. Specifically, this chapter had 4 specific aims:

(i) to examine if *T. temporalis* individuals exhibit laterality in exploratory behaviour.

(ii) to assess if laterality carries a cost.

(iii) to assess if laterality carries a benefit.

(iv) to examine whether laterality and any associated costs and benefits vary with reproductive state.

1.11.4 Chapter 5: Parental care behaviours are lateralised in a biparental cichlid fish

To date, there is evidence of lateralisation in parent-offspring communication in mammals. Offspring prefer to keep parents on their left side suggesting right brain hemisphere control of social processing (Karenina et al. 2017) however, little is known regarding lateralisation of parental care behaviours other than mother-offspring interactions, especially in non-mammalian animal taxa. Chapter 5 had two specific aims:

(i) determine whether parental care behaviours are lateralised in a biparental substrate brooder, *T. temporalis.* Specifically, whether individuals exhibits consistent directional biases in brood defence behaviours and hemisphere use during care.

(ii) assess whether any lateral biases in parental care behaviours are aligned at the population level in a biparental fish species who coordinate and cooperate behavioural responses during reproduction.

1.11.5 Chapter 6: Flexibility in laterality of parental care behaviours in a biparental cichlid fish

Current evidence suggests that laterality, expressed at the behaviour level, may be more flexible than previously thought, but demonstrations of any flexibility in this trait have only considered individual level responses (Broder and Angeloni 2014; Ferrari et al. 2015; Chivers et al. 2016). Chapter 6 aimed to examine whether there is flexibility in aligned directional biases in parental care behaviours in a population of biparental cichlid fish, *T. temporalis,* in contexts where the social and predation pressures faced are variable. Specifically, to determine if laterality in brood defence behaviour and visual hemisphere use during parental care varies when i) biparental care is performed, and there is a strong selective pressure to coordinate and align directional biases in care behaviours, and the predation pressure of brood defence is shared amongst two parents and ii) when uniparental care is performed following the loss of a mate, and there is little or no selective pressure to coordinate and align directional biases in care behaviours, and an enhanced predation pressure results from brood defence not be shared amongst two parents.

1.12 Ethical note

Fish were housed in aquaria approved and licenced by Home Office regulations under the Animals (Scientific Procedures) Act 1986. All experiments, prior to commencement, were approved by the ethical review committees of the Faculty of Science and Engineering at the University of Hull (reference numbers FEC_69_2017, FEC_25_2018 and FEC_2019_141). Care was taken to minimise stress of experimental fish by the provision of careful handling and adequate acclimation in novel experimental tanks.

Chapter 2: Consistency in the strength of laterality in male, but not female, guppies across different behavioural contexts

Gravid female guppies (*Poecilia reticulata*)

© user: Steph McLean | Personal photo

This chapter has been published as:

McLean, S., & Morrell, L. J. (2020). Consistency in the strength of laterality in male, but not female, guppies across different behavioural contexts. *Biology letters*, *16*(5), 20190870

Abstract

Laterality, the division of brain functions into separate hemispheres, is widespread across animal taxa. Lateralised individuals exhibit cognitive advantages yet substantial variation in laterality exists, particularly between the sexes. Why variation is maintained is unknown as few studies consider differences in lateralised behaviours between the sexes, and their underlying selection pressures, across different contexts. We investigated if *Poecilia reticulata* exhibited sex differences in the direction, strength and consistency of laterality. We assessed the turning preferences of individuals detouring around a barrier to view visual stimuli representative of different behavioural contexts: an artificial object of familiar colour, an opposite sex conspecific and a no stimulus control. While no sex differences were evident in the direction or strength of laterality, consistency in the strength of laterality across contexts varied between the sexes. Individuals of both sexes consistently detoured in one direction, but the strength of laterality exhibited by males was more predictable than females across contexts. This suggests that predictability of laterality across ecologically relevant scenarios represents an important, but previously unexplored, source of variation in laterality between the sexes.

2.1 Introduction

Cerebral lateralisation or ‘laterality’, the partitioning of different cognitive processes into specific brain hemispheres (Walker 1980), is found in vertebrate and invertebrate taxa (Güntürkün 1997; Bisazza et al. 1998a; Frasnelli et al. 2012) and often observed as side biases in behaviour (Bisazza et al. 1998a; Frasnelli et al. 2012; Brown and Magat 2011a). Laterality occurs at population and individual levels. Population level laterality results when at least 50% of a population have aligned directional biases in laterality, and is thought to arise from strong selection pressures for a specific side to become specialised for a precise function (Lehman 1981). However, individuals can also exhibit consistent biases for a particular side regardless of directional preference (individual level laterality; Lehman 1981). Although the evolutionary basis of laterality at this level is unclear (Lehman 1981), the need for lateralisation of an individual’s brain to function efficiently suggests it is of strong importance for individual fitness.

Advantages of laterality include an enhanced neural capacity, hemisphere dominance and simultaneous processing of cognitive functions (Vallortigara and Rogers 2005), which result in lateralised individuals outcompeting non-lateralised conspecifics in several behaviours (Sovrano et al. 2005; Dadda et al. 2015). However, despite these advantages substantial variation in laterality persists within and between species. Why variation is maintained in a seemingly advantageous trait is unclear, especially since laterality influences fitness-related behaviours, including predator avoidance, whereby lateralised individuals exhibit enhanced performance using their preferred side (Bisazza et al. 1998a). Thus, understanding how variation in laterality develops and why it is maintained will allow a better understanding of how this variation could impact behavioural performance and ultimately fitness.

Sex is a recognised source of variation in laterality (Alonso et al. 1991; Reddon and Hurd 2009a), yet many studies remain limited to a single sex, or species where sex cannot be non-invasively identified. In species where sex differences in laterality have been studied, the patterns of laterality have been influenced by variation between males and females (Alonso et al. 1991; Reddon and Hurd 2009a). This variation has sometimes been attributed to individual traits such as boldness (Reddon and Hurd 2009b) and aggression (Reddon and Hurd, 2008), but largely the factors responsible for causing and maintaining sex-specific variation in laterality are poorly understood.

Behavioural variation between males and females within singular behavioural contexts is prevalent (Hughes 1968; Cooper et al. 2015). In many behaviours the sexes exhibit different roles and thus are subject to different selection pressures (Schuett and Dall 2009). For example, during reproduction both sexes are under strong but differing selection pressures: male fitness is determined by number of successful matings and female fitness by access to resources for gamete production (Bateman 1948). To date, few studies have considered the influence of sex differences in behaviour with regard to laterality.

Here we investigated i) the detour test as a reputable method to assess laterality at the behaviour level and ii) sex differences in the pattern (direction, strength and consistency) of laterality exhibited in three behavioural contexts using guppies (*Poecilia reticulata*), a species in which sex differences in behaviour are well established (Magurran 2005). We examined the turning preferences of individuals detouring around a barrier to view a visual stimulus, a proxy for preferences in eye use (Brown et al. 2004) and thus cerebral lateralisation. Visual stimuli represented common behavioural contexts: an artificial object to assess exploratory behaviour, an opposite sex conspecific to examine sexual motivation and a no stimulus control.

2.2 Materials and Methods

2.2.1 Animals

Sixty-seven adult wild-type guppies (34 males, 33 females) were selected from a laboratory population maintained at the University of Hull. To allow for individual identification, subjects were housed in small mixed sex tanks (20 x 20 x 18 cm) containing three differently sized individuals captured from stock tanks following visual identification. Male and female biased sex ratios were equally balanced. Tanks were maintained at 25° ± 1°C on a 12L:12D photoperiod and fed daily with commercial feed.

2.2.2 Assessing behavioural laterality

Behavioural lateralisation was assessed using a detour test (Appendix 2.1, Figure S2.1; Bisazza et al. 1997). The apparatus consisted of a rectangular tank with a double-ended T-maze joined by an opaque plastic runway. A barrier (10 x 16 cm) made of vertical cylindrical bars (0.25 cm diameter) spaced 0.25 cm apart, was placed at one end of the tank in front of a stimulus, partially obscuring it. A removable opaque plastic door separated the runway from the end sections of the T-maze. Behavioural laterality was assessed with three visual stimuli: an artificial object of familiar colour (orange test-tube bung), an opposite sex conspecific (each conspecific was unique and contained in a 8 x 11 cm transparent cylindrical tank) and an empty environment (control) presented 48 h apart, with the order randomly determined at the housing tank level. Water in the tank was maintained at 25°C, was 11 cm deep and was replaced after every fish to avoid changes in temperature and dissolved oxygen levels, which can affect laterality (Domenici et al. 2014). The tank was evenly lit and all trials were video recorded (Lifecam Studio, Microsoft, Washington, USA, connected to a computer) from above.

Each individual was allowed to acclimatise to the test arena for 3 min before being confined to one end using the door. The barrier and visual stimulus were then placed behind the runway at the opposite end of the tank and the door removed. Individuals were allowed 30s to independently approach the runway, after which they were gently encouraged using a small dip net. Fish swam down the runway towards the barrier forcing them to detour left or right. The detour direction was recorded and the individual confined at the end of the tank while the barrier and stimuli were moved to the alternate end (accounting for asymmetry in the set-up) and the procedure repeated for 10 consecutive trials.

For each individual, the direction of laterality (population level) was assessed using a relative laterality index (L_R): (right − left)/(right + left) x 100 (Bisazza et al. 1997). L_R ranges from −100 to 100 indicating a preference to consistently detour leftward or rightward respectively. The strength of laterality irrespective of direction (individual level), was assessed using the absolute laterality index L_A, calculated as $|L_R|$. L_A ranges from 0 (equal number of left and right detours) to 100 (turning consistently in one direction).

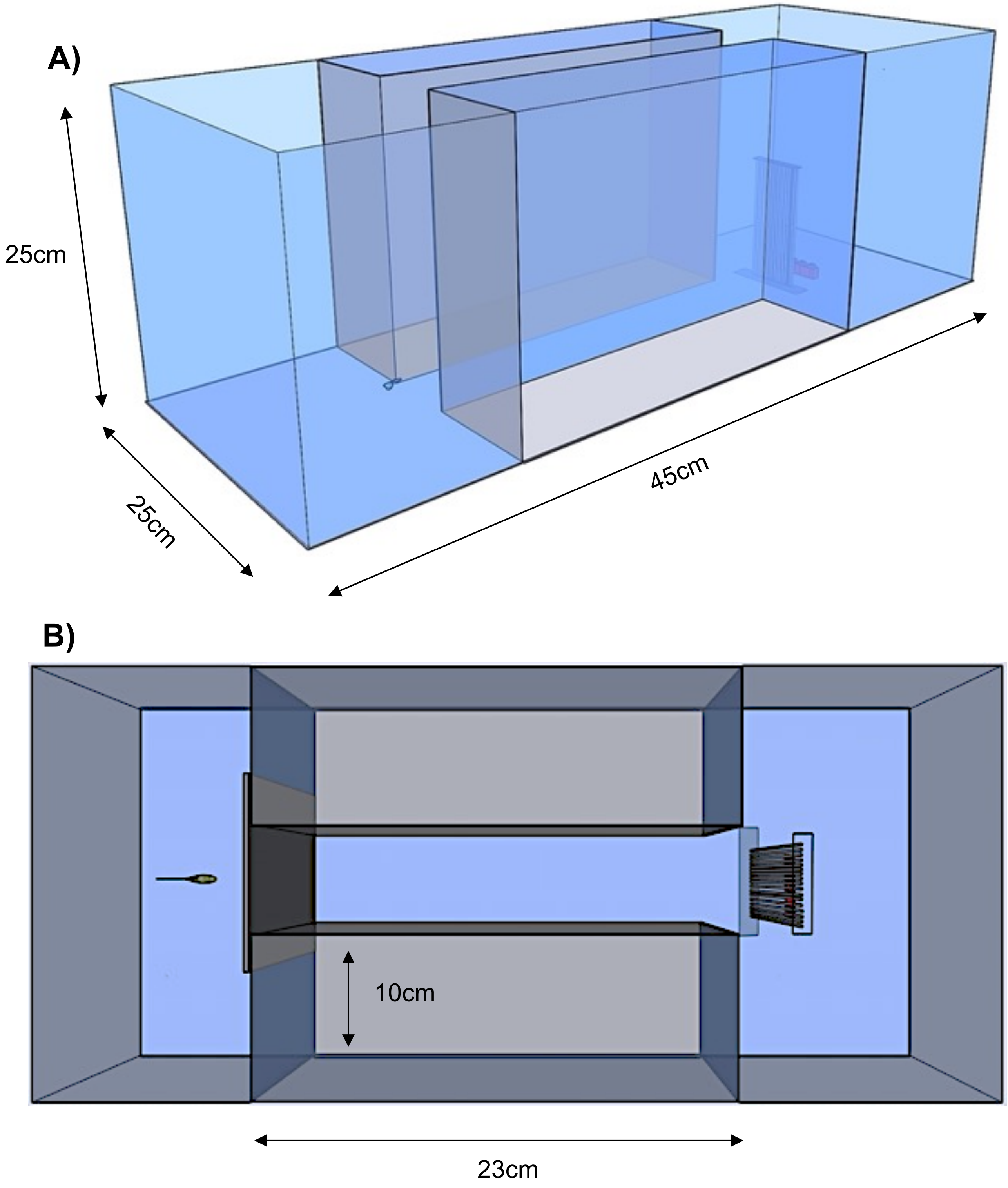


Figure 2.1: Diagrammatic representation of the detour test used to examine behavioural laterality: A) side view and B) from above. Fish swam along a runway (alternatively in opposite

directions) to detour around a barrier while viewing a visual stimulus (here an artificial object). The portable door used to confine individuals at either end of the T-maze before beginning each new trial is depicted in B).

2.2.3 Data analyses

Statistical analyses were conducted using R version 3.6.2 (R Core Team 2019). To examine whether guppies exhibited directional preferences in laterality in each behavioural context, two-tailed t-tests were used to determine whether L_R scores deviated from random choices (0). Chi-squared tests were used to examine whether fish exhibited significant individual-level laterality in each context, by determining whether there were more individuals with extreme L_A scores in the tails of the distribution than expected by chance (based on a normal approximation to the binomial distribution at $p = 0.5$). $\chi 2$ was calculated as $((N - 1) \times \mathrm{var}(X1)/(n \times 0.5 \times 0.5)$, where N is the number of individuals, n is number of trials per individual and X1 is the number of right (or left) turns per individual (Roche et al. 2020). The false discovery rate method (Benjamini and Hochberg 1995) was used to correct for multiple testing and adjusted p-values are reported. Linear mixed effects models (LMM), fitted using lme4 (Bates et al. 2015), were used to examine the effect of sex, stimulus, body length, housing sex ratio and biologically relevant two-way interactions on L_R and L_A. Individual ID nested within group was included as a random factor to account for repeated measures and for each housing tank experiencing the stimuli in the same order. Residuals were assessed for homoscedasticity and a normal distribution by visual inspection of residual-fit and Q-Q plots. The model (R code format) that best explained the data for L_R and L_A, based on model simplification using likelihood ratio tests, was: $L_R/L_A \sim 1$.

We assessed consistency of laterality using two measures: repeatability (group level) of L_R and L_A, and predictability (individual level) of L_A only. Between context agreement repeatability estimates were calculated for the L_R and L_A of each sex using the rpt function (rptR package; Stoffel et al. 2017), from variance components of a LMM with individual ID as a grouping random factor. Statistical significance of repeatability was determined by likelihood ratio tests comparing the deviances of models with the grouping factor of interest to models without. Observed likelihood ratios were compared to distributions of likelihood ratios determined from parametric bootstrapping (x1000) to obtain p-values (Faraway 2016). To compare predictability of L_A between sexes, we used the residuals from simplified LMMs containing context as a fixed effect and individual ID as a random intercept, for each sex separately, to calculate a measure of intra-individual variation (IIV; Stamps et al. 2012). Context was retained in the model regardless of significance, since it was directly relevant to experimental design. IIV was calculated as the residual individual standard deviation (riSD), $\surd(\Sigma(Y_{ij} - E_{ij})^2)/N_i - 1$. Here, Y_{ij} and E_{ij} represent observed and expected values for each individual (*i*) at each observation (*j*) and N_i represents the number of observations (Stamps et al. 2012). Male and female IIV were compared using a two-sample t-test to determine if predictability of L_A varied between sexes.

2.3 Results

L_R and L_A were not influenced by sex, stimulus, housing sex ratio, body size or their interactions (Table 2.1). Thus, males and females exhibited similar patterns of laterality in the detour test regardless of behavioural context.

Table 2.1: The effect of sex, stimulus, body length, housing sex ratio and biologically relevant two-way interactions on A) L_R and B) L_A. The intercept represents females, from female biased tanks, in the conspecific treatment.

	estimate	**s.e.**	**df**	**t**	**p**
A) Direction of laterality, L_R					
Intercept	26.408	39.855	63.365	0.663	0.510
Sex (male)	82.242	80.546	62.853	1.021	0.311
Stimulus (control)	-7.058	8.109	130.000	-0.870	0.386
Stimulus (artificial object)	-1.176	8.109	130.000	-0.145	0.885
Body length	-0.922	1.348	61.370	-0.684	0.497
Sex ratio (male-biased)	-7.299	8.581	26.592	-0.851	0.403
Sex (male) x Stimulus (control)	-5.668	11.554	130.000	-0.491	0.625
Sex (male) x Stimulus (artificial object)	-3.066	11.554	130.000	-0.265	0.791
Sex (male) x Body length	-2.713	2.806	61.986	-0.967	0.337
B) Strength of laterality, L_A					
Intercept	38.224	20.045	59.328	1.907	0.061
Sex (male)	-61.213	40.631	57.143	-1.507	0.137
Stimulus (control)	-4.705	5.470	129.995	-0.860	0.391
Stimulus (artificial object)	-9.411	5.470	129.995	-1.720	0.087
Sex ratio (male-biased)	5.255	5.600	23.440	0.938	0.357
Body length	-0.078	0.669	54.250	-0.118	0.906
Sex (male) x Stimulus (control)	5.311	7.795	129.995	0.681	0.496
Sex (male) x Stimulus (artificial object)	13.654	7.795	129.995	1.752	0.082
Sex (male) x Body length	1.860	1.413	55.877	1.317	0.193

Individuals exhibited significant laterality (L_A) in the detour test (Table 2.2B). Thus, guppies tended to detour consistently leftward or rightward in each behavioural context (Figure 2.2). However, individual preferences were not aligned among individuals as no overall population-level directional bias (L_R) was observed in any behavioural context (Table 2.2A).

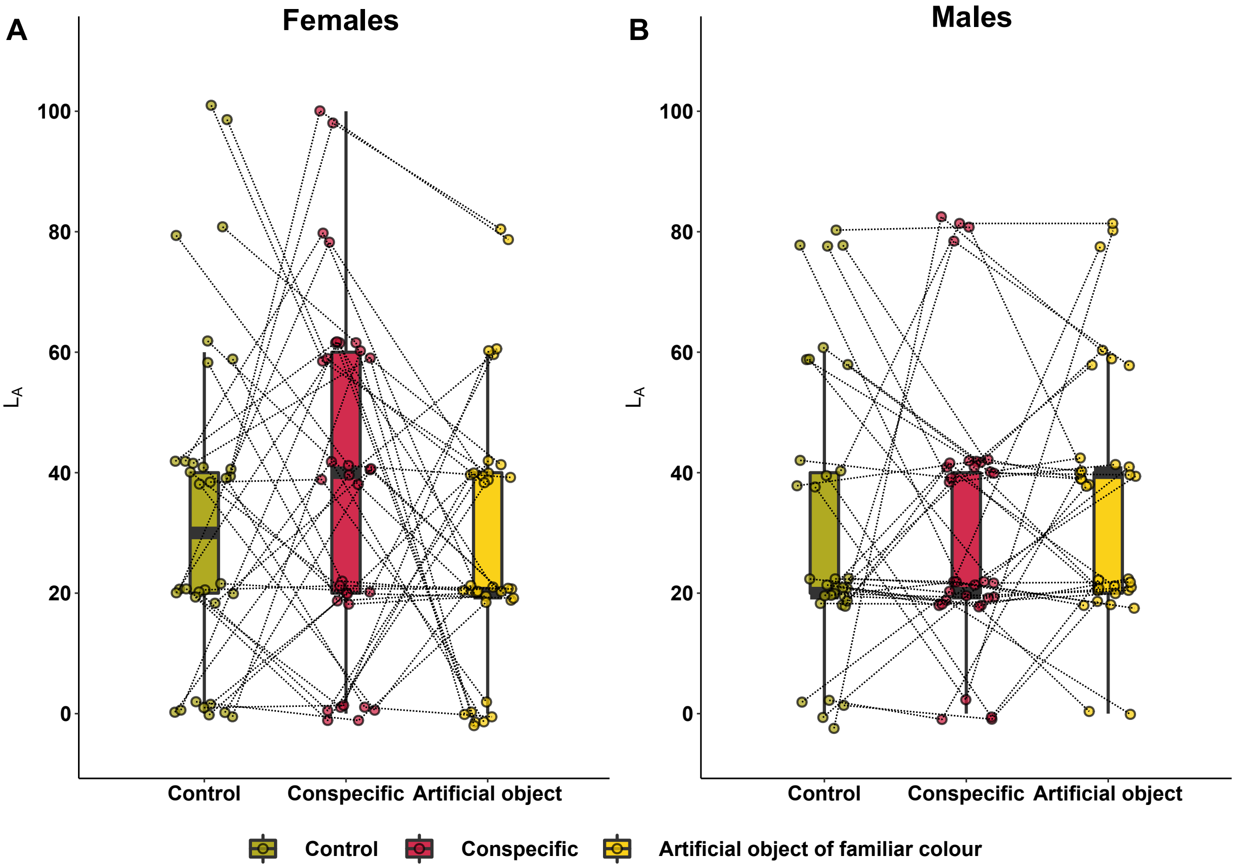


Figure 2.2: L_A for A) female and B) male guppies detouring around a barrier to approach an empty environment control (green), an opposite sex conspecific (red) or an artificial object (yellow). Boxplots display the median and IQR for each sex while individual points represent the L_A of each individual in each context and dashed lines represent between context consistencies.

L_R was repeatable across contexts in both sexes (males: R = 0.324 ± 0.116, 83% CI = 0.162 – 0.471, p = 0.001; females: R = 0.363 ± 0.115, 83% CI = 0.19 – 0.506, p < 0.001; Figure 2.3A) however; L_A was only significantly repeatable in males, not females (males: R = 0.252 ± 0.11, 83% CI = 0.081 – 0.390, p = 0.011; females: R = 0.124 ± 0.095, 83% CI = 0 – 0.255, p = 0.134; Figure 2.3A). Females were significantly less predictable (larger riSD) at the individual level than males (t = 3.267, d.f. = 62.169, p = 0.001; Figures 2.2 and 2.3B).

Table 2.2: One-sample t-tests and χ^2 tests examining if A) L_R and B) L_A differed from random choices in each behavioural context. Adjusted p-values are reported and if significant highlighted in bold.

		A) Direction of laterality, L_R		**B) Strength of laterality, L_A**	
Stimulus	**df**	**t**	**p**	**χ^2**	**p**
Conspecific	66	-0.224	0.823	124.704	**< 0.001**
Control	66	2.224	0.089	109.026	**0.001**
Artificial object	66	-0.821	0.621	98.591	**0.006**

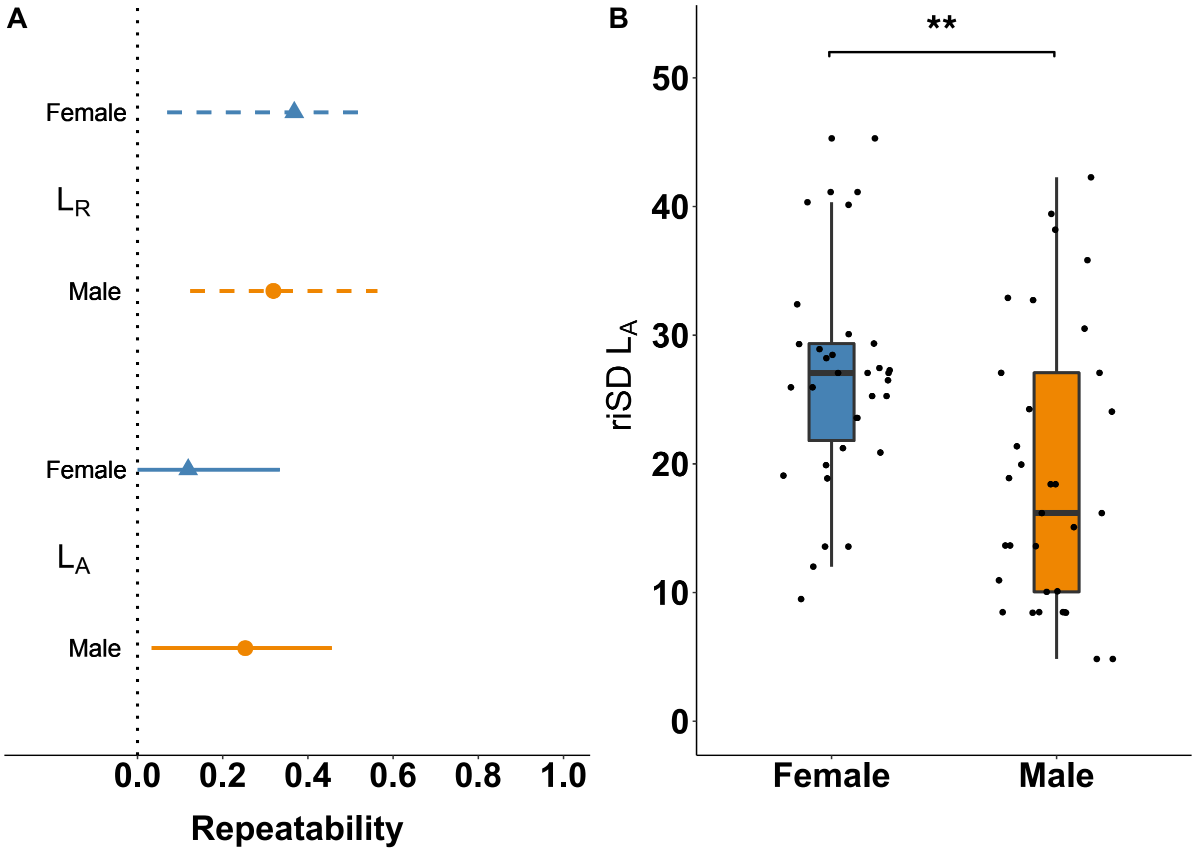


Figure 2.3: A) repeatability of L_R and L_A for females (blue triangles) and males (orange circles) with associated 83% CI (L_R = dashed, L_A = solid) as recommended by Payton et al (2003). B) riSD of L_A for females (blue) and males (orange). Asterisks indicate significant differences between groups at $p<0.05$.

2.4 Discussion

Our results provide the first evidence of a sex difference in the consistency of laterality across different behavioural contexts. Individuals of both sexes consistently turned left or right, but males were more predictable than females in the strength of laterality exhibited across contexts. Although our study does not allow consistency across contexts to be distinguished from consistency in general (i.e. both within and across contexts), previous work has shown within-context consistency of laterality in female guppies (Brown and Irving 2014). Little is known about the relationship between laterality and other behaviours in guppies, but we propose three key factors that may drive the observed difference in consistency: laterality as the subject of sexual selection, variation in sexual motivation, and sex differences in the strength of selection resulting from predation.

In line with other studies in Poecilids (Bisazza et al. 1997; 1998b) and fish more generally (Bisazza, 1998a; Nepomnyashchikh and Izvekov 2006) we identified consistent directional biases at the individual level when detouring around a barrier while viewing a visual stimuli in three behaviour contexts (exploratory behaviour, sexual behaviour and when no stimulus is present; Figure 2.2). We found no evidence that individual biases were aligned among individuals at the population level in any behavioural context. This is not surprising given that population level biases in the absence of a visual stimulus or with a novel object representative of exploratory behaviour are not documented in poecilids, while the available evidence of asymmetries in eye use when viewing an opposite sex conspecific or group of opposite sex conspecifics is contradictory. Males of some species of poecilid fish (*Gambusia holbroki, Gambusia nicaraguensis* and *Poecilia reticulata*) exhibit a rightward detour bias when viewing a group of females while other species (*Brachyrhaphis roseni* and *Girardinus falcatus*) exhibited a leftward bias in the same context (Bisazza et al 1997). However, other studies in fish have reported consistent directional biases in eye use at the individual level despite there being no overall population-level bias for a specific direction (Cantalupo et al. 1995). Thus, we confirm that the detour test, a widely used methodology to assess laterality in fish (Bisazza et al. 1997; 1998a; 1998b; Nepomnyashchikh and Izvekov 2006; Reddon and Hurd 2008; 2009a; 2009b), reptiles (Csermely et al. 2010), birds (Vallortigara et al. 1999a) and molluscs (Domenici et al. 2017) is a reputable method to assess laterality at the behavioural level.

Sex differences in the consistency of parental care behaviours are found in house sparrows (*Passer domesticus*), whereby males exhibit strong consistency not observed in females (Nakagawa et al. 2007). Male consistency in this case acts as an indicator of male quality for female mate choice and has direct implications for sexual selection (Nakagawa et al. 2007). The increased consistency of L_A in male guppies could result from laterality being under direct sexual selection, or correlated with sexually selected traits, but this is currently unknown.

Secondly, sexual motivation could impact the strength of laterality exhibited in the detour test and thus the consistency of L_A across contexts. In guppies, both sexes are under strong sexual selection (Magurran 2005): male fitness is driven by number of successful matings (Bateman 1948) while female fitness is driven by access to resources for gamete production (Bateman 1948). As guppies live-bear, gravid females are likely less sexually motivated than non-gravid females (Magurran and Seghers 1994a, 1994b; Macbeth and Luine 2010), while selection for males to pursue mating opportunities results in high levels of sexual harassment towards females (Brewster and Houde 2003). Sexual motivation across males is thus likely more consistent, while in females sexual motivation likely varies with reproductive status.

In female guppies deprived of males, strong lateral biases have been shown that are not present in females with access to males (Bisazza et al. 1998b). Here, female reproductive status and associated sexual motivation was unknown, but could have influenced variation in female L_A both within and across contexts. For example, live bearing is associated with enhanced cognition especially in tasks involving spatial learning (Magurran and Seghers 1994b) that are important for successful foraging and offspring survival (Pawluski et al. 2006). Thus, heavily gravid females may exhibit stronger lateralisation with artificial objects or in empty environments relative to non-/less gravid counterparts, as a result of enhanced cognition in spatial tasks associated with carrying young.

Finally, male guppies experience stronger predation pressures than females owing to brighter colouration and smaller size (Bisazza 1993). Increased predation pressure could have a strong stabilising effect on L_A in males, driven by natural selection, which causes them to exhibit consistency in L_A across behavioural contexts. In some fishes, increased predation pressure is associated with stronger laterality (Brown et al. 2004, 2007b), which has been linked to enhanced escape reactivity (Dadda et al. 2010) suggesting that individuals exhibiting strong predation pressures would benefit from consistent hemispherical biases regardless of context.

Despite no overall sex differences in the direction and/or strength of laterality, our results demonstrate variation between the sexes in the consistency of L_A across contexts. Several factors could underpin this variation, the majority of which have yet to be studied in the context of laterality. Thus exploration of the effects of sexual selection, reproductive state and predation pressure on the evolution and expression of laterality within and between contexts in guppies is needed. Future studies should also investigate the generality of this finding by examining laterality in both sexes across a variety of behavioural contexts and species.

Chapter 3: Sex Differences in Laterality Are Associated with Reproduction in Threespine Stickleback

Male three spine stickleback defending eggs in a nest (*Gasterosteus aculeatus*)

© user: Steph McLean | Personal photo

This chapter has been published as

McLean S & Morrell L. J. (2021) Sex Differences in Laterality Are Associated with Reproduction in Threespine Stickleback. *The American Naturalist*, *197*(6).

Abstract

Laterality, the partitioning of information processing into specific brain hemispheres, is widespread across animal taxa. Substantial unexplained variation in this trait exists, particularly between the sexes, despite multiple identified advantages of lateralisation. Here, we demonstrate a relationship among laterality (measured as directional biases), reproduction, and experience of mating and parenting. Using threespine sticklebacks (*Gasterosteus aculeatus*), a species with uniparental male care, we showed that individuals of the caring sex (males) were more strongly lateralised than the non-caring sex (females) during reproduction and that laterality was reduced outside the breeding season in males. Additionally, males with experience of mating and parenting were more strongly lateralised than males without this experience. Our findings suggest that fitness related behaviours that vary between the sexes, such as reproductive behaviours including courtship, spawning, and parenting, are significant but previously unidentified sources of variation in laterality.

3.1 Introduction

Cerebral lateralisation or "laterality", the partitioning of cognitive functions into specific brain hemispheres, was originally thought to be a trait unique to humans because of its tight association with complex brain functions (Bisazza et al. 1998a; Corballis 2012). However, laterality is now recognised as a ubiquitous trait, exhibited by vertebrate (Walker 1980; Güntürkün 1997; Bisazza et al. 1998a) and invertebrate (Frasnelli et al. 2012; Niven and Bell 2018) taxa that is often observed at the behavioural level as preferential use of one side of a bilateral characteristic (Koboroff et al. 2008; Brown and Magat 2011a) or as side biases in behaviours (Bisazza et al. 2001a). Such biases stem from cerebral lateralisation and are an indicator of the degree of lateralisation in an individual's brain (Vallortigara and Rogers 2005; Reddon et al. 2009a; Gutiérrez-Ibáñez et al. 2011; Jozet-Alves et al. 2012b).

Several advantages of cerebral lateralisation have been identified, including an increased neural capacity (Pascual et al. 2004; Magat and Brown 2009), enhanced ability to multi-task (Dadda and Bisazza 2006a, 2006b), greater spatial and numerical discrimination abilities (Bisazza and Dadda 2005; Sovrano et al. 2005; Dadda et al. 2015), and improved performance when using the preferred side in fitness-related behaviours (Takeuchi et al. 2012). These advantages are thought to arise from lateralised individuals being better able to cope with divided attention resulting from simultaneous information processing (Rogers et al. 2004). However, laterality also has costs, including a reduced efficiency in tasks requiring hemispheric communication and cooperation (Dadda et al. 2009), increased predictability from consistent biases in behaviours (Cantalupo et al. 1995; Takeuchi 2012), and reduced efficiency in responses to stimuli viewed in the non-preferred visual hemifield (Vallortigara and Rogers 2005).

Substantial unexplained variation in laterality persists both within and between species, especially at the individual level (Bisazza et al. 1997). Sex is an increasingly recognised source of variation in laterality, with males and females often differing in the patterns of laterality they exhibit (Lemur spp. [Milliken et al. 1991]; Gallus *gallus domesticus* [Vallortigara and Andrew 1991]; *Archocentrus nigrofasciatus* [Reddon and Hurd 2008]). While in some cases this variation has been attributed to prenatal steroid hormones (Schaafsma and Groothuis 2011) and intrinsic traits such as boldness (Irving and Brown 2013) and aggression (Reddon and Hurd 2008), the underlying drivers of sex-specific variation in laterality remain poorly understood.

The influence of reproductive experience on both brain and behaviour is well documented (Franssen et al. 2011; Reichert et al. 2012; Royle et al. 2012; Stein et al. 2016), yet it has rarely been considered in the context of laterality, which is surprising given that it can be highly variable between the sexes. Some reproductive behaviours are lateralised, including courtship (Ventolini et al. 2005; Vidal et al. 2018) and male mate choice (Templeton et al. 2012), but how reproductive experience could influence laterality is largely unknown. The performance of reproductive behaviours could select for different patterns of cerebral lateralisation in individuals or between the sexes, especially in cases where these behaviours are sex specific. Courtship behaviours, for example, often involve auditory, visual, and chemical signals and include dance, posture, and/or calls (Bastock 1967), whereby individuals could have enhanced performance when these signals are processed in a specific brain hemisphere. Similarly, parental care is a key reproductive behaviour in many taxa that is extremely diverse between both species and sexes (Royle et al. 2012). Caring parents must perform multiple functions simultaneously—including, for example, predator avoidance or foraging—while concurrently caring for offspring (Royle et al. 2012). The cognitive advantages of laterality, particularly an enhanced ability to perform simultaneous information processing (Dadda and Bisazza 2006a, 2006b), could mitigate the cognitive costs of reproduction, ultimately driving variation in laterality that results from selection for different patterns of cerebral lateralisation between sexes or between individuals with differing experience of reproduction because of the potential fitness benefits that could be obtained.

Here, we test the hypotheses that variation in laterality is driven by reproduction and the performance of reproductive behaviours. Specifically, we examine whether in a uniparental species (i) the caring sex is more strongly lateralised than the non-caring sex and (ii) whether laterality is linked to the performance of reproductive behaviours, including nest construction and courting, mating, and parenting. Threespine sticklebacks (*Gasterosteus aculeatus*) are a model system for examining these questions, as they perform uniparental male care with a number of distinctive stages to the reproductive cycle, including territory defence, construction of a nest, attraction of a mate, and care for the offspring before and after hatching (Tinbergen 1952). We predict that males should exhibit stronger laterality than females, reproductive males should be more strongly lateralised than non-reproductive males, and males that have experience of performing reproductive behaviours, including nest construction and courting, spawning, and parenting, should be more strongly lateralised than males absent in this experience because of the cognitive advantages associated with cerebral lateralisation.

3.2 Materials and Methods

3.2.1 Animals

A total of 153 adult *Gasterosteus aculeatus* were purchased from CarpCo in February 2019, sourced from naturally breeding populations in clay-based ponds filled from the River Bourne in Hadlow, Kent, United Kingdom. The fish were held in mixed sex groups in two large stock tanks (75 x 75 x 40 cm) that were provided with enrichment (gravel substrate, plastic plants, and shelter) and maintained on aerated freshwater under temperature and lighting conditions that encouraged reproductive development (17 ° C ± 1° C on a 16L:8D photoperiod). Once individuals were in a reproductive state, identifiable by male nuptial coloration (an orange/red throat and blue eyes; Hiermes et al. 2016), the sexes were isolated into two single-sex stock tanks (75 x 75 x 40 cm); 69 males, 84 females) for one week prior to behavioural trials. All individuals were fed to excess three times daily frozen bloodworm (chironomid larvae) and *Daphnia sp*.

Males and females were assessed for behavioural laterality (see below) at multiple stages during a reproductive cycle outlined in Figure 3.1, as follows:

stage i. In a reproductive state but before any reproductive behaviours were performed (males and females) stage ii. After performing pre-spawning reproductive behaviours but before courting and spawning (males only)

stage iii. After performing post-spawning reproductive behaviours, including courting and spawning (males and females)

stage iv. In a non-reproductive state after a reproductive cycle (males and females.

3.2.2 Stages of Reproduction

Stage i: Reproductive State

A total of 83 *G. aculeatus* (50 males and 33 females) were haphazardly selected from the stock population and initially assessed for laterality (see below) once they were in a reproductive state (males showing nuptial coloration and females showing evidence of being gravid; Figure 1i) but before any reproductive behaviours had been performed. Reproductive males were then assigned to one of two treatment groups, breeding males and non-breeding males, using a random number generator but with an equal distribution across treatments ensured (n per treatment = 24). The breeding treatment represents males performing reproductive behaviours, including nest construction, courtship, spawning, and parenting (Kynard 1978), while non-breeding males are a control for males performing such behaviours (Figure 3.1).

Stage ii: Pre-spawning

Breeding males were transferred to individual nesting aquaria (n = 24) comprising a plastic tank (45 x 30 x15 cm) with gravel substrate, a plastic plant, and nesting materials: a small plastic dish (18 cm diameter) filled with sand and two hundred 6-cm-long black polyester threads. Sticklebacks readily use polyester threads as nesting materials in laboratory studies, since they resemble unicellular algae and wild vegetation (Barber et al. 2001; Johannesen et al. 2012). To encourage nest construction, each breeding male was provided with visual access to a different heavily gravid female (confined to a small area [28 x 14 x14 cm] of the nesting aquaria by a clear, perforated plastic divider) for 30 min twice daily until a nest with a visible entrance, representative of completion (Van Iersel 1953), was present (~1 day). Breeding males were then measured for laterality (Figure 3.1ii). Non-breeding (control) males were transferred to individual nesting aquaria (n = 24) identical to those of breeding males but absent of nesting materials. Control males were also given visual access to a different heavily gravid female for 30 min twice daily for 1 day (average number of days to complete nest construction by breeding males); thus, the only difference between treatments was nest construction. Following visual exposure to gravid females, non-breeding males were measured for laterality (Figure 3.1ii).

Stage iii: Post-spawning

Breeding males with a constructed nest were then given access to the heavily gravid female, which they were previously exposed to during nest building (n = 24). Once females had successfully laid eggs and males had spawned, females were isolated and measured for laterality within 24 h (Figure 3.1iii). Males were allowed to perform parental care behaviours (territory defence, nest maintenance, and egg fanning and guarding) until free swimming larvae were observed, before being measured for laterality (Figure 3.1iii).

Non-breeding control males were provided with nest materials (a small 18-cm-diameter plastic dish filled with sand and two hundred 6-cm-long black polyester threads) and visual access to a gravid female to encourage nest construction. Once nest construction was complete, control males were measured for laterality (Figure 3.1iii). Thus, our experimental treatment differed from our control treatment in mating (courting and spawning with a female) and parenting experience. We recognise that our experiment does not separate mating and parenting experience. However, disentangling the two effects would have involved (i) experimental destruction and removal of the nests and fertilised eggs of control males and (ii) removal of breeding males from their breeding territory after spawning to perform measurements of laterality, both of which could have influenced the subsequent behaviour of these individuals.

Stage iv: Non reproductive State

Following a reproductive cycle, breeding and non-breeding males were individually housed within a large stock tank (75 x 75 x 40 cm) to allow for individual identification, while a stock population of females were kept separately from males but in a group to ensure they could be sexed in a non-reproductive state. Temperature and photoperiod were adjusted over a period of 7 days to be representative of United Kingdom winter conditions when this species is not reproductive (10 ° C ± 1° C on a 12L:12D photoperiod and fed once daily ad lib.). Fish were maintained under winter conditions for 6 weeks, by which time males and females showed no signs of nuptial coloration and egg production, respectively, before both sexes (males: n = 48; females: n = 32) were measured for laterality (Figure 3.1iv).

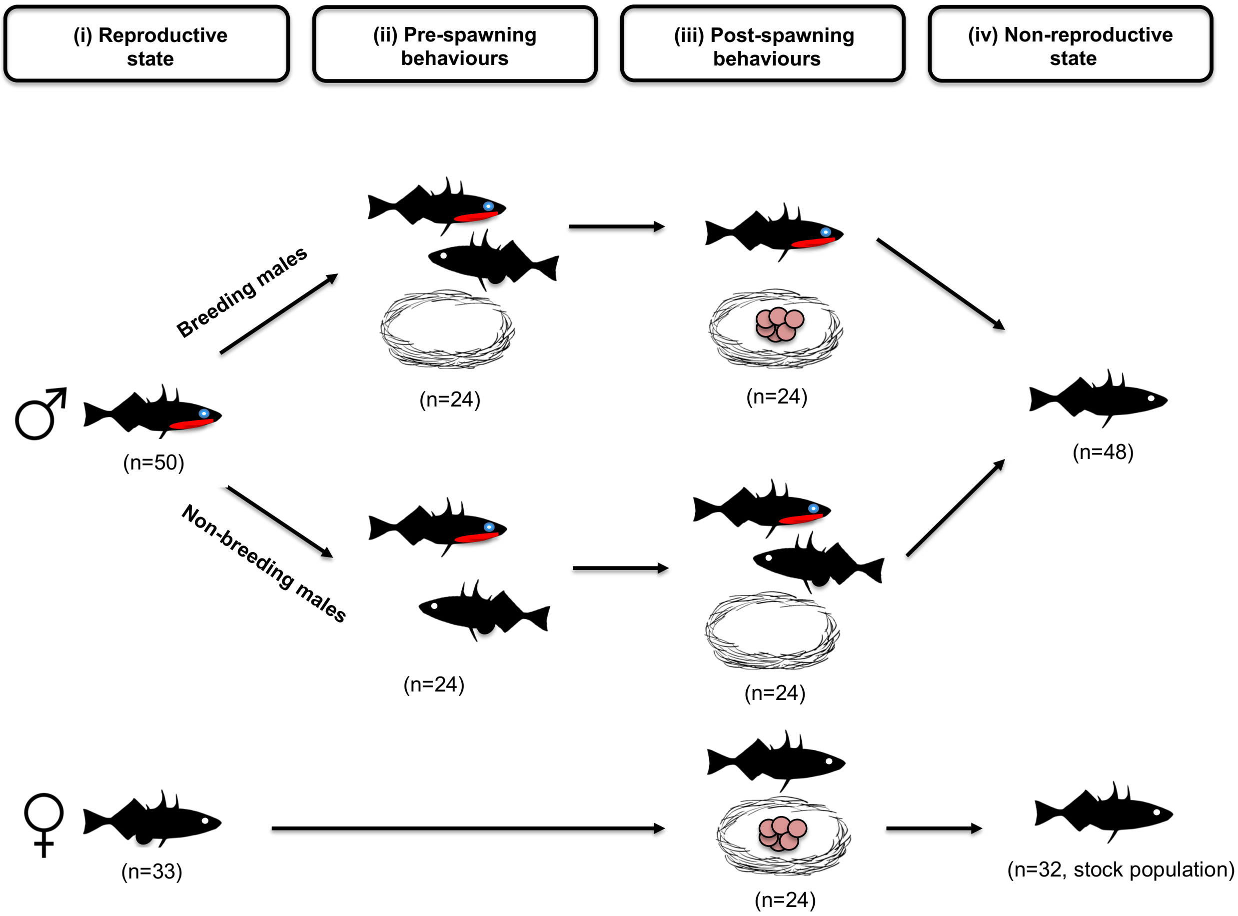


Figure 3.1: Diagrammatic representation of the reproductive stages when males (♂) and females (♀) were measured for laterality: (i) reproductive state—showing nuptial coloration (♂) or gravid (♀); (ii) pre-spawning—nest construction (♂); (iii) post-spawning—courtship, spawning, and performance of parental care behaviours (♂) or egg laying (♀); (iv) non-reproductive—no signs of nuptial coloration (♂) or egg production (♀).

3.2.3 Assessing Behavioural Laterality

Behavioural laterality was measured in a standard detour test (Bisazza et al. 1997; Figure 3.2), which assesses directional preferences in detour behaviour. The setup consisted of a large glass tank (90 x 50 x 40 cm; water depth of 12 cm maintained at 17 ° C ± 1° C [reproductive] and 10 ° C ± 1° C [non-reproductive]), lit evenly from above, that contained a central runway joining two t-shaped compartments (Figure 3.2). Each compartment could be partially obscured by a barrier (10 x16 cm) made of plastic cylindrical bars (0.25 cm diameter) placed 0.25 cm apart, designed to hinder but not eliminate viewing of a stimulus placed behind the barrier. Here, the stimulus was a shelter consisting of half a plant pot (7.5 cm diameter) placed behind an artificial plant (13 cm tall), since threespine sticklebacks often seek refuge before performing ecologically relevant behaviours (e.g., foraging) in new environments (Krause et al. 1998). Fish have laterally placed eyes with little frontal overlap; thus, each eye receives an independent view of the surrounding environment with slow and incomplete interocular information transfer (McClearly 1960). As a result, information obtained from each eye is sent almost exclusively to the contralateral brain hemisphere (Irving and Brown 2013); thus, the direction detoured represents a proxy for preferences in eye use and associated hemispheric processing of the visual stimulus.

An individual fish, captured using a small dip net, was allowed to acclimatise to the empty experimental setup for 3 min prior to commencing a behavioural trial. The individual was then confined to one of the t-shaped compartments using an opaque plastic door while the barrier and visual stimulus were placed in the alternative t-shaped compartment at the opposite end of the runway. Assessment of laterality commenced when the door was lifted, allowing the individual access to the runway. Each individual was given 30s to independently approach the runway, after which it was gently encouraged from behind with a small dip net. Fish then swam down the runway toward the barrier, forcing them to detour left or right. For each individual this procedure was repeated for 10 consecutive detours (one behavioural trial) on alternating ends of the runway to account for any asymmetry in the setup, and the direction detoured was recorded. Water changes were conducted between trials, since changes in temperature and dissolved oxygen levels are known to affect behavioural laterality (Domenici et al. 2014).

For each individual at each reproductive stage, a relative lateralisation index (L_R) was calculated using the following formula: [(right detours - left detours)/(right detours + left detours) x 100]. The L_R index assesses directional biases of fish at the population level and ranges from -100 to +100, representing a population that detoured consistently leftward or rightward, respectively (Bisazza et al. 1997).

An absolute laterality index (L_A) was also calculated to determine the strength of laterality at the individual level. The L_A index was calculated as $|L_R|$ and ranges from 0 (individuals that turned left and right an equal number of times) to 100 (individuals that turned consistently in one direction; Bisazza et al. 1997). Additionally, we also calculated a measurement of body size (standard length; cm) for each individual using ImageJ (Schneider et al. 2012).

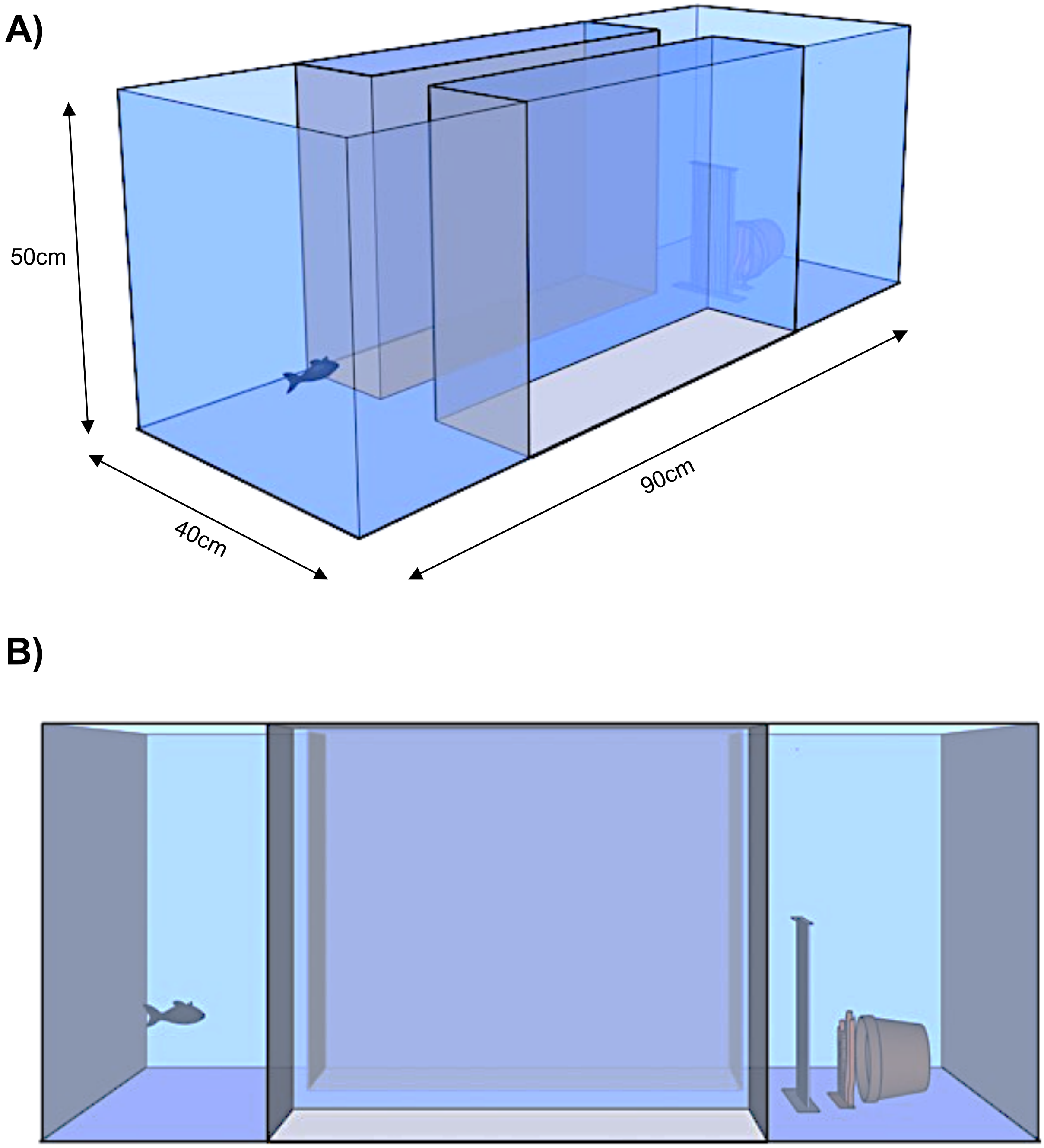


Figure 3.2: Diagrammatic representation of the detour test used to examine behavioural laterality: A) diagonal view and B) side view. Fish swam along a runway (alternatively in opposite directions) to detour around a barrier while viewing a visual stimulus (here a plant pot and artificial plant representative of a shelter).

3.2.4 Data Analyses

Data analyses were conducted using R version 3.6.2 (R Core Team 2019).

3.2.4.1 Is There Evidence for Laterality in Sticklebacks?

One sample t-tests were used to determine whether the L_R of male and female populations at each reproductive stage differed significantly from a random expectation of no laterality (0). To test for individual-level laterality, we examined the sample variance using a χ^2 test that compared the observed variance to the expected variance to determine whether male and female individuals exhibited more extreme L_A scores than expected by chance and thus exhibited significant laterality, on the basis of a normal approximation to the binomial distribution at p = 0.5 (Roche et al. 2020; McLean and Morrell 2020). We calculated χ^2 as ((N - 1) x var(X1)/(n x 0.5 x 0.5), where N is the number of individuals, n is number of trials per individual, and X1 is the number of right (or left) detours per individual (for a detailed description of the methodology, see the supplementary information in Roche et al. 2020).

3.2.4.2 Are There Sex Differences in Laterality When Reproductive and Not?

Next, we used a linear mixed effects model (LMM), fitted using lme4 (Bates et al. 2015) to assess the effects of sex, reproductive stage, and their interaction on both L_R and L_A. Only individuals in a reproductive (stage i) and non-reproductive (stage iv) state were included, as only these are common states across both sexes. Body size was included as a covariate and individual ID as a random effect to account for the repeated measures design for males across all reproductive stages and females before and after egg laying. Females in a non-reproductive state (stock population) were assigned a unique ID also included in the model. The model was fitted using a Gaussian error distribution following previous work on laterality (Bisazza et al. 1997), and assumptions of normality and homoscedasticity of residuals were assessed using visual inspection of residual-fit plots and quantile-quantile plots.

3.2.4.3 Is Laterality Linked to the Performance of Reproductive Behaviours?

Finally, L_A data were split by sex to examine the effect of reproductive stage, fitted as a categorical predictor variable, and body size within each sex separately using LMMs (Bates et al. 2015), including individual ID as a random effect and assuming Gaussian errors. Assumptions of normality and homoscedasticity of residuals were assessed as before, but for females these assumptions were violated. Consequently, we used a generalised linear model (GLMER) with a binomial error distribution (fitted with lme4; Bates et al. 2015) to assess whether the reproductive behaviours performed by females influenced the proportion of turns to the preferred side. The fitted GLMER included individual ID as a random factor and was not overdispersed (Harrison 2014). For males we incorporated a priori planned comparisons into our LMM to test hypotheses about the performance of reproductive behaviours on laterality (Sokal and Rohlf 1981; Ruxton and Beauchamp 2008)—specifically, whether there were differences in L_A between males that were reproductive, had performed nest construction, and had experience of mating and parenting relative to their respective control treatments.

3.3 Results

3.3.1 Is There Evidence for Laterality in Sticklebacks?

There was no evidence for a population-level directional bias in either sex at any stage of reproduction (Table 3.1A; $p > .05$ in all cases). However, at an individual level, significant turning preferences were evident in males (caring sex) but not in females (non-caring sex) across several stages of reproduction (Table 3.1B). In four of the six stages examined in males (reproductive state; pres-pawning—built nest; pre-spawning—no nest; post-spawning—mating and parenting experience), individuals consistently detoured in a specific direction, regardless of directional preference, more often than expected by chance.

3.3.2 Are There Sex Differences in Laterality When Reproductive and Not?

There was no effect of body size, sex, stage of reproduction, or their interaction on directional biases in laterality at the population level (L_R; Appendix 3.1, Table S3.1A); however, there was a significant interaction between sex and reproductive state on the strength of laterality (L_A; sex x stage interaction: $F_{1, 154} = 5.499$, $p = .020$; Appendix 3.1, Table S3.1B). Males were more strongly lateralised than females when reproductive; however, no variation was evident between the sexes in non-reproductive state (Figure 3.3).

Table 3.1: Results of A) one-sample t-tests examining if L_R differed from random expectation of no laterality (0), thus, representing that greater than 50% of sampled individuals exhibited aligned directional biases at the population level (L_R), and B) chi-squared tests examining if L_A values were more extreme than expected, thus representing individual level consistent directional biases (L_A) in the detour test, for males and females at each stage of reproduction. P-values are corrected for multiple testing using the false discovery rate method (Benjamini and Hochberg 1995). * $p < 0.05$. ** $p < 0.01$.

			A) L_R		**B) L_A**	
Sex	**Stage**	**df**	**t**	**p**	**χ^2**	**p**
Male	Reproductive	49	1.695	0.867	92.192	**0.002**
	Pre-spawning – built nest	23	0.092	0.927	45.183	**0.001**
	Pre-spawning – no nest	23	1.193	0.506	52.733	**0.008**
	Post-spawning – mated & parented	23	1.175	0.453	54.333	**0.001**
	Post spawning – not mated or parented	23	1.533	0.416	31.933	0.152
	Non-reproductive	43	0.637	0.678	47.154	0.345
Female	Reproductive – gravid	32	1.629	0.509	32.872	0.424
	Post-spawning – laid eggs	23	0.414	0.768	35.733	0.079
	Non-reproductive	31	0.820	0.627	36.800	0.280

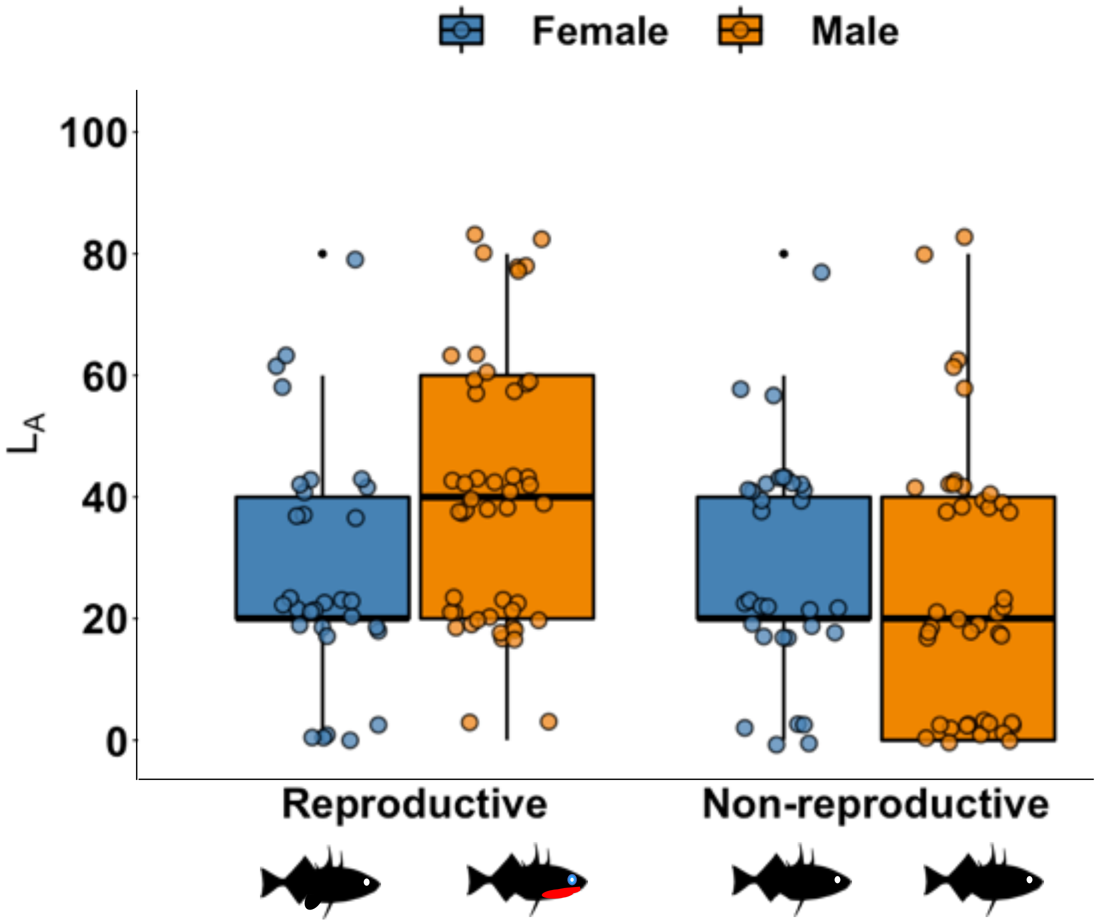
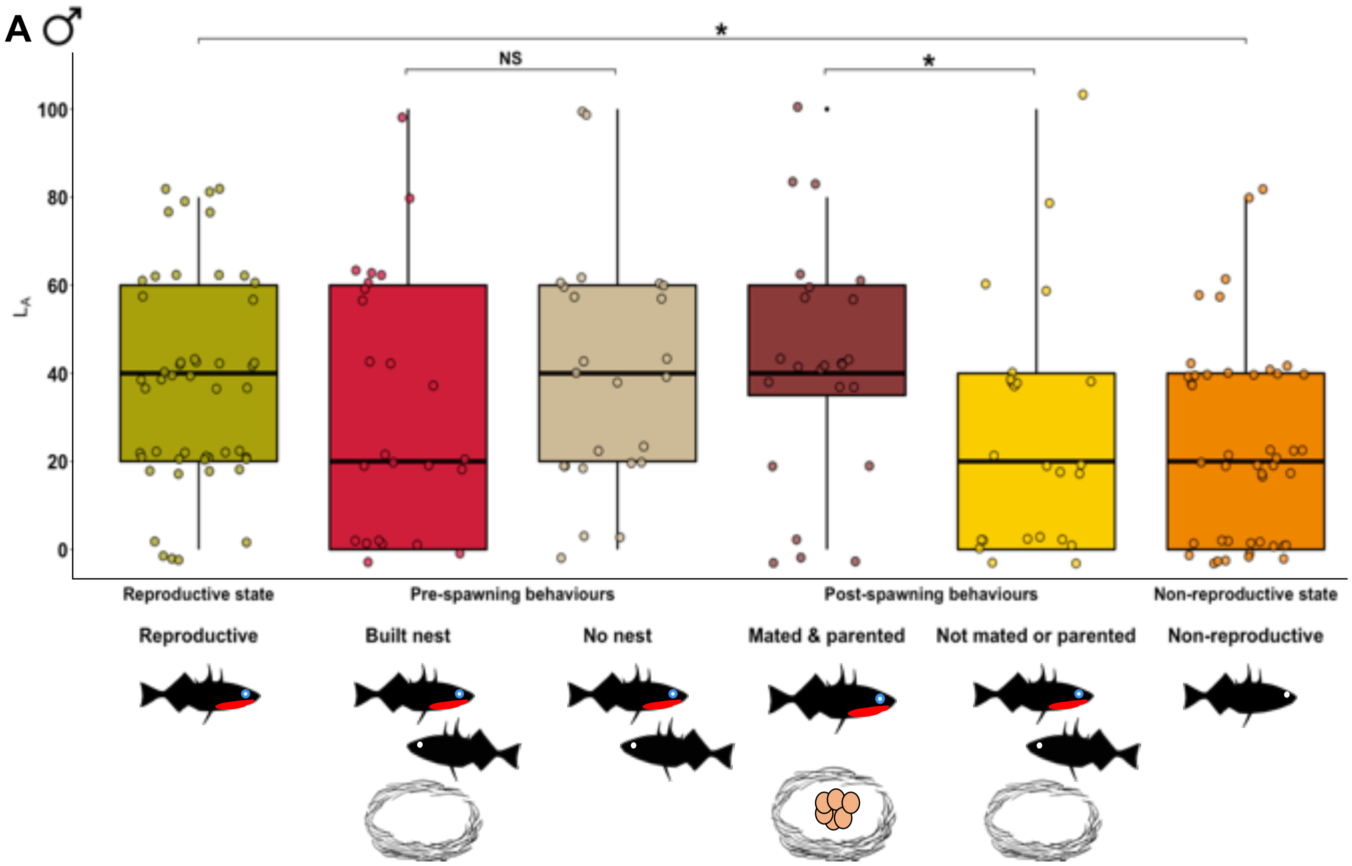


Figure 3.3: L_A of females (blue) and males (orange) in a reproductive and non-reproductive state.

3.3.3 Is Laterality Linked to the Performance of Reproductive Behaviours?

In males, the strength of laterality exhibited differed with experience of reproduction (male L_A LMM stage main effect: $F_{5, 183} = 2.551$, p = .029; Figure 3.4A), while in females it did not (Female L_A LMM stage main effect: $\chi^2 = 0.257$, df = 2, p = .879; Figure 3.4B).


A ♂
*
NS
*
100
80
60
40
20
0
L_A
Reproductive state
Pre-spawning behaviours
Post-spawning behaviours
Non-reproductive state
Reproductive
Built nest
No nest
Mated & parented
Not mated or parented
Non-reproductive

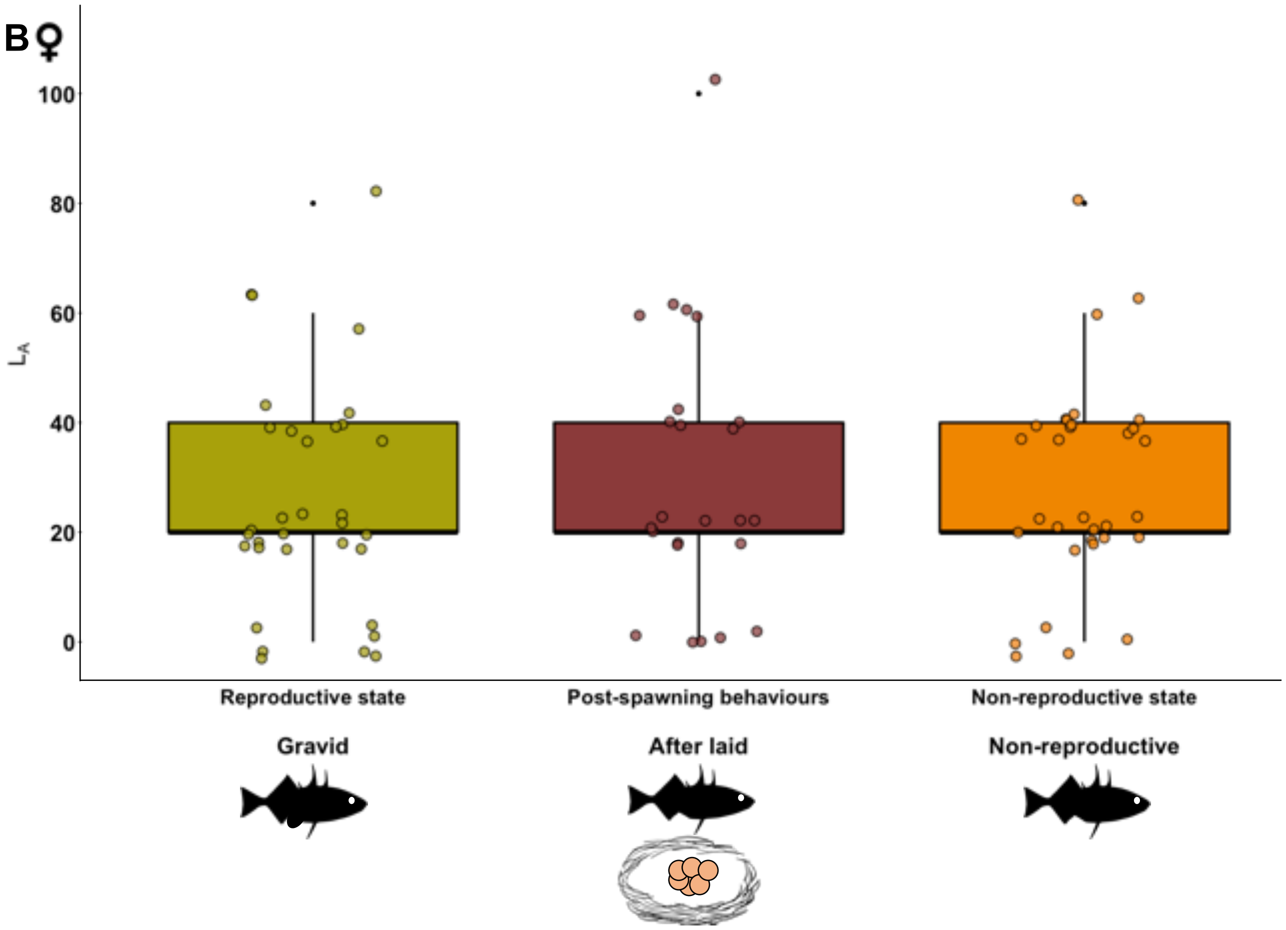

B ♀
100
80
60
40
20
0
L_A
Reproductive state
Post-spawning behaviours
Non-reproductive state
Gravid
After laid
Non-reproductive

Figure 3.4: L_A of A) males and B) females at each stage of reproduction. Asterisks indicate significant differences in planned comparisons of L_A for individuals performing different reproductive behaviours: * represents $p \leq 0.05$ and ** represents $p \leq 0.01$.

Specifically, reproductive males were more strongly lateralised than non-reproductive males (t = 2.540, df = 141, p = :012; Figure 3.4A), and males that had experience of mating and parenting (Figure 3.3A; post-spawning behaviours—mated and parented) were more strongly lateralised than males that had built nests but had no experience of mating or parenting (t = 1.966, df = 183, p = :050; Figure 3.4A; postspawning behaviours—not mated or parented). However, there were no differences between males that had constructed nests and those that had not (t = 21.039, df = 183, p = .300; Figure 3.4A; pre-spawning behaviours—built nest vs. no nest).

3.4 Discussion

Our results provide the first evidence of a link between laterality, reproduction, and experience of mating and parenting. Individuals of the caring sex (males) were more strongly lateralised than the non-caring sex (females) in a reproductive state but not outside the breeding season in a non-reproductive state. Furthermore, males that had experience of mating and parenting were more strongly lateralised than males absent in this experience, and laterality was reduced outside the breeding season in males. Together, these findings suggest that the costs and benefits of laterality may be modified by asymmetries in reproductive investment between the sexes and between individuals, which could in turn influence the expression of laterality.

Stronger laterality in reproductive males may be linked to the benefits associated with laterality, specifically the performance of simultaneous information processing, which enables multi-tasking (Rogers et al. 2004; Dadda and Bisazza 2006a; 2006b). In threespine sticklebacks, the cost, complexity, and cognitive demand of reproduction is greater for males than for females. Females perform mate choice and invest heavily in egg production, while males invest in several cognitively demanding behaviours, including territory defence, nest construction, male-male competition, courtship behaviours, and parental care (Bell and Foster 1994). Multi-tasking would allow for males to simultaneously perform reproductive behaviours including parental care while concurrently performing tasks necessary for survival, such as foraging and predator avoidance. Consequently, reproductive breeding males would presumably benefit from a more strongly lateralised brain (expressed as stronger behavioural laterality seen here; Figure 3.4A) than either females or non-breeding males.

Laterality is associated with costs, including a reduced efficiency in tasks requiring interhemispheric communication (Rogers 2000). Female stickleback perform mate choice whereby males may present in either visual hemisphere; thus, interhemispheric communication is likely beneficial to females, especially during reproduction (Facchin et al. 1999). As a result, the costs of laterality may outweigh the benefits, explaining the absence of laterality in this sex (Figure 3.4B). Additionally, threespine sticklebacks exhibit sexual dimorphism in brain size where males have larger brains, associated with the cognitive demands of reproduction and parental behaviours in this species (Kotrschal et al. 2012;

Samuk et al. 2014), than females. A larger brain could allow for greater cerebral lateralisation; however, to date only asymmetries in brain structure have been linked to behavioural laterality in fish (Reddon et al. 2009), but correlations between brain size and laterality are evident in mice (Cassells et al. 1990). Furthermore, when individuals are repeatedly tested learning may occur (Kieffer and Colgan 1992; Kabadayi et al. 2018), and thus the observed sex difference in laterality could result from sex-specific variation in learning. However, if learning occurred, we would anticipate an increase in L_A over time as individuals learned to detour in a particular direction and for both sexes to show equivalent levels of L_A on initial testing (when reproductive), neither of which were observed in the current study.

Males absent in mating and parenting experience were less strongly lateralised than males with this experience, and laterality was reduced outside the reproductive cycle in the caring sex (Figure 3.4A), indicating that laterality may be a plastic trait. Side biases in behaviours, especially those tightly associated with fitness (Rogers 2000), could be detrimental for males when not combined with reproduction. For example, individuals exhibiting consistent side biases may suffer from increased predictability and vulnerability, especially where biases can be learned and/or exploited by predators (Cantalupo et al. 1995; Takeuchi 2012). Thus, plasticity in behavioural laterality, as a result of changing environmental cues, would allow individuals to maximise their fitness during reproduction.

The observed difference in L_A between males with and without mating and parenting experience could have arisen from a loss of reproductive motivation in non-breeding males; however, this is unlikely given that all males had visual access to females and males during the entirety of the experiment representative of mating opportunities and male-male competition, respectively. Whether laterality and motivation are linked and whether this could influence learning in laterality are fruitful avenues for further research. Although we are unable to determine whether the experience of courtship, mating, or parenting independently or in combination are responsible for driving stronger laterality in breeding males, no differences were observed between breeding and non-breeding males that had and had not performed nest construction, respectively, highlighting the influence of mating and performance of post-spawning reproductive behaviours on L_A. Future research would benefit from identifying which specific aspect or aspects are responsible for driving this variation, but methods adequate to disentangle mating experience from parenting experience that minimise or eliminate effects on subsequent behaviour are necessary.

Despite being an established method of assessing laterality (Bisazza et al. 1997; 1998a; Reddon et al. 2009; Domenici et al. 2014), the detour test has recently been criticised (Roche et al. 2020), as laterality measured using this technique was found to be variable within individuals under repeated testing in the same conditions. In contrast, McLean and Morrell (2020), using the same test, found that individual female guppies were consistent in both the direction and the strength of laterality, while males were consistent in direction. In the present study, we expect low within-individual consistency, particularly as we are measuring individuals with different reproductive experience and in different reproductive states; thus, variation may be expected, as these individuals could respond differently to stimuli in the detour test (McLean and Morrell 2020; Roche et al. 2020). In addition to the overall changes in laterality observed between the caring and non-caring sex within and outside the reproductive season, we found that 78% of males had an equivalent or lower L_A when non-reproductive relative to their L_A when reproductive, and 71% of males had an equivalent or increased L_A following mating and parental care experience relative to the same males after nest construction.

The mechanisms driving the observed variation in laterality are unknown; however, changes in hormones and gene expression, which influence both the motivation and the performance of behaviours simultaneously by regulating and controlling the brain and muscles (Garland et al. 2016), could be influential. Reproductive male sticklebacks exhibit an increase in plasma levels of 11-ketotestosterone, an androgen produced by the testes that is responsible for secondary sexual characters, such as nuptial coloration, and nest construction (Mayer and Borg 1995; Páll et al. 2002). Thus, levels of this androgen are higher in males than in females during reproduction (Mayer and Borg 1995). Postnatal exposure to a related hormone, testosterone, has been associated with the expression of laterality in male *Aequidens rivulatus* (Schaafsma and Groothuis 2011). Thus, 11-ketotestosterone levels could be important in the expression of laterality; however, this hormone is down-regulated during the parental phase in sticklebacks (Páll et al. 2002). Additionally, Bukhari et al. (2019) have shown that patterns of gene expression during the parenting period for caring and non-caring male sticklebacks differ. Specifically, oxytocin, important for social affiliation and parental care in mammals and fish, is up-regulated when male sticklebacks are caring for eggs in a nest, but whether this gene is linked to laterality is currently unknown. The relationship among hormone regulation, gene expression, and laterality remains poorly understood, particularly with regard to sex and reproduction.

We found a link among laterality, reproduction, and experience of mating and parenting, indicating that reproduction and associated reproductive behaviours are key but previously unidentified sources of variation in laterality both between the sexes and within a single sex. The caring sex and individuals that had mating and parenting experience were more strongly lateralised than both the non-caring sex and individuals of the caring sex with no mating or parenting experience. These differences could arise from the benefits of laterality outweighing the costs across different stages of reproduction, but currently these costs and benefits are unknown. Future work should focus on disentangling the specific aspect or aspects of mating and/or parenting that drive variation in laterality, the mechanistic basis of this variation and its implications for performance in both reproduction and parenting behaviours, and performance in other behavioural domains that will ultimately impact fitness. This information is critical to understanding how variation in cerebral lateralisation evolved and is maintained.

Chapter 4: The costs and benefits of laterality in *Telmatochromis temporalis* in different reproductive states

Male *Telmatochromis temporalis*

© user: Steph McLean | Personal photo

Abstract

Laterality, the partitioning of differential information processing into specific brain hemispheres, is associated with costs and benefits that underpin both selection for this trait and its overall expression. The costs and benefits experienced by lateralised individuals may be context specific and thus highly variable, but to date they have only been considered under fixed conditions. Here, we assess laterality and its associated costs and benefits in a biparental cichlid fish, *Telmatochromis temporalis,* in different reproductive and social contexts: while reproductive and actively performing parental care behaviours, and in a non-reproductive non-social state. Specifically, we examined laterality in exploratory behaviour using a standard detour test and then examined performance when attention is required to be shared between two tasks performed simultaneously (an identified benefit of laterality): prey capture and predator vigilance, and performance in a task requiring communication and cooperation between brain hemispheres (an identified cost of laterality): choosing between groups of conspecifics seen in different visual hemispheres simultaneously. We find that lateralisation of exploratory behaviour is associated with poorer performance in the group choice task, but that this did not vary with reproductive context. Due to a strong inherent side bias, lateralised individuals were unable to identify the larger conspecific group representing the greatest competitive threat in two consecutive trials. Lateralised fish did not show enhanced performance in the prey capture task, but strongly lateralised fish were quicker to undertake the task than non-lateralised fish. This suggests a trade-off between the costs and benefits of laterality whereby the advantages of cerebral lateralisation must outweigh the costs regardless of varying reproductive and social conditions since *T.*

temporalis individuals exhibited lateralised exploratory behaviour in both a reproductive and non-reproductive state.

4.1 Introduction

Cerebral lateralisation, specialisation of different brain hemispheres for the performance and regulation of specific brain functions and behaviours, is widely documented across animal taxa (Vallortigara and Andrew 1994; Denenberg 1981; Bradshaw and Rogers 1993; Bisazza et al. 1998a; Niven and Frasnelli 2018). Cerebral lateralisation is often demonstrated at the behavioural level as side biases in behaviours (Bisazza et al. 2001a) or as preferential use of one side of the body or half of a bilateral characteristic (Koboroff et al. 2008; Brown and Magat 2011a), a phenomenon known as behavioural laterality. The observed behavioural biases stem from cerebral lateralisation and are an indicator of the degree of lateralisation of an individual's brain (Vallortigara and Rogers 2005). Behavioural laterality can occur at either the population or individual level (Lehman 1981): population level laterality refers to the majority of individuals in a population exhibiting aligned biases for a specific direction (left or right), whereas individual level laterality refers to an individual exhibiting a consistent directional bias, regardless of directional preference, in a population that contains both lateral phenotypes (Lehman 1981).

Cerebral lateralisation is associated with both costs and benefits that can influence individual fitness (Bisazza and Brown 2011). Lateralisation is hypothesised to enhance cognitive ability since it permits partitioning of different information types into specific brain hemispheres, allowing separate and parallel processing, and thus, an increased efficiency of information processing (Rogers 2000, 2002). One advantage of laterality is that it allows individuals an enhanced capacity in situations where individuals are required to process different information types simultaneously, 'multi-tasking', since lateralised individuals are better able to cope with divided attention (Vallortigara and Rogers 2005). For example, in contexts where individuals must multi-task, such as foraging in the presence of a predator or performing schooling behaviours, lateralised individuals have been shown to outcompete non-lateralised conspecifics (Bisazza and Dadda 2005, 2006a). However, laterality is not without costs, which largely result from an inability to transfer and incorporate information that reaches both brain hemispheres (Rogers 2002; Vallortigara and Rogers 2005). For example, lateralised individuals are less efficient than their non-lateralised counterparts in tasks that require cooperation and communication between brain hemispheres, such as finding the central door in a row of nine (bisection test; Dadda et al. 2009). Additionally, strongly lateralised individuals also exhibit poorer performance in spatial tasks, likely resulting from an inability to overcome strong inherent directional biases (Brown and Braithwaite 2005).

For laterality to evolve, we expect the benefits of being lateralised to outweigh the costs, providing individuals with a selective advantage. Wild caught female *Brachyraphis episcop*i, from areas with high predation pressures are more strongly lateralised than their counterparts from areas with low predation pressures (Brown et al. 2007b). This increased lateralisation is thought to arise from the advantage of having an enhanced ability to perform simultaneous information processing in an environment where individuals are frequently required to be vigilant for predators while performing additional tasks necessary for survival (Brown et al. 2007b). Similarly, in 23 species of Australian parrots the strength of laterality was found to be associated with foraging method. Large bodied species that use extensive coordinated beak-foot actions to extract seeds tended to be strongly lateralised, while the smaller bodied grazing species tended to be non-lateralised, with the loss of laterality thought to be associated with a change in diet (Brown and Magat 2011b). Thus, the costs and benefits of laterality are likely context specific and highly variable (Bibost et al 2013). While previous work has identified costs and benefits that lateralised individuals experience under fixed conditions (Dadda and Bisazza 2006a, 2006b; Dadda et al. 2009), the natural environment of most animals is highly unpredictable. Thus, under varying conditions, different patterns of laterality may be selected for, due to a trade off in the costs and benefits that are experienced by lateralised individuals, and the potential impact of laterality on behavioural performance, and ultimately fitness.

Substantial variation in laterality persists both within and between species and sexes (Bisazza et al 1997, 1998a; Reddon and Hurd 2008; Niven and Frasnelli 2018). Much of the variation within and between species has been attributed to varying factors including age (Jozet-Alves et al. 2012a), early life experience (Deng and Rogers 1997; Broder and Angeloni 2014), ecological influences (Brown et al. 2004) and parental effects (Lucon-Xiccato et al. 2016a), but this is in stark contrast to investigations of the underlying factors driving variation between the sexes, which remain poorly understood. Initially studies of cerebral lateralisation were strongly biased towards ecologically relevant behaviours, such as foraging or predator avoidance but more recently research interest towards laterality in social behaviours has grown. Specifically, there has been an increasing number of studies investigating laterality in reproductive-specific social behaviours, which are highly variable between the sexes (Ariyomo and Watt 2013), with evidence of variation in laterality in both courtship and mate choice (Ventolini et al. 2005; Vidal et al. 2018; Templeton et al. 2012).

Here, we aim to examine laterality and its associated costs and benefits under different reproductive and social conditions: in individuals while reproductive and actively performing parental care behaviours, and in a non-reproductive, non-social state. Parental care is a reproductive social behaviour with a high cognitive demand (Reddon and Hurd 2009a). Caring parents must perform several functions simultaneously to survive, including caring behaviours and functions critical for existence such as foraging and predator avoidance (Royle et al. 2012). The advantages of laterality, particularly an enhanced ability to multi-task (Rogers et al. 2004; Dadda and Bisazza 2006a, 2006b), suggest that the benefits of laterality would likely outweigh the costs for the sex/sexes performing parental care, rendering cerebral lateralisation selectively advantageous for caring parents. However, whether the same pattern of laterality and balance of costs and benefits would be experienced by caring parents in a non-reproductive, non-caring state is unknown. Consistent directional biases could instead be detrimental, especially from an ecological perspective, if they increase the vulnerability of individuals in an unpredictable environment, particularly in situations where such biases could be learned and exploited as a result of continued exposure (Rogers 2002; Ghirlanda and Vallortigara 2004).

We used a biparental substrate brooding cichlid, *Telmatochromis temporalis,* to assess the relationship between laterality, its associated costs and benefits and reproduction/parental care. Specifically, we examined turning preferences of individuals detouring around a barrier to view a visual stimulus (novel object), a proxy for preferences in eye use and thus cerebral lateralisation (Bisazza et al. 1997, 1998b) and then examined the performance of fish in two tasks previously identified to assess the costs and benefits of laterality for individuals in a reproductive state while performing parental care, and in a non-reproductive state. First, a task requiring communication and cooperation between the left and right brain hemispheres (Dadda et al. 2009), which represents a cost of laterality. Second, a task requiring simultaneous information processing (Dadda and Bisazza 2006a, 2006b) to explore the benefits of laterality. In the first task we determined how efficiently an individual choose between two visual stimuli that varied in quality, here two differently sized groups of fish representative of varying degrees of intraspecific competition, that were presented in different visual hemifields in two consecutive trials. In the second task, we determined the time an individual took to peck at live prey in the presence (dual task, 'multi-tasking'), and absence (single task, control) of a dummy predator.

We predict that:

I. *T. temporalis* individuals, in common with other species of fish, show laterality in exploratory behaviour (the eye used to view a novel object in the detour test), since this could allow individuals to partition information about novelty into one brain hemisphere leaving the remaining brain hemisphere available to perform additional functions thus, enhancing the overall efficiency of cerebral processing.

II. If laterality carries a cost, an inability to transfer and incorporate information that reaches both brain hemispheres, lateralised individuals will have a reduced ability to make a correct instantaneous choice between a large and a small group of conspecifics, instead, being more likely to choose the stimulus on their 'dominant side' due to strong inherent side biases.

III. If laterality carries a benefit, an enhanced ability to perform simultaneous information processing, it should positively impact on an individual's ability to 'multi-task' when simultaneously viewing a predator and foraging. This will be manifested as a decrease in the time taken to peck at live prey, particularly in the presence of a predator, relative to less/non-lateralised conspecifics as a result of lateralised individuals partitioning information relating to differing entities into separate brain hemispheres allowing parallel and simultaneous cerebral processing. .

IV. If the costs and benefits of laterality vary with reproductive state as a result of an increased demand to cope with divided attention, e.g. during parental care, laterality and its associated costs and benefits may be more pronounced for reproductive relative to non-reproductive individuals. Particularly, when reproductive and performing care individuals may be more strongly lateralised than in a non-reproductive state due to the benefits of laterality outweighing the costs for caring parents.

4.2 Materials and methods

4.2.1 Animals

T. temporalis breed monogamously following the formation of a pair bond and perform elaborate biparental care of offspring until independence (Kuwamura 1997; Mboko 1998). In this species, a male and female form a pair bond, approximately one week prior to spawning, during which time the female remains in or close to the nest site (Mboko 1998). Spawning occurs in a nest of a small hole or burrow under a stone (Mboko and Kohda 1995; Kuwamura

1997): the female deposits eggs inside the nest site and males ejaculate sperm at the nest site. Paired males have territories around the nest site, which they defend against conspecifics, while females stay in closer proximity to the nest, and guard their broods from smaller predators or those in the immediate proximity of the nest site (Mboko 1998; Mboko and Kohda 1999). As both sexes perform equivalent roles during parental care and the labour of care is shared between parents in this species, we would not expect any sex-differences or confounding effects of sex on lateral biases. Thus, *T. temporalis* are a model system to investigate laterality and its associated costs and benefits under different social conditions: while reproductive and actively performing parental care behaviours, and in a non-reproductive state in both male and female parents.

24 adult *T. temporalis* (13 males and 11 females), descendants of wild caught fish from Lake Tanganyika in East Africa maintained at the University of Hull since 2012, were housed in a single species stock tank (40 x 47 x 60cm) in recirculating aerated fresh water at 26 ± 1 ° C for one month prior to experiments. All individuals were provided plastic tubing (approximately 9cm length x 4cm diameter) for enrichment, were maintained on a 12L:12D photoperiod and fed daily using commercial feed.

4.2.2 Breeding regime

For all behavioural trials (detailed below) individuals of both sexes were assayed twice: once in a non-reproductive state (hereafter “non-reproductive”) and once in a reproductive state while actively performing parental care behaviours, within the first 7 days of egg hatching (hereafter “reproductive”). All focal individuals were initially assessed in all behavioural trials while in a non-reproductive state before being individually housed in a large housing tank (40 x 47 x 60cm) for at least one week before reproductive pairings commenced.

To encourage pair bond formation and reproduction, a single male and female *T. temporalis* were assigned to an experimental tank (40 x 47 x 60cm; 1x *T. temporalis* breeding pair per tank to ensure individual identification) that contained a variety of other stock cichlid species (mean n per tank = 17 ± 2) and assorted tubing (approximately 9cm length x 4cm diameter) that could be used as a resource for reproduction. The other cichlid fish within the experimental tank represented a predatory threat to the brood when in proximity to the nest site, encouraging individuals who had successfully spawned to perform parental care behaviours including brood defence. Seven days post egg hatching after parental care behaviours had been performed, focal individuals were then assayed in all behavioural trials in a reproductive state.

4.2.3 Data collection

4.2.3.1 Examining the relationship between laterality, reproduction and parental care

To assess the relationship between laterality, reproduction and parental care, we measured behavioural lateralisation using a standard detour test, which examines the direction detoured around a barrier when viewing a visual stimulus (Bisazza et al. 1997, 1998a; Figure 4.1). Directional biases in the detour test represent a proxy for preferences in eye use and hemispheric processing since fish have laterally placed eyes with little frontal overlap (Brown et al. 2004) and slow and incomplete inter-ocular information transfer (McCleary 1960). Thus, fish detouring rightward used their left eye to view the visual stimuli and their contralateral brain hemisphere for information processing and vice versa for fish detouring leftward. We examined laterality in exploratory behaviour by assessing the direction detoured when viewing a novel object in the detour test, for individuals in both reproductive states.

(i) Apparatus

The detour test consisted of a glass tank (60 x 120 x 60cm) containing a double-ended T-maze joined by an opaque plastic runway (14 x 40cm), which was separated from the end sections of the maze by a removable opaque door (Figure 4.1). A barrier (15 x 30cm) consisting of vertical cylinder bars (0.5cm diameter) spaced 0.5cm apart was placed at one end of the tank in front of a visual stimulus, to obscure but not eradicate viewing. The visual stimulus used was a yellow Duplo© cube measuring 3 x 6 x 4cm to ensure uniformity in the size, shape and colour of an unfamiliar object that was not associated with ecologically relevant behaviours in this species. Water in the tank was 25cm deep, maintained at 26 ± 1 ° C and aerated using two airstones placed behind the side panels comprising the runway, as changes in temperature and dissolved oxygen levels can affect laterality and neurological function (Domenici et al. 2007, 2014). The experimental tank was covered externally in white plastic sheeting to minimise disturbance from the observer. To minimise light induced detour biases, a fluorescent lamp was fitted directly above the tank. All behavioural trials were performed in a closed room to ensure uniformity in surrounding conditions and were video recorded from directly above the experimental tank (GoPro Hero 6, GoPro, California, USA).

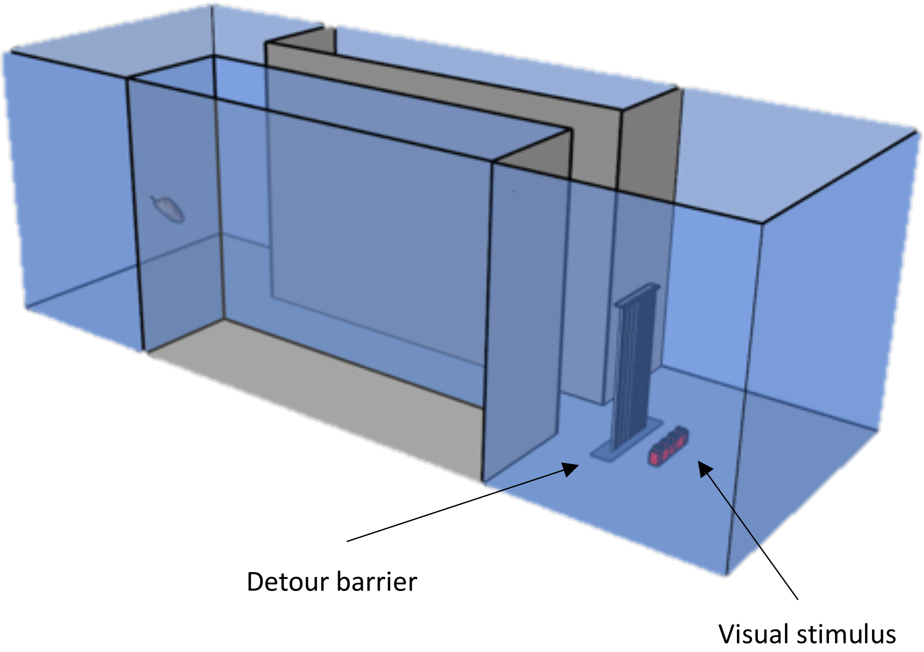


Figure 4.1: Diagrammatic representation of the detour test apparatus. Note the visual stimulus pictured is a red duplo © cube however, a yellow duplo © cube was used in this experiment. The removable opaque door is not pictured.

(ii) Procedure

To begin a trial, an individual was placed in the detour tank in the absence of the barrier, visual stimulus and opaque door and allowed to acclimatise for five minutes, prior to confinement at one end of the tank using the door. The barrier and visual stimulus were then placed behind the runway at the opposite end of the tank and the door confining the individual was removed. If the individual had not independently approached the runway within 30s a small dip net was used to gently encourage them. At the end of the runway fish approached the barrier forcing them to detour leftward or rightward. For each individual, 10 consecutive detours (1x trial) were performed at opposing ends of the runway (to account for any asymmetry in the experimental tank) and the direction of detour recorded.

For each individual, a relative lateralisation index (L_R) was calculated as: [(right detours – left detours) / (right detours + left detours) x 100] (Bisazza et al. 1997, 1998a, 1998b). L_R ranges from -100 to 100 indicating a preference to consistently detour leftward or rightward respectively and can be assessed at both the individual and population level (mean level) to examine directional biases in behavioural lateralisation. Additionally, to examine the strength of laterality, irrespective of directional preference, an absolute lateralisation index (L_A) was calculated for each individual as $|L_R|$. L_A ranges from 0, individuals making an equal number of detours leftward and rightward, to 100, individuals turning consistently in one direction (left or right; Bisazza et al. 1997, 1998a, 1998b).

4.2.3.2 Examining the costs of laterality

Tests of efficiency in bilateral information processing generally adopt shoal choice experiments, whereby individuals choose between two shoals differing in attractiveness (e.g. a numerically larger or size-matched shoal over a numerically smaller or mismatched shoal, whereby the former provides greater protection from predation), and are predicted to choose the shoal of higher quality (Dadda et al. 2009; Gómez-Laplaza and Gerlai. 2011; Hager and Helfman 1991). The rationale being that lateralised individuals, especially those with strong directional biases, are less efficient at determining the most attractive shoal when two shoals differing in quality are viewed simultaneously in different visual hemispheres due to strong inherent directional biases and a reduced ability to transfer and incorporate information that reaches both brain hemispheres. *T. temporalis* are a non-grouping solitary living species who do not cooperate (Mboko and Khoda 1999; Heg and Bachar 2006) and are highly aggressive to both familiar and unfamiliar conspecifics while both reproductive and non-reproductive (Hick et al. 2014; Balshine et al. 2017). We used the same experimental design where two differing sized groups of same species conspecifics (4 vs. 2 individuals), were presented simultaneously in different visual hemifields, but predicted that the larger group would represent the greatest threat with regard to interspecific competition and thus should be the primary target of antagonistic interactions to maximise fitness (Scharf et al. 2011; Figure 4.2).

(i) Apparatus

The apparatus consisted of a square glass tank (64 x 64 x 25cm) divided into four sections: a holding box, a decision zone, and two tanks for housing stimulus fish (Figure 4.2). The holding box was a rectangular opaque plastic box (12 x 20 x 18cm) with a removable plastic insert, designed to create a narrow exit (4.5cm), that was concealed by an opaque door. The door separated the holding box from the decision zone and was on a pulley allowing it to be opened remotely to ensure minimum observer disturbance. Each stimulus group was held in separate square glass tanks (20 x 20 x 18cm) on either side of the decision zone. When the door was lifted fish entering the decision zone through the narrow exit could see each stimulus group in separate visual hemispheres (Figure 4.2). The experimental tank was covered externally in white plastic sheeting to minimise disturbance from the observer. Water in the experimental tank was 12cm deep, aerated and maintained at 26 ± 1°C. To ensure uniformity in lighting and surrounding environmental conditions, all trials were performed in a closed room. Each behavioural trial was video recorded from directly above the experimental tank (GoPro Hero 6, GoPro, California, USA).

(ii) Procedure

To begin a trial, the focal fish was placed into the holding box and the two stimulus groups were placed in the holding tanks. The focal fish was allowed to acclimatise for five minutes before the door of the holding box was lifted remotely allowing the individual to independently enter the decision zone. Preliminary trials showed that focal fish made an immediate decision to interact with a stimulus group without stopping or turning back on themselves. A successful decision of which stimulus to interact with was determined as a focal individual being within one body length of a holding tank containing one of the groups of stimulus fish. This decision was measured twice for each focal individual, a minimum of two hours apart, whereby the position (left or right visual hemisphere presentation) of stimulus groups was reversed between trials. Half of the focal individuals were measured with the larger group of stimulus fish initially visible in their left visual hemisphere (determined by a random number generator) while the other half were measured with the larger group of stimulus fish initially visible in their right visual hemisphere.

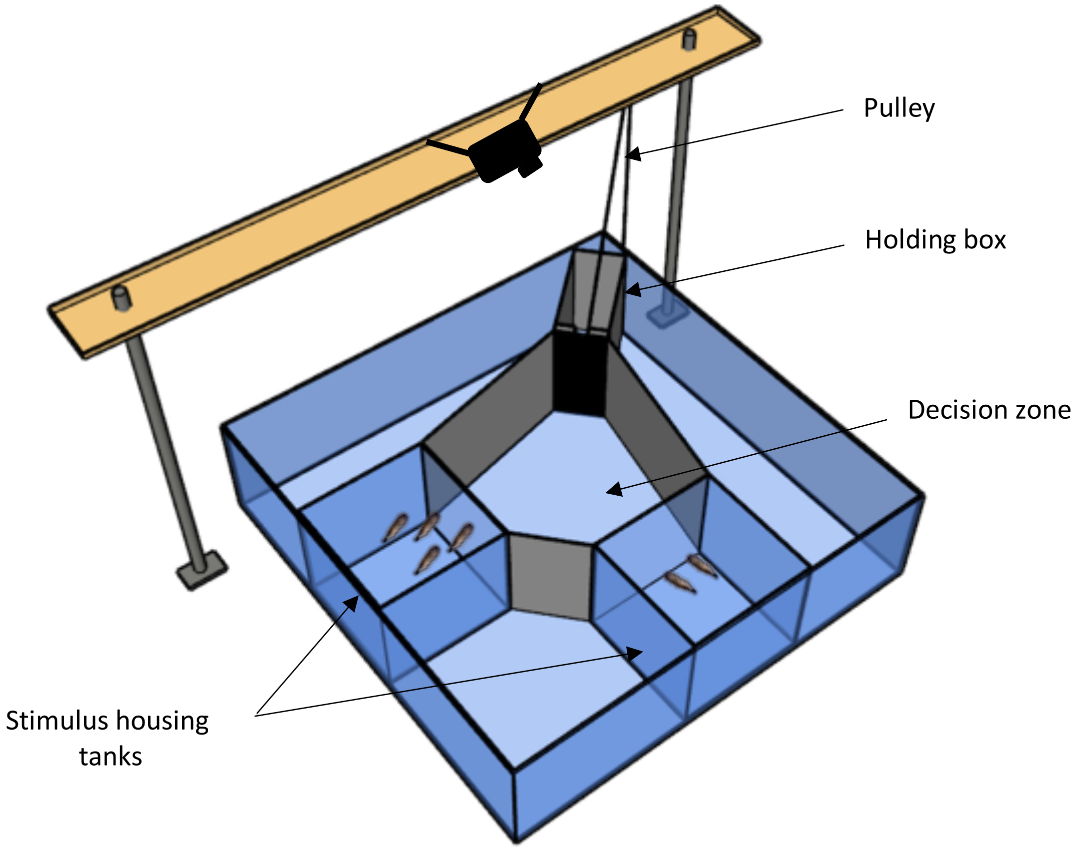


Figure 4.2: Diagrammatic representation of apparatus used to assess efficiency in a bilateral information processing task. Here, the smaller stimulus group would be viewed in the focal individuals left visual hemifield and the larger stimulus group in the right visual hemifield respectively upon entering the decision zone.

4.2.3.3 Examining the benefits of laterality

To examine efficiency in tasks requiring simultaneous information processing ('multi-tasking'), we examined the time taken to peck at live prey in both the presence (dual task, 'multi-tasking') and absence (single task, control) of a dummy predator (Figure 4.3). All fish were deprived of food for 48 hours prior to feeding trials to ensure standardisation of hunger levels.

(i) Apparatus

The apparatus consisted of a rectangular glass tank (30 x 100 x 20cm) divided into 3 sections (Figure 4.3). One end section, holding zone (30 x 30 x 20cm), was used to confine and acclimatise individuals to the test arena for five minutes before a trial commenced. The holding zone was connected to the central section, the feeding zone (30 x 40 x 20cm), by an opaque plastic door on a pulley. This allowed fish to enter the feeding zone where live prey were presented in a centrally-positioned transparent weighted cylindrical tube (3.5 x 15cm) with minimal disturbance from the observer. The feeding zone was adjacent to the predator zone (30 x 30 x 20cm), where a large model predator (18cm standard length) was suspended behind a transparent plastic screen (Figure 4.3). In control trials, the predator was removed and the transparent screen replaced by an opaque screen. During trials water in the tank was 15cm deep, aerated and maintained at 26 ± 1 ° C. The experimental tank was covered externally in white plastic sheeting to minimise disturbance from the observer. All trials were performed in a closed room to maintain uniformity in lighting and surrounding environment conditions and were video recorded from above the experimental tank (GoPro Hero 6, GoPro, California, USA).

(ii) Procedure

To begin a trial, live prey (fifteen cherry shrimp, *Neocaridina davidi*), were added to the cylindrical tube. A focal individual was then placed in the holding zone for five minutes before the door confining the individual was lifted remotely allowing the individual to independently enter the feeding zone. In a few cases where the individual did not emerge from the holding zone within five minutes (n=2), a small dipnet was used to encourage the individual towards the feeding zone. For each individual, efficiency in foraging behaviour was measured as the time taken to first peck at live prey following emergence (time taken to peck at live prey – time taken to emerge into the feeding zone). Any individuals failing to peck at live prey within 30 min of emerging into the feeding zone were excluded from further observations. Efficiency in the feeding trial was measured twice for each individual (48 hours apart), once in the presence of a predator and once in the absence of a predator (control), whereby half of the focal fish were initially measured in the presence of a predator and the other half were initially measured in the control treatment determined by a random number generator.

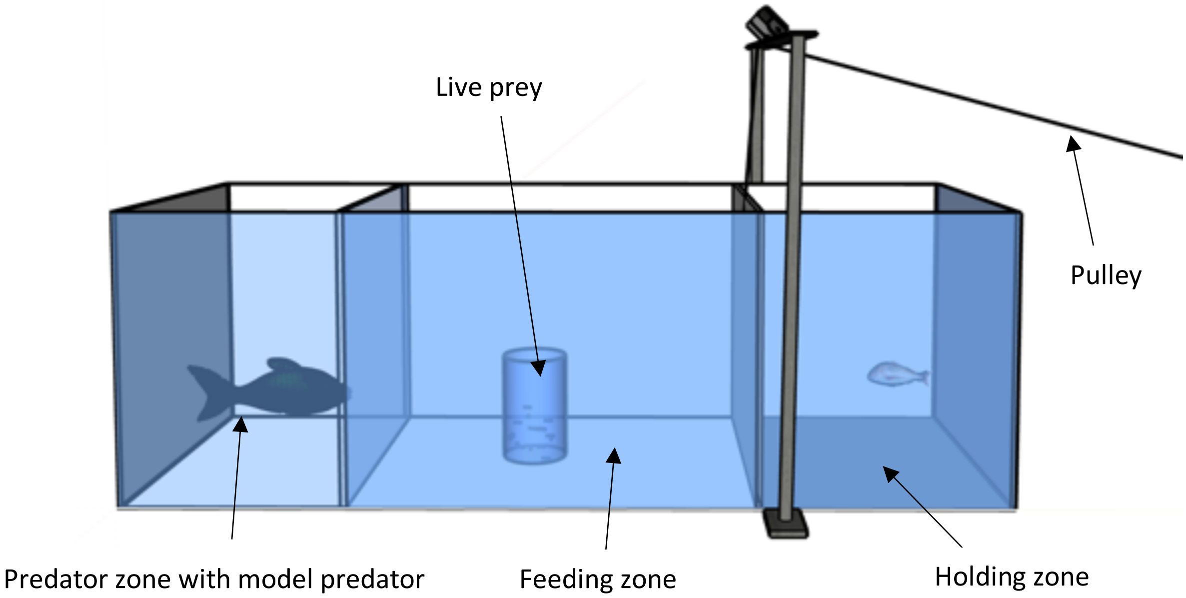


Figure 4.3: Diagrammatic representation of apparatus used to assess efficiency in simultaneous information processing, 'multi-tasking', here, foraging in the presence (dual task) and absence (single task, control) of a predator. Foraging in the presence of a predator (dual task) is depicted. In the control treatment, focal individuals forage in the absence of a predator (predator is removed and an opaque screen is inserted in front of the predator holding zone).

4.2.4 Data analyses

All statistical analyses were conducted using R version 3.6.2 (R Core Team, 2019).

4.2.4.1 Examining the relationship between laterality, reproduction and parental care

To examine the relationship between laterality, reproduction and parental care, generalised linear mixed models (GLMER) fitted with binomial error distributions (lme4: Bates et al. 2015) were used to assess the effects of sex, reproductive state and their interaction on L_R and L_A independently. All models included individual ID as a random factor to account for the repeated measures design and were not over-dispersed (Harrison 2014). For all models, model simplification was performed using maximum likelihood estimation, conducting stepwise removals of variables proceeding with interaction terms with the smallest t-values. Only variables that resulted in significantly larger Akaike information criterion (AIC) values, following their removal, were retained in the minimal model. Here we report the minimal adequate models that contain all main effects, regardless of significance.

To assess whether individuals exhibited significant individual level laterality (L_A) and to examine whether any aligned biases in laterality were left or right dominant (L_R), a mean L_A and mean L_R were calculated for each individual across all non-significant variables from the above GLMER models. One-sample t-tests were used to examine whether these means differed from a null expectation of no laterality ($L_A = 25$, $L_R = 0$), calculated based on the mean L_A and L_R scores from a simulated number of left and right turns drawn from a binomial distribution (1000 simulation runs with a 50% chance of turning left or right) assuming no laterality when performing 10 detours.

4.2.4.2 Examining the costs of laterality

To examine the costs of laterality, a chi-squared test (χ^2) was used to assess whether there were differences in i) the number of times the larger stimulus group representative of the greatest competitive threat was chosen and ii) directional preference in stimulus shoal choice by assessing the number of the times the shoal presented in the right visual hemisphere was chosen, when the large and small stimulus groups were presented in opposing visual hemifields in two trials. These differences were assessed for left, right and non-lateralised individuals, whereby non-lateralised individuals were those that did not exhibit an L_A score significantly different from an expectation of random choice/no laterality (L_A = 25). Contrastingly, lateralised individuals were those who exhibited significant lateral biases, i.e. they exhibited an L_A score that differed significantly from a random expectation of no laterality, whereby an individual's directional bias, i.e. whether they were left or right lateralised, was determined by their L_R score such that negative and positive L_R scores were indicative of left and right lateralised biases respectively.

To examine factors influencing preference for the larger stimulus group and directional preference in stimulus group choice, GLMER models with a binomial distribution (lme4: Bates et al. 2015) were fitted to assess the effect of sex, reproductive state, L_A and L_R independently, and all plausible two-way interactions on i) the number of times the larger stimulus group was chosen in two trials and ii) the number of times the shoal viewed in the right visual hemifield was chosen in two trials respectively. The fitted GLMER models included individual ID to account for the repeated measures design and were not over-dispersed (Harrison 2014).

4.2.4.3 Examining the benefits of laterality

The factors affecting efficiency in tasks requiring simultaneous information processing 'multi-tasking' were assessed using an LME model (lme4; Bates et al 2015) with time to peck at live prey as the dependent variable, absence/presence of a predator, sex, reproductive state, laterality (L_A and L_R) and all plausible two-way interactions as independent variables. Additionally, we also assessed whether latency to enter the feeding zone, a context that requires multiple stimuli to be processed, was influenced by the presence/absence of predator, sex, reproductive state, laterality (L_A and L_R) and all biologically plausible two way interactions. For all above LME models, individual ID was fitted as a random factor to account for the repeated measures design and separate models were fitted to investigate the effects of L_A and L_R independently due to co-linearity between these variables. Assumptions of normality and homoscedasticity of residuals were assessed using visual inspection of residual-fit plots and Q-Q plots and latency to enter the feeding zone was $\log_{10}$-transformed to confirm to model assumptions.

4.3 Results

4.3.1 Examining the relationship between laterality, reproduction and parental care

There was no significant effect of sex, reproductive state or their interaction on either L_R or L_A (Table 4.1), indicating that both the direction and strength of laterality were similar for both sexes and reproductive states. However, individual *T. temporalis* consistently detoured in one direction, regardless of directional preference, when detouring around a barrier to view a novel object (L_A: $t = 4.251$, $df = 23$, $p < 0.001$; Figure 4.4A). These individual biases were aligned at the population level, with greater than 50% of individuals exhibiting a preference to detour rightward around the barrier (L_R: $t = 2.311$, $df = 23$, $p = 0.030$; Figure 4.4B), thus using their left eye to view the novel object and their right brain hemisphere for information processing.

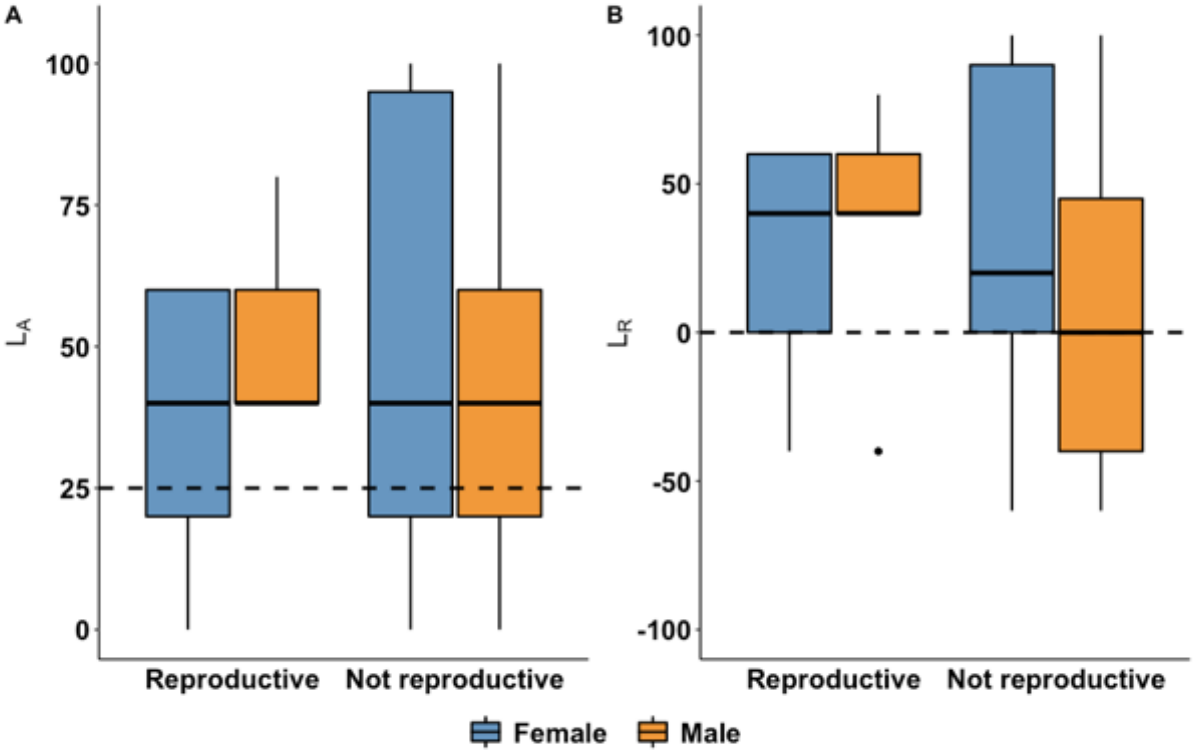


Figure 4.4: The A) L_A and B) L_R of females (blue) and males (orange) in a reproductive and non-reproductive state. Boxplots show the median and IQR of the sample population and horizontal dashed lines indicate a null expectation of no laterality, A) $L_A = 25$ and B) $L_R = 0$.

Table 4.1: Results of GLMER models fitted with binomial error distributions investigating the effect of sex and reproductive state on A) L_A and B) L_R. For all models individual ID was included as a random factor. Significant variables are highlighted in bold.

	estimate	s.e.	z	p
A) Strength of laterality, L_A				
Intercept	0.995	0.216	4.607	**<0.001**
Sex (male)	0.044	0.261	0.170	0.865
Reproductive state (reproductive)	-0.021	0.228	-0.092	0.927
B) Direction of laterality, L_R				
Intercept	0.573	0.319	1.794	0.072
Sex (male)	-0.245	0.430	-0.569	0.569
Reproductive state (reproductive)	0.257	0.223	1.153	0.249

4.3.2 Examining the costs of laterality

There was a significant difference in the number of times *T. temporalis* chose the larger stimulus group, representative of the greatest competitive threat, when stimulus groups were presented in opposing visual hemispheres in two trials ($\chi^2 = 17.61$, df = 2, $p < 0.001$). The majority of individuals choose the larger stimulus group (greatest competitive threat) in one trial and the smaller stimulus group (less competitive threat), in the other (Figure 4.5A). There was no significant effect of sex, reproductive state, laterality (L_A and L_R) or their interactions on preference for the larger stimulus group (proportion of times the larger of the two groups was chosen in two trials; Table 4.2) suggesting that the costs of laterality experienced by individuals were relatively consistent. Additionally, there was no significant effect of sex, reproductive state, L_A or their interactions on directional preference in stimulus group choice (number of times the group presented in the right visual hemisphere was chosen; Table 4.3). However, we found that L_R significantly influenced the number of times the group presented in the right visual hemisphere was chosen (L_R: χ^2 = 7.070, df = 1, p = 0.007), whereby left and right lateralised individuals choose more often the group presented in the eye corresponding to their lateral preference (Figure 4.5B), suggesting that directional biases in the detour test affect directional preferences in other tasks that require communication and cooperation between brain hemispheres due to inherent directional biases.

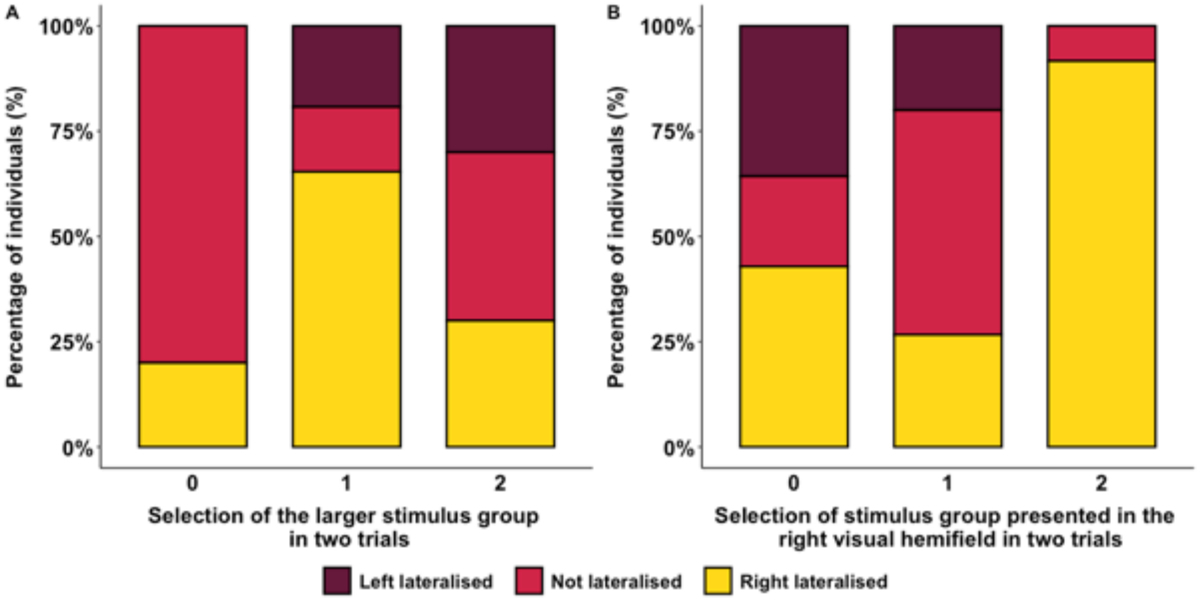


Figure 4.5: Examining the costs of laterality: bilateral information processing task. A) proportion of left lateralised (purple, n = 8), right lateralised (yellow, n = 21) and non-lateralised (pink, n = 12) individuals (%) based on individual L_R scores that choose the larger stimulus group, representative of a greater competitive threat, in neither trial (0), a single trial (1) or both trials (2) and B) the proportion of left lateralised (purple), right lateralised (yellow) and non-lateralised (pink) individuals (%) that picked the stimulus group presented in their right visual hemisphere in neither trial (0), a single trial (1) or both trials (2).

Table 4.2: Results of GLMER models examining factors affecting the number of times the larger stimulus group, representative of the greatest competitive threat, was chosen when stimulus groups were presented in opposing visual hemifields in two trials. Separate models were constructed with A) L_A and B) L_R (fitted as an independent variable) as indices of laterality. For all models individuals ID was included as a random factor. Significant variables are highlighted in bold.

	estimate	**s.e.**	**z**	**p**
A) model with L_A as an independent variable				
Intercept	-0.095	0.518	-0.185	0.853
Sex (male)	0.464	0.453	1.026	0.305
Reproductive state (reproductive)	-0.430	0.454	-0.947	0.343
L_A	0.006	0.008	0.818	0.414
B) model with L_R as an independent variable				
Intercept	0.278	0.400	0.696	0.486
Sex (male)	0.449	0.454	0.990	0.322
Reproductive state (reproductive)	-0.414	0.455	-0.912	0.362
L_R	-0.003	0.004	-0.635	0.525

Table 4.3: Results of GLMER models examining factors affecting the number of times the stimulus group presented in the right visual hemifield was chosen when stimulus groups were presented in opposing visual hemifields in two trials. Separate models were constructed with A) L_A and B) L_R (fitted as an independent variable) as indices of laterality. For all models individuals ID was included as a random factor. Significant variables are highlighted in bold.

	estimate	s.e.	z	p
A) model with L_A as an independent variable				
Intercept	-0.484	0.559	-0.867	0.386
Sex (male)	-0.226	0.480	-0.471	0.638
Reproductive state (reproductive)	0.219	0.470	0.468	0.640
L_A	0.008	0.008	1.045	0.296
B) model with L_R as an independent variable				
Intercept	-0.416	0.415	-1.002	0.316
Sex (male)	-0.058	0.464	-0.127	0.899
Reproductive state (reproductive)	0.069	0.466	0.148	0.882
L_R	0.012	0.005	2.501	**0.012**

4.3.3 Examining the benefits of laterality

We found no evidence that laterality influenced an individual's ability to perform simultaneous information processing, 'multi-tasking'. The time taken to peck at live prey was not influenced by laterality (L_A or L_R), sex, reproductive state, predator presence or the two-way interactions between these variables (Table 4.4), suggesting that the time taken to peck at live prey was consistent in the presence and absence of a predator regardless of strength or direction of lateral biases, the sex of the individual and whether or not they were reproductive. However, we found that latency to enter the feeding zone was influenced by L_A ($F_{1,60.306} = 4.523$, $p = 0.037$) but not by L_R, reproductive state, the presence or absence of a predator, or two-way interactions between these variables (Table 4.5). More strongly lateralised individuals were quicker to emerge into the feeding zone than their non/less lateralised counterparts (Figure 4.6), suggesting that laterality may allow individuals an enhanced ability to cope with divided attention since the feeding zone is a context that requires processing of multiple stimuli.

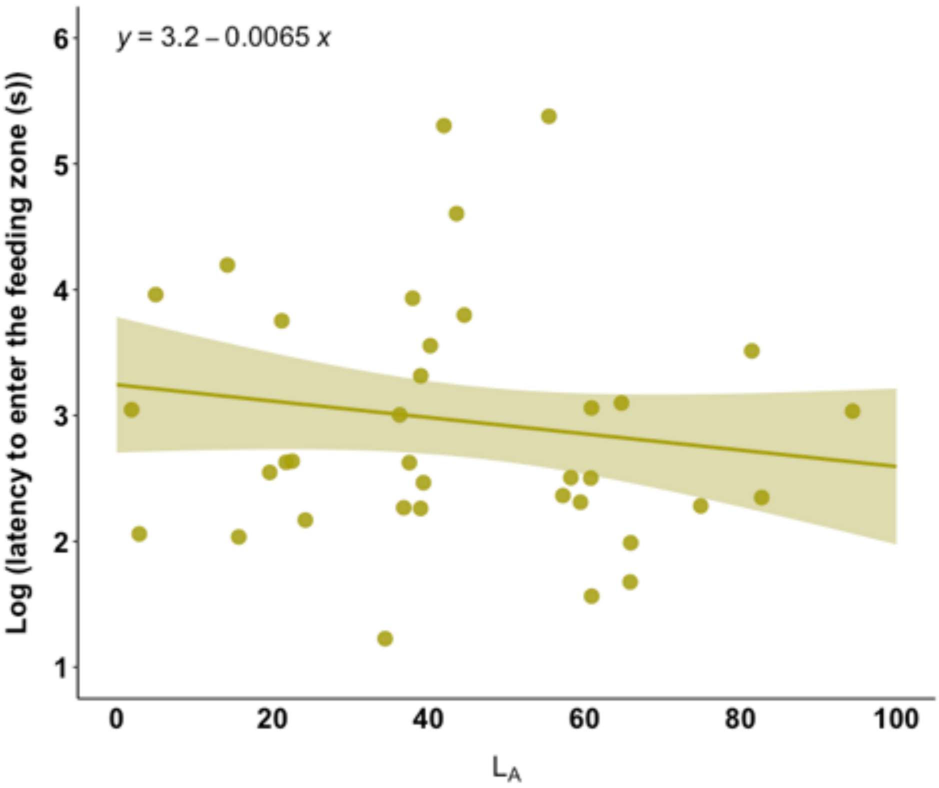


Figure 4.6: Relationship between log latency to enter the feeding zone in seconds and L_A.

Table 4.4: Results of LME models examining factors affecting the time to peck at live prey. Separate models were constructed with A) L_A and B)L_R (fitted as an independent variable) as indices of laterality. For all models individuals ID was included as a random factor. Significant variables are highlighted in bold.

	estimate	s.e.	df	t	p
A) model with L_A as independent variable					
Intercept	615.126	135.145	32.352	4.552	**<0.001**
Predator (present)	65.829	81.432	48.698	0.808	0.423
Sex (male)	-13.576	122.645	14.719	-0.111	0.913
Reproductive state (reproductive)	-46.167	85.027	59.290	-0.543	0.589
L_A	-2.842	1.878	45.573	-1.513	0.137
B) model with L_R as independent variable					
Intercept	516.926	109.237	27.501	4.732	**<0.001**
Predator (present)	65.829	82.508	48.183	0.798	0.429
Sex (male)	-34.807	123.733	14.008	-0.281	0.783
Reproductive state (reproductive)	-237.444	86.790	61.203	-0.431	0.668
L_R	-1.115	1.137	38.657	-0.980	0.333

Table 4.5: Results of LMER models examining factors affecting the time to emerge into the feeding zone, a context requiring the processing of multiple stimuli. Separate models were constructed with A) L_A and B)L_R (fitted as an independent variable) as indices of laterality. For all models individuals ID was included as a random factor. Significant variables are highlighted in bold.

	estimate	s.e.	df	t	p
A) model with L_A as independent variable					
Intercept	3.171	0.322	39.777	9.833	**<0.001**
Predator (present)	-0.227	0.187	55.151	-1.212	0.230
Sex (male)	0.394	0.297	20.019	1.327	0.199
Reproductive state (reproductive)	0.081	0.196	63.566	0.413	0.680
L_A	-0.009	0.004	55.401	-2.151	**0.035**
B) model with L_R as independent variable					
Intercept	2.776	0.270	34.935	10.262	**<0.001**
Predator (present)	-0.227	0.191	56.307	-1.184	0.241
Sex (male)	0.362	0.313	20.919	1.158	0.260
Reproductive state (reproductive)	0.093	0.203	65.904	0.458	0.648
L_R	-0.001	0.002	51.632	-0.550	0.584

4.4 Discussion

Our results show that lateral biases in exploratory behaviour exhibited in the detour test in *T. temporalis* are associated with a potential cost, poorer performance in a bilateral information processing task, and behaviour in a simultaneous information processing task. Directional biases in laterality meant that individuals were more likely to choose the stimulus group presented in their ‘dominant’ visual hemisphere, resulting in a preference for the larger stimulus group (greater competitive threat) in one trial and the smaller stimulus group (lesser competitive threat) in another. More strongly lateralised fish were also quicker to enter a context requiring the processing of multiple stimuli (the feeding zone in the predation task), than their less strongly or non-lateralised counterparts. This may result from an enhanced ability to cope with divided attention resulting from more efficient information processing, although we found no evidence that more strongly lateralised individuals were better able to multi-task than their less or non-lateralised counterparts (i.e. the time taken to peck at prey was unaffected by laterality or predator presence/absence).

Male and female *T. temporalis* exhibited significant laterality in exploratory behaviour and these biases were aligned at the population level, with no sex or reproductive state differences. *T. temporalis* preferentially turned rightward around the barrier in the detour test, thus using their left eye to view the novel object and their right brain hemisphere for information processing (Figure 4.4). Population level laterality is thought to be selected for when interactions occur between individuals or where there is a need for coordination between individuals or amongst groups in a social species or performing social behaviours, such that the fitness of a lateralised individual depends on the actions of other lateralised individuals (Rogers 2000; Ghirlanda and Vallortigara 2004). Although *T. temporalis* are generally non-social, this species performs biparental care with a need for interaction and coordination between parents (Snekser et al. 2011). Both sexes perform brood defense: males hold territories around the nest site, which they guard against brood predators, while females stay in closer proximity to the nest site and guard against immediate and/or smaller predatory threats (Mboko 1998; Mboko and Kohda 1999). Biparental care and antagonist interactions, such as brood defense, represent selective pressures that could favour the evolution of population level lateral biases amongst individuals. Additionally, although generally non-social *T. temporalis* are a highly aggressive, territorial species and thus aggressive interactions, even in a non-reproductive state, could represent a selection pressure that favours the evolution of aligned biases in laterality, which may explain the aligned directional biases at the population level observed in this study in both a reproductive and non-reproductive state (Figure 4.4B). Aligned directional biases in the processing of information regarding a novel environment would leave the opposing brain hemisphere free and available to process information about, for example, predatory threats or conspecifics. This would likely be beneficial to individuals, especially those that perform parental care where the execution of several tasks, and thus processing of multiple stimuli, simultaneously is critical for survival. Indeed, previous work investigating emotive lateralised responses in animals have shown right brain hemisphere control of negative responses such as attack and fear responses and left brain hemisphere control of positive responses such as foraging and approach behaviour (Andrew and Rogers 2002; Vallortigara and Rogers 2005). To explore this trend and potential hypotheses further, additional experiments examining laterality in behaviours performed during parental care in a biparental species with a clear division of sex roles could prove fruitful. This would allow us to explore whether lateralised individuals exhibiting lateral biases in response to one entity that is shared during biparental care, e.g. brood defense, use the remaining hemisphere to partition information about another entity of care, such as foraging during care, and whether these biases differ between the sexes based on the parental care roles or behaviours they perform.

Our study showed that lateralised individuals suffered a cost of cerebral lateralisation whereby lateralised individuals were less efficient in the group choice task that requires cooperation and communication between brain hemispheres as information was presented in separate visual hemispheres simultaneously. Lateralised *T. temporalis* choose the greater competitive threat (larger group of individuals) in fewer trials than their non-lateralised counterparts, due to strong inherent directional biases, regardless of sex or reproductive state (Figure 4.5). In nature, an increased density of individuals increases competition for resources, mates and territories (Connell 1983; Hixon et al. 1991; Kvarnemo et al. 1996), thus more individuals in a fixed area represent a greater competitive threat. In this study, individuals would perform aggressive behaviours such as biting and contest interactions towards conspecifics within the stimulus groups, suggesting that *T. temporalis* individuals perceived the stimulus groups as competitive threats, and that laterality carries a cost, whereby the efficiency of identifying the greatest competitive threat is comprised.

Fish have laterally placed eyes with a very small region of frontal overlap, around 10% (Collin and Shand 2003), thus in the wild different stimuli are likely to be seen in different visual hemifields, and the side they are presented on is likely highly unpredictable (Vallortigara 2006). As several behaviours in cichlids are lateralised, including foraging (Lee et al. 2012; Takeuchi and Oda 2017) and intraspecific and interspecific interactions such as lateral displays (Arnott et al. 2011) and aggression (Reddon and Hurd 2008), it is likely that individuals may make incorrect or suboptimal decisions in contexts where stimuli are presented in different visual hemifields and a quick assessment of the surrounding environment is required (Dadda et al. 2009). Such situations are likely to occur frequently during the performance of various behaviours, whereby incorrect or suboptimal decisions could have detrimental implications for individual fitness. For example in fish species that perform parental care, brood defense is one of the most predominant forms of care and predatory threats to broods are likely to occur in both visual hemispheres simultaneously. Thus incorrect or suboptimal decisions could be critical for offspring survival and ultimately parental fitness. Future work should investigate the costs of laterality on behavioural performance where stimuli can be presented in both visual fields simultaneously and cooperation between brain hemispheres is required to make an accurate decision, in a number of fitness related behaviours to better understand the true extent of the costs of lateralisation from an ecological and evolutionary perspective.

In contrast to previous work that has shown that lateralised individuals are better able to 'multi-task', pecking at live prey twice as fast as their non-lateralised counterparts in the presence of a predator, but displaying no differences when a predator is absent (Dadda and Bisazza 2006a), we found no evidence that lateralised individuals were better able to perform multiple tasks simultaneously (foraging while performing predator vigilance) than their non/less lateralised conspecifics. We suggest the discrepancies between our findings and those of Dadda and Bisazza (2006a) result from focal individuals in our study exhibiting some neophobia while foraging in a novel environment, which likely impacted foraging efficiency (Soma and Hasegawa 2004; Ensminger and Westneat 2012). While lateralised individuals were not better able to 'multi-task' per se, we found that more strongly lateralised individuals were quicker to emerge from the holding zone into the feeding zone than non/less strongly lateralised individuals (Figure 4.6). This suggests that lateralised individuals were able to more efficiently process information about a context containing multiple stimuli (here, prey, a novel environment and/or a predator; Vallortigara and Rogers 2005) before engaging with these stimuli, than non-lateralised individuals. This difference may result from lateralised individuals having an enhanced ability to cope with divided attention, another known advantage of laterality (Rogers 2000, 2002; Vallortigara and Rogers 2005; Vallortigara 2006). This advantage results from an increased efficiency in information processing by partitioning different types of information into specific brain hemispheres, thus, enabling separate and parallel processing (Rogers 2000, 2002). Situations requiring the processing of multiple stimuli are likely common across a number of behaviours and contexts, and so an enhanced efficiency of information processing would likely be beneficial for improved performance in such behaviours especially those tightly associated with fitness that could ultimately provide individuals with a selective advantage. For example, in the wild where prey can be extremely unpredictable, a lateralised individual may be quicker to approach and attack than their non-lateralised counterparts resulting in enhanced foraging success.

Overall, *T. temporalis* exhibited lateral biases in exploratory behaviour that were consistent between the sexes and in different reproductive states. While we identified that these consistent directional biases incurred at least one potential cost, a reduced ability to process information obtained in separate visual hemispheres, we did not identify a benefit with regard to an enhanced ability to multi-task. However, consistent lateral biases did result in lateralised individuals emerging more quickly into a context containing multiple stimuli than their non lateralised counterparts that may result from an enhanced ability to cope with divided attention. How these costs might impact behavioural performance for fitness related behaviours remains unclear and provide an interesting avenue for future research. Specifically, consideration of social behaviours that are tightly associated with individual fitness or are context specific such as parental care behaviours or intraspecific competition for mates would be beneficial. In the context of laterality, there is a strong research bias towards foraging and predator avoidance behaviour, and understanding the impacts of the costs and benefits of laterality across a wider range of behaviours will provide a more thorough understanding of how these costs and benefits are traded-off, helping us to better understand the substantial levels of variation in behavioural laterality widely observed across animal taxa.

Chapter 5: Parental care behaviours are lateralised in a biparental cichlid fish

Pair bonded *Telmatochromis temporalis* pair

© user: Steph McLean | Personal photo

Abstract

Social and cooperative interactions represent a key factor mediating the evolution of laterality, the partitioning of different cognitive functions into separate brain hemispheres, at the population level. Reproduction and reproductive behaviours can be highly social and often require varying levels of communication between male and female parents or between parent(s) and offspring, yet little is known about the lateralisation of these behaviours. Here, we examined whether parental care behaviours were lateralised in a biparental cichlid fish species, *Telmatochromis temporalis*, by assessing directional biases in brood defence behaviour and visual hemisphere use when monitoring offspring and brood predators during parental care. We show that both male and female *T. temporalis*, that are highly social and cooperative during care, exhibited consistent directional biases in brood defence and visual hemifield use during care at the individual and population level. Specifically, the majority of individuals exhibited a leftward bias in the performance of care behaviours directed at brood predators (brood defence attacks and the eye used to monitor brood predators during care) and a rightward bias in the performance of behaviours directed at offspring (the eye used to view the brood following an attack and monitor the brood during care). However, no population level bias was evident in the eye used to detect a brood predator prior to attack. Directional biases in parental behaviours could influence behavioural performance during care, which could in turn have implications for parent and offspring life history traits and individual fitness.

5.1 Introduction

Asymmetry of brain hemispheres either structurally or functionally, known as cerebral lateralisation or laterality, are often demonstrated as side biases in behaviours (Bisazza et al. 2001a; Vallortigara and Rogers 2005) and are widely documented in both vertebrates and invertebrates (Walker 1980; Bisazza et al. 1998a; Frasnelli et al. 2012). Laterality is a selectively advantageous trait that enables individuals to cope with divided attention via three mechanisms (Bibost and Brown 2014). Firstly, lateralised individuals have an enhanced neural capacity since hemispheric specialisation leaves one of two possible brain hemispheres available to perform additional functions (Rogers 2002; Vallortigara 2006), thus reducing the need for expensive neural tissue and circuitry associated with repeating the same function in both hemispheres (Levy 1977). Secondly, lateralised individuals exhibit hemisphere dominance, which decreases interference between different functions (Rogers 2002; Vallortigara 2006) and prevents simultaneous initiation of incompatible responses in organisms with laterally placed eyes (Cantalupo et al. 1995). Lastly, lateralisation enables separate and parallel processing in each brain hemisphere, which increases the brains ability to conduct simultaneous information processing, sometimes known as ‘multi-tasking’ (Rogers 2002; Vallortigara 2006). As a result of these advantages, lateralised individuals have been found to outcompete their non-lateralised counterparts in several behaviours including numerical discrimination (Dadda et al. 2015), spatial reorientation (Sovrano et al. 2005) and catching prey while simultaneously performing predator vigilance (Dadda and Bisazza 2006a).

Laterality can occur at the individual level, whereby each individual has a consistent directional bias, but the population is composed of both lateral phenotypes (left biased and right biased individuals; Denenberg 1981; Rogers 2002), or at the population level, whereby the majority of individuals in a population have aligned biases for a specific direction (left or right; Denenberg 1981; Rogers 2002). Although the advantages of laterality are clear for individual efficiency, many populations exhibit aligned directional biases at the population level across a wide range of behaviours (Bisazza et al. 1998a). This is surprising given that such biases increase the predictability of behaviour, presenting a clear disadvantage for individuals with the common lateral phenotype (Hori 1993). Aligned directional biases are thought to arise when individuals interact with one and other such that the fitness of an asymmetric individual depends on what other asymmetrical individuals do (Rogers 2000; Ghirlanda and Vallortigara 2004). Situations that require coordination amongst individuals such as interactions within social species, living within social groups or performing social behaviours represent selection pressures that favour the evolution of population level laterality (Rogers 2000; Ghirlanda and Vallortigara 2004).

There is growing evidence that reproductive behaviours, including courtship and copulation, that require interaction and often coordination between individuals are lateralised. In black winged stilts, *Himantopus himantopu,* males are likely to perform courtship displays when a female is detected in the left visual field (Ventolini et al. 2005), while in parasitic wasps (*Leptomastidea abnormis*) males exhibit right-biased antennal tapping towards mates (Romano et al. 2016). Additionally, house sparrows (*Passer domesticus*; Nyland et al. 2003), male rice weevils (*Sitophilus oryzae*; Benelli et al. 2017a) and confused flour beetles (*Tribolium confusum*; Benelli et al. 2017a) all show left biases when mounting females during copulation, which have been linked to increased mating success in the latter (Benelli et al. 2017a).

Parental care is a key reproductive behaviour across many animal taxa that is extremely diverse, both between species and between sexes. The degree of sociability in parental care behaviour is highly variable (Royle et al. 2012), whereby parental care can be performed by a single parent (male or female uniparental care), both parents (biparental care) or by non-parents such as siblings (alloparental care; Kokko and Jennions 2008). Caring for young is a cognitively demanding behaviour that requires individuals to perform multiple tasks simultaneously to survive (Smith and Wootton 1994; Royle et al. 2012). For example, caring parents must perform all aspects of parental care while simultaneously performing behaviours critical for survival such as foraging and predator avoidance (Royle et al. 2012). The advantages associated with cerebral lateralisation, including an enhanced ability to cope with divided attention, suggest that caring parents could obtain a potential fitness benefit from having a lateralised brain. Furthermore, in parental care systems where more than one individual cares for the young (biparental or alloparental care), and thus there is a need to interact and coordinate behavioural responses, aligned directional biases in laterality at the population level could provide a selective advantage. Such biases could have implications for the performance of both parental care behaviours and other fitness related behaviours performed simultaneously while caring, which could in turn have implications for life history traits in both parents and offspring (Rogers 2002; Ventolini et al. 2005). However, very little is known regarding lateralisation of parental care behaviour(s), especially in species with high levels of social interaction during the caring period.

Here we address this gap by examining laterality of parental care behaviours in a biparental substrate brooding cichlid, *Telmatochromis temporalis*. Specifically, we assessed brood defense behaviour and visual hemisphere use when monitoring the brood and brood predators during three stages of parental care: 1-3 days after egg laying, and at 1-3 days and 10-13 days after the eggs had hatched to determine whether i) parental care behaviours are lateralised and ii) whether any lateral biases in parental care behaviours are aligned at the population level in a biparental fish species. We predicted that i) as a result of the potential fitness benefits that caring parents could obtain from cerebral lateralisation, individuals of both sexes would exhibit laterality in parental care behaviours at the individual level and ii) as a result of the need to interact and coordinate behavioural responses during biparental care, which is a selective force thought to drive aligned directional biases in laterality, lateral biases would be aligned at the population level, with no variation in directional biases between the sexes resulting from equivalent sex roles and investment in parental care.

5.2 Materials and Methods

5.2.1 Animals

T. temporalis breed monogamously following the formation of pair bonds and perform elaborate biparental care of offspring until independence (Mboko 1998). At least one week prior to spawning, pair bonds are formed during which time females remain in or close to a nest site (Mboko 1998). *T. temporalis* spawn in a nest of a small hole or burrow under a stone, whereby females deposit eggs inside the nest site and males ejaculate sperm at the nest site (Mboko and Kohda 1995; Kuwamura 1997). Young will hatch out approximately 3 days after spawning and are protected by both parents who guard the nest site by chasing and attacking (biting) potential brood predators they consider a threat. Although brood defence is equivalent for biparental parents in this species, paired males have territories around the nest site, which they defend against threat from males of the same species and other predatory species, while females stay in closer proximity to the nest and guard their broods only from predators small enough to enter the nest site or those considered an immediate threat (Mboko 1998; Mboko and Kohda 1999). Thus, *T. temporalis* represent a model species to examine laterality in parental care behaviours.

30 *T. temporalis* (13 males, 17 females) originally sourced from Lake Tanganyika in East Africa, were purchased from Mikes Rifts (Newton-le-Willows, UK) in October 2019, and were housed in single species stock tanks (40 x 47 x 60cm) prior to behavioural observations. A single male and female *T. temporalis* (breeding pair) were then assigned to an experimental tank (40 x 47 x 60cm) to encourage pair bond formation. Each experimental tank contained a variety of other cichlid species (min n per tank = 11, max n per tank = 28, mean n per tank = 17) of varying sex that would be considered a predatory threat to the eggs and developing offspring when in proximity to the spawning site. All tanks were maintained at 23 ± 1°C on a 12L:12D photoperiod, fed daily with commercial feed and provided with enrichment (plastic tubing: approximately 9cm length x 4cm diameter) that could be used for shelter and/or a resource for reproduction. Due to the uneven sex ratio and a small number of mortalities (n=2), we assessed a total of 12 pair bonded breeding pairs. The majority of these pairs were comprised of unique males and females however, 3 of the 12 pairs contained shared individuals (1 female and 2 males). Where individuals reproduced twice, laterality in parental care behaviours of repeated measures individuals were only examined in the initial breeding attempt (n=21). All individuals were deprived of food on the days that behavioural observations were obtained in order to remove competition for food resources, ensuring that

any defensive behaviours associated with foraging could be disentangled from defensive behaviours directly related to brood defence.

5.2.2 Data collection

Breeding pairs of *T. temporalis* were video recorded using a camera (GoPro Hero6, GoPro, California, USA) positioned directly above the experimental tank at three stages during parental care: 1-3 days after egg laying once the eggs were fertilised (hereafter “post egg laying”; egg colouration turns from transparent to opaque when fertilised; personal observation), 1-3 days after the eggs hatched (“early post hatch”) and 10-13 days after hatching (“late post hatch”). During each stage of parental care, each experimental tank was video recorded for 1h in the morning (between 09:00-12:00) and 1h in the afternoon (between 13:00-17:00). Lateral biases in two aspects of parental care behaviour were assessed: brood defence behaviour and visual hemisphere use during care.

Brood defence behaviour

All videos were later analysed to assess laterality in brood defence behaviour across the three stages of parental care (post egg laying, early and late post hatch). A single brood defence event in *T. temporalis* is comprised of three components: (i) detection of a potential brood predator, defined as an individual within the male’s territory or in close proximity (~1.5 body lengths) to the nest site that was then attacked (Kuwamura 1986; Katoh et al. 2005), (ii) attacking the perceived brood predator via chasing out and/or biting (Kuwamura 1986) and (iii) viewing the nest site/brood after the attack while returning to either a territory surrounding the nest site (males) or to the nest site (females) to defend the brood from further predatory threat (Kuwamura 1986). Laterality is commonly observed at the behavioural level as side biases in behaviours or as use of one side/half of a bilateral characteristic (Bisazza et al. 2001a; Koboroff et al. 2008). Specifically in fish, biases in visual hemisphere use are common, since fish have laterally placed eyes with little frontal overlap and complete decussation at the optic chiasma (Brown et al. 2004), thus each eye receives an independent view of the surrounding environment, which is processed by the contralateral brain hemisphere. Furthermore, evidence suggests that different emotive stimuli are processed in different brain hemispheres (negative and positive emotions are processed in the right and left brain hemispheres respectively; Vallortigara and Rogers 2005; Quaranta et al. 2007). Thus, we assessed laterality in each of the three components of brood defence behaviour independently as it is likely that lateral biases, if present, could vary between the different components of brood defence behaviour when different emotive stimuli are considered, such as brood predators and offspring.

We assessed laterality in parental care behaviours performed within a 30m duration (00:05 – 00:35m of the 1h video, allowing the first five minutes of observation to be excluded in order to minimise any influence of experimenter disturbance when commencing the video recording) by examining directional biases in brood defence behaviour. For each event of brood defence behaviour performed by an individual, we scored lateral biases in each of the three components independently (eye a predator is detected in prior to attack, the side of a predator that is preferentially attacked and the eye used to view the nest site containing the brood post attack) as left, right or 0, whereby left and right indicate directional biases in behaviours and 0 is indicative the absence of such biases (i.e. an individual may perform two consecutive brood defence attacks without viewing the nest site or returning to a territory/nest site). Only instances where focal individuals chased predators away from the area they were detected in or where brood predators were bitten were considered successful brood defence events. Between 1 and 98 brood defence events were recorded for each individual in each 30 minute period (mean = 21.174, SD = 19.930). For directional biases in the eye used to detect a brood predator prior to performing a brood defence attack and the eye used to view the nest site containing a brood post attack, we estimated use of the left or right visual hemifield based on the position and angle of the fish that defined monocular viewing of the detected brood predator or nest site respectively (Figure 5.1). Thus, it is possible that both the brood predator and the brood were visualised in the same visual hemifield. Where brood predators and or the nest site were not viewed in a single monocular field of vision, individuals were scored as 0, no directional bias. Conversely, directional biases in the side of a predator that was attacked were directly assessed by determining the side of a predator that was primarily targeted during a chase or was bitten, as *T. temporalis* typically chase out or attack a specific side of a brood predator. Attackers typically target brood predators that are present on a specific side of the attacker, and thus viewed in a specific visual hemifield during the attack, or they attack head on, whereby the brood predator is viewed in both visual hemifields or a single visual hemifield depending on the position of the attacker during the attack. A new attack was defined as an attack on a potential brood predator following a previous predatory threat being chased out and/or attacked.

Visual hemifield use during parental care

We also assessed visual hemifield use during the post egg laying, early post hatch and late post hatch periods of parental care by examining biases in the visual hemisphere used to monitor potential brood predators and the nest site containing a brood independently, since brood defence attacks do not always occur simultaneously. To assess biases in visual hemifield use, still frames of each 30m recording were analysed at 30s intervals (±10s to obtain a clear image of each sex) to assess the positioning and associated angle of the fish that defined monocular viewing relative to the most immediate predatory threat to the brood and the nest site containing a brood (Figure 5.1). We defined the most immediate predatory threat as an individual that was subsequently attacked or the brood predator within the closest proximity to the paired breeding male, since paired males guard a territory around the nest site, and to the nest site in females, since paired females guard the nest site from immediate predatory threat. Where predatory threats and/or the nest site were not visible in either visual hemifield during an observational time point (e.g. if the female was inside the tubing containing the brood and not able to be seen), individuals were scored as 0. This produced 120 observations (60 observations in the morning and 60 observations in the afternoon), of visual hemisphere use when monitoring brood predators and 120 observations of visual hemisphere use when monitoring the nest site containing a brood for each individual during each stage of parental care.

5.2.3 Data analyses

All data analyses were conducted using R version 3.6.2 (R Core Team 2019). To examine laterality in parental care behaviours, a standard relative laterality index (L_R) was calculated for each individual for each component of brood defence behaviour and for visual hemisphere use towards both brood predators and the nest site containing a brood independently. L_R was calculated excluding instances scored as 0 as: [(right– left)/(right + left) x100], where right and left represent directional biases in behaviours, and provide an assessment of directional biases of fish at the population level (Bisazza et al. 1997). L_R ranges from -100 to +100, representing a population exhibiting consistent leftward or rightward biases respectively. For each individual, an absolute laterality index (L_A) was calculated as $|L_R|$ to provide an assessment of the strength of laterality at the individual level. L_A ranges from 0 (individuals exhibiting an equal left and right preference) to 100 (individuals exhibiting a consistent preference for a specific direction; Bisazza et al. 1997).

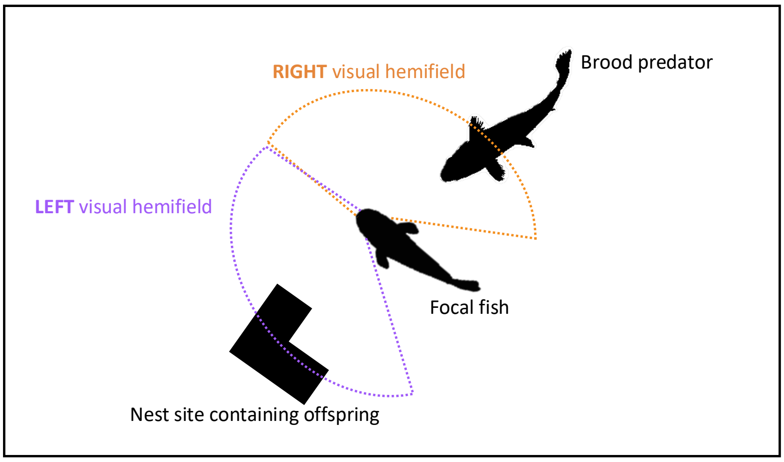


Figure 5.1: Schematic representation showing the position of a focal fish and the angles of viewing that define monocular vision in the right (orange) or left (purple) visual hemifield. Here, the nest site containing offspring is independently viewed in the left visual hemifield while the potential brood predator is independently viewed in the right visual hemifield. Note: the nearest brood predator could also be positioned in the same hemifield as the nest site.

General linear mixed effects models (LMMs), fitted using the lme4 package (Bates et al. 2015) were used to assess the effects of sex, parental care behaviour, stage of parental care and all plausible two-way interactions on L_R and L_A separately. For all models individual ID and time (AM or PM) were included as random effects to account for the repeated measures design, with the number of potential brood predators in each experimental tank also included as a random factor to account for variation in the number of co-habitants present in breeding tanks. Assumptions of normality and homoscedasticity of residuals were assessed using visual inspection of residual-fit plots and Q-Q plots. Non-significant interactions were removed and the minimum adequate model containing all main effects, regardless of significance, is reported here. Where independent variables significantly influenced response variables, differences in the laterality indices amongst the levels of these variables were examined using Tukeys honestly significant difference (HSD) test (Lenth 2016).

To examine whether individuals exhibited significant individual level laterality in parental care behaviours and to examine whether any aligned biases in parental care behaviours were left or right dominant, a mean L_R and L_A were calculated for each individual across all non-significant variables from the above LMM models. One-sample t-tests were used to examine whether these means differed from a null expectation of no laterality (L_R = 0, L_A = 19 (brood defence attacks) and L_A = 10 for visual hemifield use during care; see Appendix 5.1 for the calculation of null expectation of no laterality). The false discovery rate method was used to correct for multiple testing and the adjusted p-values are reported here (Benjamini and Hochberg 1995). Where laterality was influenced by independent variables in the above LMM models (sex, parental care behaviour, stage of care or their interactions), one sample t-tests were used to determine if laterality indices differed from the null expectation of no laterality, accounting for the influence of significant explanatory variables (see Appendix 5.1 for how a null expectation of no laterality was calculated).

5.3 Results

5.3.1. Lateral biases at the population level, L_R

There were significant differences in the relative laterality indices (L_R) exhibited across the different parental care behaviours examined (LMM Behaviour main effect: $F_{4,\,546.99}$= 17.685, $p < 0.001$; Table 5.1; Figure 5.2A), but L_R did not differ between the sexes (Sex main effect: $F_{1,20.15}$ = 0.503, p = 0.486; Table 5.1) , or at different stages of reproduction (Stage main effect: $F_{2,533.30}$ = 1.752 , p = 0.174;Table 5.1). *T. temporalis* exhibited a leftward bias when attacking a brood predator and a marginally significant (after correcting for multiple testing) rightward bias in the visual hemisphere used to view the nest site containing a brood after an attack (Table 5.2A; Figure 5.2A). Additionally, opposing biases in visual hemisphere use during parental care were evident in *T. temporalis* whereby, potential brood predators were preferentially monitored in the left visual hemifield, while the nest site containing a brood was preferentially monitored in the right visual hemifield (Table 5.2A, Figure 5.2A), suggesting that *T. temporalis* partition the processing of information related to brood predators and their offspring in different brain hemispheres. However, no directional biases at the population level were observed in the eye used to detect a potential brood predator before an attack (Figure 5.2A), suggesting that biases in subsequent components of parental care behaviour do not result because a brood predator is first detected in a specific visual hemifield.

Table 5.1: Results of linear mixed effects models examining factors affecting the L_R of parental care behaviours in *T. temporalis*. For each model, individual ID, time (AM or PM) and the number of brood predators in the experimental tank were included as random effects. The minimum adequate model containing all main effects, regardless of significance, is reported here. Significant p-values are highlighted in bold. The intercept represents female parents in the post-lay stage of care, detecting a brood predator prior to an attack.

Direction of laterality (L_R)	**estimate**	**s.e.**	**df**	**t**	**p**
Intercept	8.567	7.290	434.661	1.175	0.240
Behaviour					
predator attacked	-26.148	9.654	545.219	-2.709	**0.006**
return to brood	4.888	9.649	545.196	0.507	0.612
monitor brood predators	-20.475	9.515	546.754	-2.152	**0.031**
monitor nest site	2.183	9.515	546.754	0.229	0.818
Sex					
male	-11.998	8.646	301.510	-1.388	0.166
Stage					
Early post-hatch	-1.942	9.405	555.024	-0.206	0.836
Late post-hatch	-11.249	9.502	555.489	-1.184	0.236

Table 5.2: Results of one-sample t-tests examining if the A) mean L_R and B) mean L_A across the three stages of parental care examined differed from random choices for each of the components of brood defence behaviour and visual hemisphere use during care that were observed. P-values are corrected for multiple testing using the false discovery rate method (Benjamini and Hochberg 1995) and highlighted in bold if significant.

		A) Direction of laterality, L_R		**B) Strength of laterality, L_A**	
Parental Care Behaviour	**df**	**T**	**p**	**t**	**p**
Brood defence attacks					
Predator detected	19	0.329	0.745	2.896	**0.004**
Predator attacked	19	-2.926	**0.021**	3.387	**<0.001**
View nest post attack	19	2.125	**0.058**	5.505	**<0.001**
Visual hemisphere use					
View predators	20	-2.711	**0.022**	8.027	**<0.001**
View nest site	20	4.171	**0.002**	7.576	**<0.001**

5.3.2 Lateral biases at the individual level, L_A

Individual *T. temporalis* exhibited consistent directional biases in all five components of parental care behaviour examined (Table 5.2B) but the strength of laterality (L_A) did not vary between care behaviours (Behaviour main effect: $F_{4,523.76} = 1.610$, $p = 0.170$; Table 5.3; Figure 5.2B). There was a significant interaction between sex and stage on the overall strength of laterality L_A exhibited across all components of parental care behaviour observed (LMM Sex * Stage interaction: $F_{2, 529.15} = 4.337$, $p = 0.013$; Table 5.3; Figure 5.3). Across all stages of parental care, individuals of both sexes exhibited consistent directional biases in care behaviours (Table 5.4) however, post-hoc tests revealed that in males, L_A was significantly reduced in the late post hatch period of care relative to the post-lay (t ratio = -2.370, p = 0.047; Figure 5.3), and early post-hatch (t ratio = 2.600, p = 0.025; Figure 5.3) periods, but there was no difference in L_A between the post-lay and early post-hatch periods (t ratio = -0.245, p = 0.967; Figure 5.3). In contrast, females exhibited stronger laterality in parental care behaviours that were performed in the early post-hatch period of care relative to post-laying (t ratio = -2.436, p = 0.040, Figure 5.3), but no other differences were apparent (post-lay vs. late post-hatch: t ratio = -1.782, p = 0.176; early vs. late post hatch: t ratio = 0.624, p = 0.807; Figure 5.3).

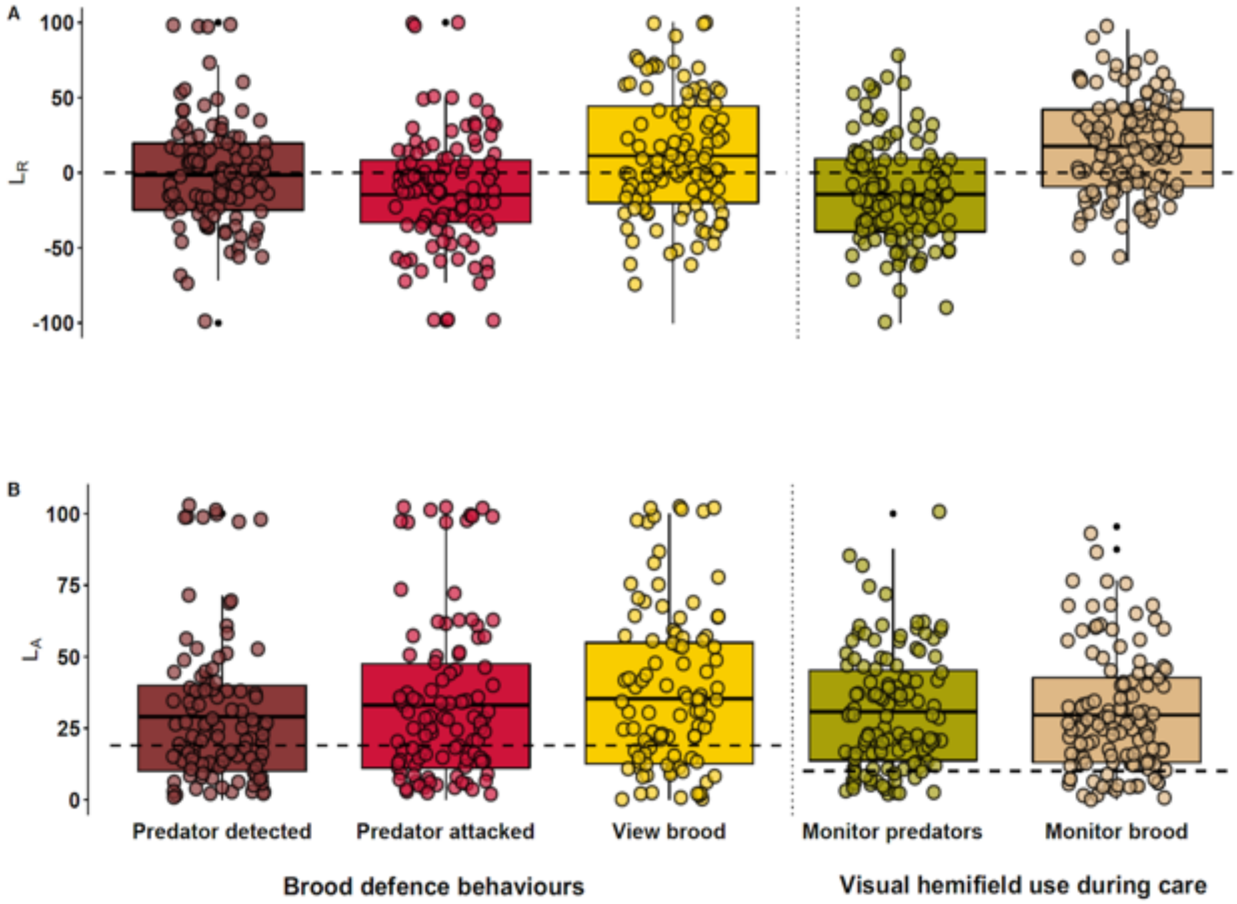


Figure 5.2: A) L_R and B) L_A of brood defence behaviours and visual hemifield use during parental care in *T. temporalis*. Boxplots show the mean and IQR of the sampled population overlaid by individual data points across all three stages of parental care: post egg laying, early

post hatch and late post hatch. Horizontal dashed lines indicate a null expectation of no laterality.

Table 5.3: Results of linear mixed effects models examining factors affecting the L_A of parental care behaviours in *T. temporalis*. For each model, individual ID, time (AM or PM) and the number of brood predators in the experimental tank were included as random effects. The minimum adequate model containing all main effects, regardless of significance, is reported here. Significant p-values are highlighted in bold. The intercept represents female parents, at the post laying stage of care, detecting a brood predator prior to attack.

Strength of laterality (L_A)	**estimate**	**s.e.**	**df**	**t**	**p**
Intercept	29.045	5.758	31.120	5.043	**<0.001**
Behaviour					
predator attacked	5.030	6.131	522.455	0.820	0.412
return to brood	1.781	6.128	522.448	0.291	0.771
monitor brood predators	-6.842	6.048	523.966	-1.131	0.258
monitor nest site	-8.493	6.048	523.966	-1.404	0.160
Sex					
Male	7.134	6.771	73.334	1.054	0.295
Stage					
early post hatch	0.792	6.006	529.236	0.132	0.895
late post hatch	2.512	6.069	529.331	0.414	0.679
Sex x Stage					
male: early post hatch	-7.481	4.901	533.056	-1.526	0.127
male: late post hatch	-14.517	4.932	533.140	-2.943	**0.003**

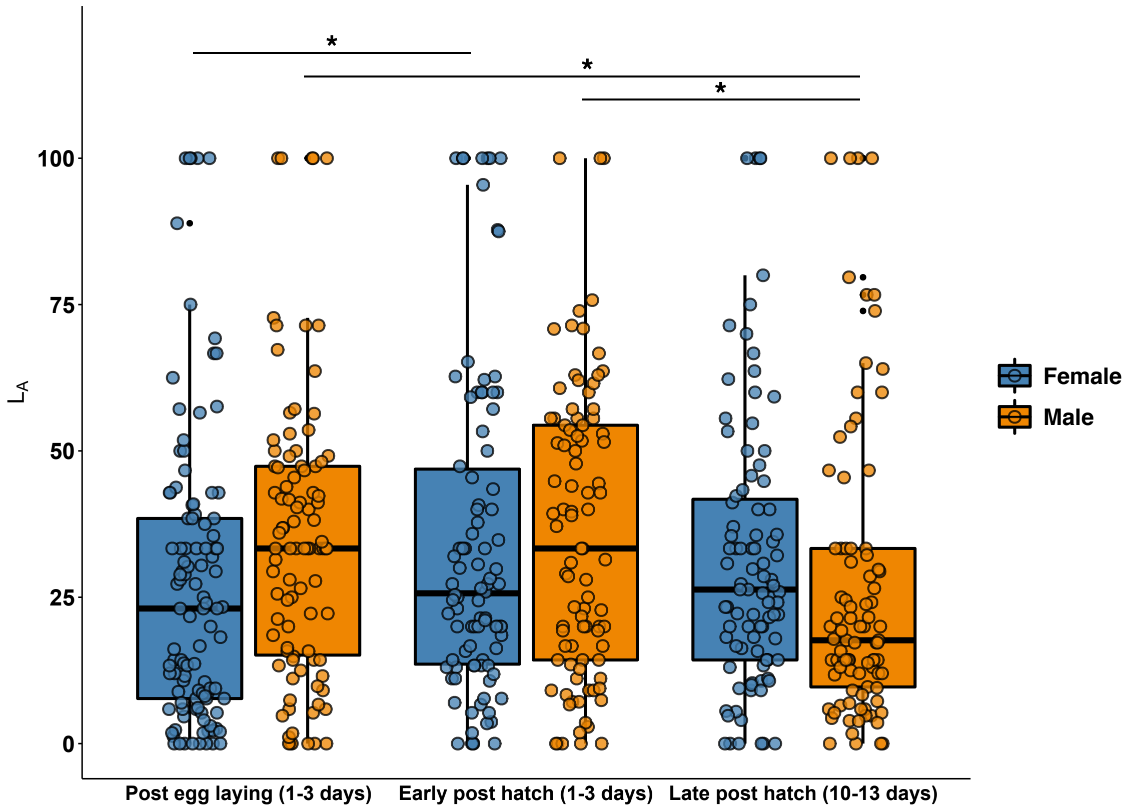


Figure 5.3: L_A of female (blue) and male (orange) *Telmatochromis temporalis* for all parental care behaviours across the three stages of parental care examined: post egg laying, early post hatch and late post hatch. Asterisks indicate significant differences between groups at $p<0.05$ (*).

Table 5.4: Results of one-sample t-tests examining if the mean L_A across all behaviours was significantly different from random choices (see Appendix 5.1 for calculation of null expectation of no laterality). P-values are corrected for multiple testing using the false discovery rate method (Benjamini and Hochberg 1995) and highlighted in bold if significant.

Sex	Stage	df	t	p
Male	Post egg laying	9	7.504	**<0.001**
	Early post hatch	8	6.354	**<0.001**
	Late post hatch	8	2.239	**0.027**
Female	Post egg laying	10	3.356	**0.005**
	Early post hatch	9	3.037	**0.008**
	Late post hatch	9	7.810	**<0.001**

5.4 Discussion

Although evidence shows that there are side biases in mother-infant interactions in a number of mammals (Hopkins and De Lathouwers 2006; Karenina et al. 2017; Karenina and Giljov 2018; Regaiolli et al. 2018), our results provide the first evidence that parental care behaviours are also lateralised in fish. Male and female biparental *T. temporalis* exhibited laterality in brood defence behaviour and visual hemisphere use during parental care at both the individual and population level. Consistent directional biases in parental care, could influence not only behavioural performance during care but also performance in other behaviours that are performed simultaneously while caring, especially those tightly associated with fitness such as foraging and predator avoidance, which could have direct implications for parent and offspring life histories. Specifically, consistent directional biases in parental care behaviours such as brood defence could improve behavioural performance and ultimately the quality and/or success of care (Dadda et al. 2010), which could in turn positively influence parental investment and reproductive success of parents and survival of offspring (Vallortigara and Rogers 2005; Dadda et al. 2010; Niven and Frasnelli 2018).

Directional biases were aligned at the population level for two of the three components of brood defence behaviour: the side of a brood predator that was preferentially attacked and the eye used to view the nest site/brood following an attack, and in both aspects of visual hemifield use during care: monitoring a brood and monitoring potential brood predators (Figure 5.2A). Evolutionary theory predicts that population level lateralisation reflects an evolutionary stable strategy that can develop when asymmetrical individuals have to co-ordinate their behavioural responses with other asymmetrical individuals in social interactions (Vallortigara 2006). Our results suggest that these conditions are met for *T. temporalis* performing biparental care whereby both sexes are responsible for performing brood defence behaviours (Mboko 1998; Mboko and Kohda 1999). Aligned directional biases in care behaviours could allow individuals to better coordinate behavioural responses, which could in turn impact offspring survival and thus, parental reproductive success. However, being more or less lateralised in the opposite direction as a result of these aligned biases, may also be advantageous for the performance of additional tasks where it is necessary to make the best use of available resources, e.g. when foraging during care (Frasnelli and Vallortigara 2018). This implies that laterality may not be a fixed feature of brain organisation, but instead is regulated by functional context.

T. temporalis individuals preferred to preferentially attack the left side of a brood predator and use the left visual hemifield to monitor potential brood predators during care but used the right eye to view the brood/nest site following an attack and the right visual hemifield to monitor the nest site/brood during care (Figure 5.2A). As fish have laterally placed eyes, stimuli viewed in the right visual hemisphere are processed with the left brain hemisphere and vice versa (Irving and Brown 2013) suggesting that *T. temporalis* partition information processing relating to predators and offspring in different brain hemispheres. These findings are consistent with previous work on the lateralisation of emotion in animals. Evidence suggests the right brain hemisphere processes negative emotion and is responsible for attack and fear responses (Andrew and Rogers 2002; Vallortigara and Rogers 2005), such as predatory threats, while the left brain hemisphere is responsible for processing positive emotions, such as control of approach behaviour, inhibition of aggression, manipulation of objects and foraging (Andrew and Rogers 2002; Vallortigara and Rogers 2005), including processing of familiar stimuli, such as offspring. However, the present study does not allow us to disentangle whether hemispheric processing is linked to emotive responses in both brain hemispheres independently or whether biases in hemispheric processing are true for a single stimuli, either predators or offspring, and biases in hemispheric processing of the remaining stimuli occur by default. Regardless of the underlying mechanism of hemispheric processing, partitioning of information processing into specific brain hemispheres is advantageous and could enable enhanced efficiency or performance in parental care behaviour. Thus, future work would benefit from exploring the costs and benefits of exhibiting laterality in parental care behaviours for caring parents using a life-history frame work that considers both parents and offspring, to enable a better understanding of how lateralisation of parental care behaviours could influence life histories and ultimately individual fitness.

T. temporalis individuals exhibited significant laterality in the visual hemisphere used to detect brood predators prior to attack at the individual level, whereby such biases may result from the positioning of individuals to preferentially monitor brood predators in a specific visual hemisphere. However, these biases were not aligned amongst caring *T. temporalis* within the population (Figure 5.2A). Alignment of directional biases in the visual hemisphere used to detect brood predators prior to attack between biparental parents at the population level could be detrimental since predators can occur in either visual hemisphere in the natural environment (Vallortigara 2006) and aligned biases amongst caring parents could be learned and exploited by potential brood predators (Hori 1993; Bisazza et al. 1998a). Thus, the costs of exhibiting aligned directional biases likely outweighs the benefits for caring parents. However, the benefits of exhibiting consistent directional biases, regardless of direction preference, at the individual level could be maintained by a population containing both left and right lateralised phenotypes and frequency dependent selection. In black-winged stilts (*Himantopus himantopus*) predatory pecks following detection with the preferred visual hemisphere were more successful than attacks following detection in the non-preferred visual hemisphere (Ventolini et al. 2005). The same is true for scale eating cichlids, *Perissodus microlepis*, attacking prey on the side corresponding to their own mouth asymmetry (Takeuchi et al. 2012). Thus, individual *T. temporalis* could exhibit an increased success in brood defence behaviour when predatory attacks follow detection in an individuals preferred visual hemisphere, but this is currently unknown.

Other parental care behaviours in *T. temporalis* were also lateralised at the individual level (L_A; Figure 5.2B), suggesting that the benefits of laterality in these behaviours must outweigh the costs for caring parents, regardless of directional preference. The selective advantages associated with having a lateralised brain for individual efficiency, namely an increased neural capacity (Denenberg 1981), hemisphere dominance (Cantalupo et al. 1995; Vallortigara 2000) and simultaneous information processing (Vallortigara 2006), could mitigate the cognitive costs of performing parental care, explaining the widespread presence of consistent directional biases observed in parental care behaviours at the individual level (Figure 5.2B). In caring male parents, the strength of laterality in parental care behaviours was reduced in the late post hatch period of care relative to the early post hatch period of care and post egg laying (Figure 5.3). This reduction could represent a change in the benefits experienced by lateralised individuals across the different stages of parental care since spawned eggs need to hatch in order to ensure offspring survival and associated reproductive success of parents, while in the late post hatch stage, when free-swimming larvae are present, the performance of care behaviours is likely reduced with increasing offspring independence. In contrast, caring females exhibited stronger laterality in parental care behaviours performed in the early post hatch period of care relative to post egg laying (Figure 5.3). Hatched larvae are more likely to reach independence than eggs, which may not be successfully fertilised and face high rates of predation (Paradis et al. 1996; Chotkowski and Marsden 1999). If exhibiting laterality in parental care behaviours is associated with improved performance, which is true for other behaviours such as foraging (Güntürkün et al. 2000), schooling (Bisazza and Dadda 2005) and predator avoidance (Dadda et al. 2010), then strong lateralisation of caring behaviours could result in enhanced offspring survival and thus, parental fitness explaining why females exhibit stronger laterality in the early post hatch period of care, once eggs have successfully hatched. Examining the costs and benefits of laterality in parental care behaviours at various stages during the care period, in species where offspring demands may differ throughout the duration of care represents an interesting avenue for future work.

We provide the first evidence of laterality in parental care behaviours in a biparental cichlid fish. *T. temporalis* exhibit consistent biases in parental care behaviours, brood defence behaviour and visual hemifield use during care, that were aligned at the population level. Such biases could have implications for both parental care performance and performance in other behavioural domains that are performed simultaneously while caring. Further research is needed to explore the influence of such biases on behavioural performance using a life history framework that considers both parents and offspring to better understand how lateral biases in parental care behaviours have evolved and why they are maintained.

Chapter 6: Flexibility in laterality of parental care behaviours in a biparental cichlid fish

Female *Telmatochromis temporalis* defending eggs at the nest site

© user: Steph McLean | Personal photo

Abstract

Behavioural laterality, the preferential use of one side of the body or bilateral trait, is widely demonstrated across animal taxa. Commonly observed as directional biases in behaviours, laterality can be exhibited at the individual or population level. While studies have demonstrated plasticity of laterality at the individual level in response to variation in selective pressures, no study has considered plasticity of aligned directional biases at the population level, which is surprising since social selective pressures are thought to be a key driver of laterality in this context. Here, we investigate plasticity of laterality in parental care behaviours in a biparental cichlid fish, *Telmatochromis temporali*s. We removed the female mate of a biparental pair to vary the social (the need to align and coordinate behavioural responses during care in biparental vs. uniparental male care) and predation pressures (shared vs. independent brood defence) experienced by the male and measured the resultant lateralisation of parental care behaviours in a uniparental context. *T. temporalis* males responded to the removal of a mate by increasing the frequency of brood defence attacks relative to biparental males. Female mate removals were also associated with flexibility in laterality of parental care behaviours at the population level. Specifically, uniparental males exhibited less of a rightward bias in the eye used to monitor a brood during care relative to biparental males. Our study suggests that in addition to other compensatory behaviours, lateralisation of parental care behaviours can vary in response to social and predation pressures in fish.

6.1 Introduction

Many vertebrates and invertebrates exhibit behavioural laterality, the differential use of one side of a bilateral characteristic or side of their body (Rogers 1989; Bisazza et al. 1998a; Niven and Bell 2018). Behavioural laterality is the expression of cerebral lateralisation, the partitioning of different cognitive functions into specific brain hemispheres, at the behavioural level and provides an indication of the degree of lateralisation within an individual’s brain (Vallortigara and Rogers 2005; Reddon et al. 2009; Gutiérrez-Ibáñez et al. 2011; Jozet-Alves et al. 2012a). Laterality is commonly observed across ecological contexts with demonstrations of visual lateralisation being the most widely documented. For example, there is evidence of asymmetries in eye use during antagonistic interactions (Hews and Worthington 2001), predator detection (Franklin and Lima 2001; Rogers and Kaplan 2006) and exploratory and migratory behaviours (Wiltschko et al. 2002; Vallortigara and Rogers 2005). However, behavioural asymmetries have also been demonstrated in other sensory inputs such as auditory processing (Basile et al. 2009), lateral line use (de perera and Braithewaite 2005) or ventral fin use (Bisazza et al. 2001a), and as ‘handedness’, the preferential use of one hand/foot/paw in the manipulation or use of tools and objects (Rogers and Workman 1993; Rogers 2009). An individual’s fitness is impacted by the degree of laterality they exhibit (Rogers 2000; Dadda et al. 2012), with lateralised individuals often found to outcompete non-lateralised individuals in a number of behaviours, including learning (Magat and Brown 2009; Bibost and Brown 2014), predator escape (Dadda et al. 2010), spatial tasks (Sovrano et al. 2005), shoaling in fish (Bisazza and Dadda 2005; Bibost and Brown 2013) and the performance of simultaneous tasks, ‘multi-tasking’ (foraging while performing predator vigilance; Dadda and Bisazza 2006a and foraging while avoiding sexual harassment from males; Dadda and Bisazza 2006b).

As lateralised individuals appear to outperform non-lateralised individuals in a range of fitness related behaviours, it has been suggested that laterality should consistently be selected for (Rogers 2000; Ghirlanda and Vallortigara 2004), yet substantial variation in this trait persists (Bisazza et al. 1997, 1998a; Vallortigara et al. 1999b). This variation, that is present among species, populations and individuals, is thought to be maintained by the prevention of selection for laterality in certain contexts resulting from the costs that are experienced by individuals exhibiting consistent directional biases (Ghirlanda and Vallortigara 2004; Vallortigara 2006; Dadda et al. 2009). For example, lateralised individuals respond differently depending on which side stimuli or cues are perceived, which is likely disadvantageous in the wild where these stimuli or cues can appear at random on either side of an individual (Vallortigara and Rogers 2005). Additionally, lateralised individuals are found to suffer a cost of reduced performance in tasks that require communication and cooperation between brain hemispheres (Dadda et al. 2009). Similar to other traits, the trade-off between, and resultant balance of, the costs and benefits associated with laterality will determine the expression of this trait in any given species or context (Broder and Angeloni 2014; Ferrari et al. 2015).

Laterality is a heritable trait (Hopinks 1999; Bisazza et al. 2000), but several studies have also highlighted a role of the environment in the expression of laterality (Vallortigara and Rogers 2005; Rogers 2006). Specifically, developmental plasticity, whereby the phenotype an individual expresses under a given genotype varies depending on rearing environment, has proved influential (Vallortigara and Rogers 2005). In pigeons (*Columba livia*; Güntürkün 1993) and goldbelly topminnows (*Gardinus falcatus*; Dadda and Bisazza 2012), light exposure during development influenced both the strength and direction of laterality, while wild caught *Brachyraphis episcopi* taken from streams that face high or low predation pressures, showed different patterns of laterality whereby individuals from environments facing strong predation pressures exhibited stronger laterality (Brown et al. 2004). Variation in exposure to hormones has also been shown to affect laterality in domestic chicks (*Gallus domesticus*; Schwarz and Rogers 1992; Deng and Rogers 2002) and in female, but not male, cichlid fish (*Aequidens rivulatus*; Schaafsma and Groothuis 2011).

Studies in goldbelly topminnows, *Gardinus falcatus*, suggest that laterality may not be as fixed as previously thought. Cantalupo et al. (1995) have shown that repeated exposure to stimulated predator attack evoked a change in the direction of laterality, while Bisazza et al. (1998b) report that restricting access to mates for two months affected the strength of laterality in females tested in a sexual context suggesting that the expression of lateralisation may be influenced by recent experience. More recently, behavioural plasticity, a change in behaviour(s) as a result of an individual’s experience or exposure to stimuli, has been considered with regard to laterality (Broder and Angeloni 2014; Ferrari et al. 2015; Chivers et al. 2016). In situations where individuals receive greater benefits as a result of expressing a particular trait in one environment or context relative to another, and individuals experience both situations, we would expect selection to favour individuals who adjust the expression of appropriate trait(s) accordingly (Ferrari et al. 2015). There is some evidence of plasticity in behavioural laterality at the individual level, which has highlighted a key role of predation pressure. In guppies (*Poecilia reticulata*), males exposed to predator chemical cues during development were more strongly lateralised than their male counterparts reared in conditions representative of low predation risk (Broder and Angeloni 2014), while in whitetail damsel fish, *Pomacentrus chrysurus*, short term exposure (four days) to predator cues representative of high and low levels of background risk, evoked stronger laterality in individuals from high background risk environments relative to their counterparts from low background risk environments (Ferrari et al. 2015). These differences were attributed to a reduction in laterality scores for individuals in low risk environments and maintenance of higher laterality scores for individuals in high risk environments. While flexibility in laterality may prove to be beneficial at the individual level, nothing is known about flexibility of aligned lateral biases at the population level.

Aligned direction biases in laterality at the population level are said to evolve when individuals need to interact with each other, and the fitness of an asymmetrical individual depends on what other asymmetrical individuals do, such as group living or during the performance of social behaviours (Rogers 2000; Ghirlanda and Vallortigara 2004). However, social conditions and their associated selection pressures are not always fixed. For example, some species are predominantly non-social solitary living species yet they will be social or perform social interactions in specific contexts. One example of this is reproduction, whereby aggressive interactions during male competition for mates, leks and the performance of biparental care represent reproductive specific social behaviours (Festa-bianchet et al. 1990; Loiselle et al. 2007; Royle et al. 2012). Thus, it is possible that aligned directional biases in laterality could provide greater benefits in certain conditions or contexts when there is a need to interact and coordinate behavioural responses, than in other conditions/contexts where the need to perform social interactions or align and coordinate behavioural responses is reduced or absent, or where the conditions experienced during the performance of these behaviours are variable.

Here, we investigate whether laterality in parental care behaviours represents a plastic trait in a biparental substrate brooding cichlid, *Telmatochromis temporalis*, experiencing different social (the need to align and coordinate behavioural responses during care) and predation (shared vs. independent brood defence) pressures. Specifically, we assessed laterality in brood defence behaviour and visual hemisphere use during parental care in two contexts: i) when care is biparental, there is a strong selective pressure to coordinate and align directional biases in care behaviours, and the predation pressure of brood defence is shared amongst two parents and ii) when care is uniparental following the loss of a mate, and there is little or no selective pressure to coordinate and align directional biases in care behaviours, and an enhanced predation pressure results from brood defence not being shared amongst two parents. We predict that in a biparental situation, aligned directional biases in parental care behaviours will result from the advantages of exhibiting such biases outweighing the costs. However, we predict that alignment of such biases may be lost in the absence of any requirement to coordinate and align behavioural responses in parental care when a biparental mate is absent and an increased predation pressure results. Furthermore, we predict that plasticity in behavioural laterality of parental care behaviours at the individual level may be affected in one of two ways: i) in line with other studies (Ferrari et al. 2015), the strength of laterality in parental care behaviours may be increased in uniparental care relative to biparental care, due to the stronger predation pressure that results from individual rather than shared brood defence, coupled with the requirement to perform tasks relating to predators and offspring simultaneously, a known benefit of laterality or alternatively, ii) the strength of laterality of parental care behaviours may instead be reduced when uniparental care is performed due to the resultant increased predation pressure when a mate is lost coupled with the need for efficiency in the performance of tasks that require communication and cooperation between brain hemispheres, a known cost of laterality.

6.2 Materials and Methods

6.2.1 Animals

T. temporalis breed monogamously following the formation of pair bonds and perform elaborate biparental care of offspring until independence (Mboko 1998) making them a model system to investigate laterality in parental care behaviours. Forty-five *T. temporalis* (25 females, 20 males) were obtained for this experiment. Fifteen *T. temporalis* (9 females and 6 males), originally sourced from Lake Tanganyika in East Africa, were purchased from Mikes Rifts (Newton-le-Willows, UK) in March 2020, 12 *T. temporalis* (8 females and 4 males) were obtained from a stock population at the University of Manchester (Manchester, UK) in October 2020, and 18 *T. temporalis* (8 females and 10 males) were offspring reared from a laboratory stock population, originally purchased from Mikes Rifts (Newton-le-Willows, UK), maintained at the University of Hull since October 2019. All experimental individuals were initially housed in three stock tanks (40 x 47 x 60cm), one for each population of fish, prior to behavioural observations.

6.2.2 Data collection

Laterality in brood defence behaviour and visual hemisphere use during parental care was assessed in biparental and uniparental male care contexts. Biparental care formed the control group using data collected for chapter 5 as a historical control, and the uniparental male care context was achieved by the removal of a biparental female mate (this chapter). Laterality of parental care was assessed at three stages: 1-3 days after laying (hereafter ‘post laying’), 1-3 days after hatching (‘early post hatch’) and 10-13 days after hatching (‘late post hatch’). Directional biases in brood defence behaviour, specifically the visual hemisphere used to detect a brood predator prior to attack, the side of a brood predator that is attacked and the visual hemisphere used to view the nest site post attack, and directional biases in visual hemisphere use during care when monitoring brood predators and a brood were assessed. Data collection and behavioural observations followed the protocols described in detail in chapter 5 (see 5.2.2 Data collection), the data from which is used here as the biparental care control group, and thus is only briefly summarised here.

To encourage pair bond formation and reproduction, a single male and female *T. temporalis* (breeding pair) were assigned to an experimental tank (40 x 47 x 60cm) containing a variety of other cichlid species (min n per tank = 11, max n per tank = 30, mean n per tank = 18) of varying sex that would be considered a predatory threat to the eggs and developing offspring when in proximity to the spawning site. All tanks were maintained at 23 ± 1°C on a 12L:12D photoperiod, fed daily with commercial feed and provided with enrichment (plastic tubing: approximately 9cm length x 4cm diameter) that could be used for shelter and/or a resource for reproduction. Due to the uneven sex ratio and a small number of mortalities (n=4), 19 pair bonded breeding pairs, each containing unique individuals, were formed. During the post laying period of parental care (1-3 days after eggs were first sighted), breeding pairs were allowed to perform biparental care, to ensure successful fertilisation and hatching of eggs. In the wild, *T. temporalis* broods fail to survive more than a few days if a paired male is removed, suggesting that male brood defence behaviour is critical to brood survival (Mboko 1998). Females also guard their brood against predation but less frequently than males, who guard an entire territory and wider radius around the nest site (Mboko 1998; Mboko and Kohda 1999). Thus, we removed the female mate to minimise the amount of compensation in care behaviours required by the remaining parent performing uniparental care in order to maximise brood survival. We performed female mate removals on the first day that hatched young were visible (mean = 3 days post laying), thus females were present and biparental care was performed in all assays during the post lay period of care. Females were visually identified (smaller body size and position relative to the nest site - in or closer to the nest site than the male parent) and removed from the breeding tank using a small dip net and placed in stock tanks (40 x 47 x 60cm) where they were individually housed to ensure identification.

Behavioural observations for males in the absence of a mate (uniparental care treatment) were performed in the early post hatch period of care, 24h after mate removal, and in the late post hatch period of care. Laterality was assessed following identical protocols to those used in chapter 5 (see 5.2.2 Data collection) except here laterality was examined in male and female parents performing biparental care in the post laying period of care and in males performing uniparental care in the early and late post hatch periods of care following removal of their female mate post egg hatching. Laterality in parental care behaviours was assessed for 17 male parents who were able to successfully defend their brood for the entirety of the experiment (up to 14 days post hatch) once their female mate was removed.

6.2.3 Data analyses

All data analyses were conducted using R version 3.6.2 (R Core Team 2019). To examine laterality in parental care behaviours, a standard relative laterality index (L_R) was calculated for each individual for each component of brood defence behaviour and for visual hemisphere use towards both brood predators and the brood independently. L_R, calculated as: [(right–left)/(right + left) x100] where right and left represent directional biases in behaviours, provides an assessment of directional biases of fish at the population level (Bisazza et al. 1997). L_R ranges from -100 to +100, representing a population exhibiting consistent leftward or rightward biases respectively. For each individual, an absolute laterality index (L_A) was calculated as $|L_R|$ to provide an assessment of the strength of laterality at the individual level. L_A ranges from 0 (individuals exhibiting an equal left and right preference) to 100 (individuals exhibiting a consistent preference for a specific direction; Bisazza et al. 1997).

(i) Assessing the validity of using a historical control: do individuals exhibit similar patterns of laterality in parental care behaviours prior to mate removal?

To ensure that data from the uniparental (this chapter) and biparental (chapter 5) contexts could be directly compared, and to examine whether chapter 5 data could be used as a historical control (biparental care treatment), general linear mixed effects models (LMMs) were fitted using the lme4 package (Bates et al. 2016) to assess whether there were differences in behaviours between these data chapters in the post-lay period of care, prior to female mate removal. Specifically, we examined whether there were differences in the rate of brood defence attacks or in the laterality of parental care behaviours (L_R and L_A independently) performed by male and female *T. temporalis*.

To assess whether the rate of attacks differed an LMM was fitted that included the number of brood defence attacks as the response variable and sex, data chapter (5 or 6) and their interaction as independent explanatory variables. To assess whether laterality of parental care behaviours performed by male and female *T. temporalis* differed, separate LMMs were used to assess the effects of data chapter, care behaviour, sex and the interaction between care behaviour and data chapter on L_R and L_A independently. For all models assumptions of normality and homoscedasticity of residuals were assessed using visual inspection of residual-fit plots and Q-Q plots. Model simplification was performed using maximum likelihood estimation, conducting stepwise removals of variables proceeding with interaction terms with the smallest t-values. Only variables that resulted in significantly larger Akaike Information Criterion (AIC) values, following their removal, were retained in the minimal model however, here we report the minimal adequate model that contains all main effects, regardless of significance.

The number of attacks performed in the post laying stage of care, when both biparental parents were present, did not differ between data chapters (chapter 5 or chapter 6), between sexes or as a result of their interaction ($p > 0.05$ in all cases; Appendix 6.2, Table S6.2.1). Similarly, L_R of parental care behaviours performed during the post lay period did not differ between data chapters or between the sexes ($p > 0.05$ in all cases; Appendix 6.2, Table

S6.2.2A). L_R did vary depending on the parental care behaviour performed ($F_{4, 504} = 8.77$, $p < 0.001$) but this variation did not differ between data chapters ($p > 0.05$; Appendix 6.2, Table S6.2.2A). Thus the attack rate during brood defence behaviour and the L_R of parental care behaviours examined in chapter 5 and chapter 6 were similar in the post lay stage of care when biparental care was performed prior to female mate removals. However, L_A was found to differ between data chapters ($F_{1, 45} = 16.34$, $p < 0.001$) and across different parental care behaviours ($F_{4, 477} = 2.90$, $p = 0.02$), but not between the sexes, or between different care behaviours across data chapters ($p > 0.05$ in all cases; Appendix 6.2, Table S6.2.2B). The overall L_A scores when all parental care behaviours were examined in combination were lower in chapter 6 than in chapter 5 during the post laying stage of care when biparental care was performed prior to female mate removals. Consequently, in our subsequent analysis of laterality (L_R and L_A) we include behaviour at the post-lay stage to control for this effect.

(ii) Do males respond to a change in social and predation pressures when a female biparental mate is removed?

To assess whether males were compensating in parental care when a female mate was removed and an increased rate of brood predation resulted, an LMM was fitted to assess the effects of mate presence, stage of care and their interaction on the number of brood attacks performed by males. For this analysis only males performing brood defence in the early and late post hatch stages of care were considered to ensure a fully cross factored design. Differences in the attack rate of males amongst levels of significant variables were examined using Tukey's honestly significant difference (HSD) test where appropriate (Lenth 2016).

(iii) Do changes in social and predation pressures affect laterality of parental care behaviours?

To examine if L_R varied in male *T. temporalis* performing biparental and uniparental care, an LMM was fitted with L_R as the response variable and parental care behaviour, mate presence, stage of care and all biologically plausible two-way interactions as explanatory variables. We incorporated a priori planned comparisons into our LMM with L_R as the response variable to test hypotheses about the L_R of parental care behaviours in the presence and absence of a biparental mate (Sokal and Rohlf 1981; Ruxton and Beauchamp 2008). Specifically, whether there were differences in the L_R of each of the parental care behaviours examined between males performing biparental care with a female mate present and males performing uniparental care following experimental removal of a female mate. The false discovery rate method was used to correct for multiple comparisons and the adjusted p-values are reported here (Benjamini and Hochberg 1995). Additionally, to examine if L_A varied in male *T. temporalis* performing biparental and uniparental care, an LMM was fitted with L_A as the response variable and parental care behaviour, mate presence, stage of care and all biologically plausible two-way interactions as explanatory variables. Differences in the L_A of males amongst levels of significant variables were examined using Tukey's honestly significant difference (HSD) test where appropriate (Lenth 2016).

6.3 Results

6.3.1 Do males respond to a change in social and predation pressures when a female biparental mate is removed?

The number of brood defence attacks performed differed between single males, paired biparental males and both biparental parents combined (male and female) but this effect was dependent on the stage of parental care (mate presence * stage interaction: $F_{4, 171} = 8.69$, $p < 0.001$). In the early post hatch period of care, the attack rate of single males was significantly greater than the attack rate of paired biparental males (t ratio = -5.20, $p < 0.001$; Figure 6.1) and biparental parents (both male and female parents combined: t ratio = -3.62, $p = 0.007$; Figure 6.1). Single males also had a greater attack rate in the late post hatch period of care, relative to paired biparental males but this difference was not significant (t ratio = -1.64, $p = 0.575$; Figure 6.1). Together, these results suggest that males respond to changes in social and predation pressures that result from the loss or absence of a biparental female mate by adjusting the rate of brood defence attacks accordingly.

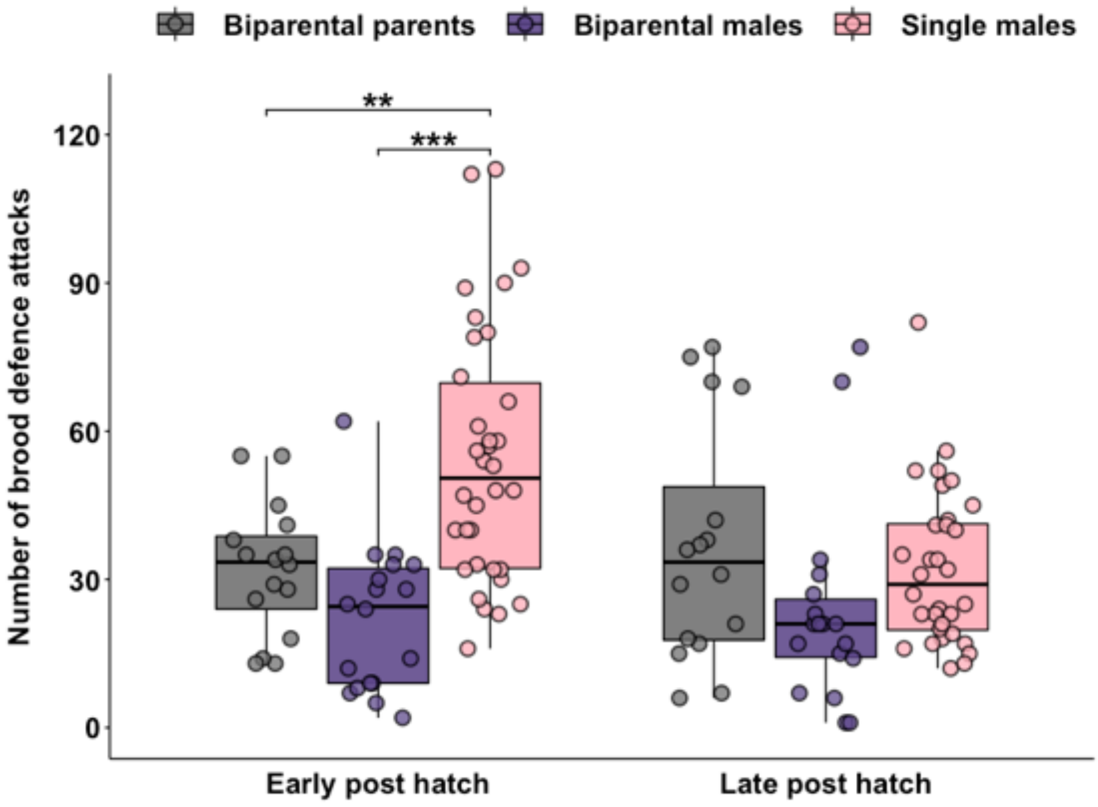


Figure 6.1: Number of brood defence attacks performed by single males (pink) following female mate removals, paired biparental males with a female mate present (purple) and by both biparental parents (male and female; grey) in the early (1-3 days post hatch) and late post hatch (10-13 days post hatch) periods of care. Asterisks indicate significant differences between groups at $p < 0.01$ ** and $p < 0.001$ ***.

6.3.2 Do changes in social and predation pressures affect laterality of parental care behaviours?

Aligned directional biases at the population level (L_R) in parental care behaviours varied with the care behaviour performed, but this effect was dependent on whether a female mate was present and biparental care was performed, or whether a female mate was absent and uniparental male care resulted (care behaviour * mate presence interaction: $F_{4, 741} = 7.28$, p < 0.001; Figure 6.2). There was a general, although not significant, trend for L_R in the majority of parental care behaviours to be reduced when a female mate was removed and males performed uniparental care relative to males performing biparental care. Additionnally, single males in the absence of their biparental female mate exhibited less of a rightward bias in the eye used to monitor the nest site containing offspring during care than paired males who performed biparental care (t ratio = -3.75, p = 0.001; Figure 6.2). Single males also showed a non-significant trend to exhibit less of a leftward bias in brood defence attacks (side of a predator that was preferentially attacked) than paired males performing biparental care (t ratio = - 2.01, p = 0.07; Figure 6.2).

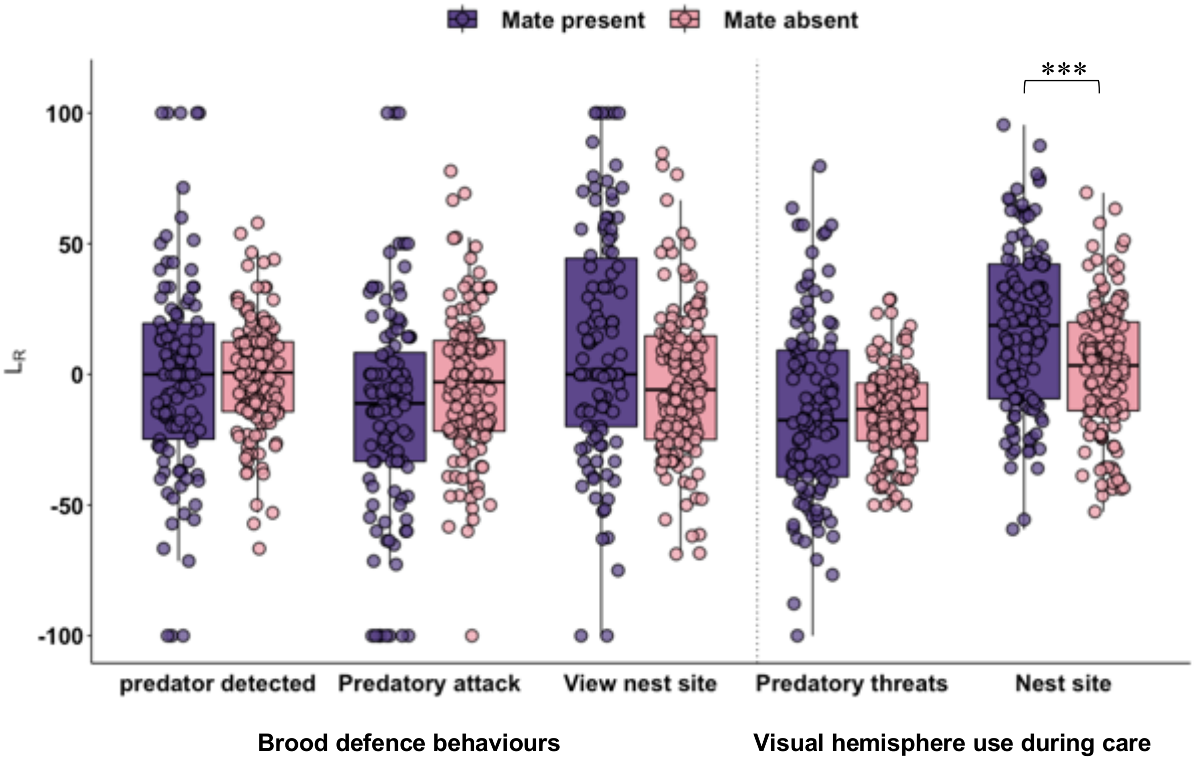


Figure 6.2: L_R of parental care behaviours (brood defence behaviours and visual hemisphere use during care) of paired male *T.temporalis* performing biparental care with a female mate

present (purple) and for single males performing uniparental care in the absence of their biparental mate following female mate removals (pink). Asterisks indicate significant differences between groups at $p \leq 0.001$ ***.

The strength of laterality in parental care behaviours of individual males varied with stage of parental care, but this effect was dependent on whether or not a biparental mate was present ($F_{2, 745} = 7.19$, $p < 0.001$; Figure 6.3). Specifically, the strength of laterality (L_A) exhibited by individuals across parental care behaviours when a female mate was present and absent differed in the post lay (t ratio = -4.66, $p < 0.001$; Figure 6.3) and early post hatch (t ratio = -5.49, $p < 0.001$; Figure 6.3) periods of care but not significantly in the late post hatch period of care (t ratio = -1.75, $p = 0.08$; Figure 6.3). L_A also differed with the care behaviour performed but this effect was dependent on the stage of care ($F_{8, 741} = 2.25$, $p = 0.02$). This effect was largely driven by differences in the L_A indices of different care behaviours varying with stage of care rather than the L_A of a single care behaviour varying across stages ($p > 0.05$ in all cases).

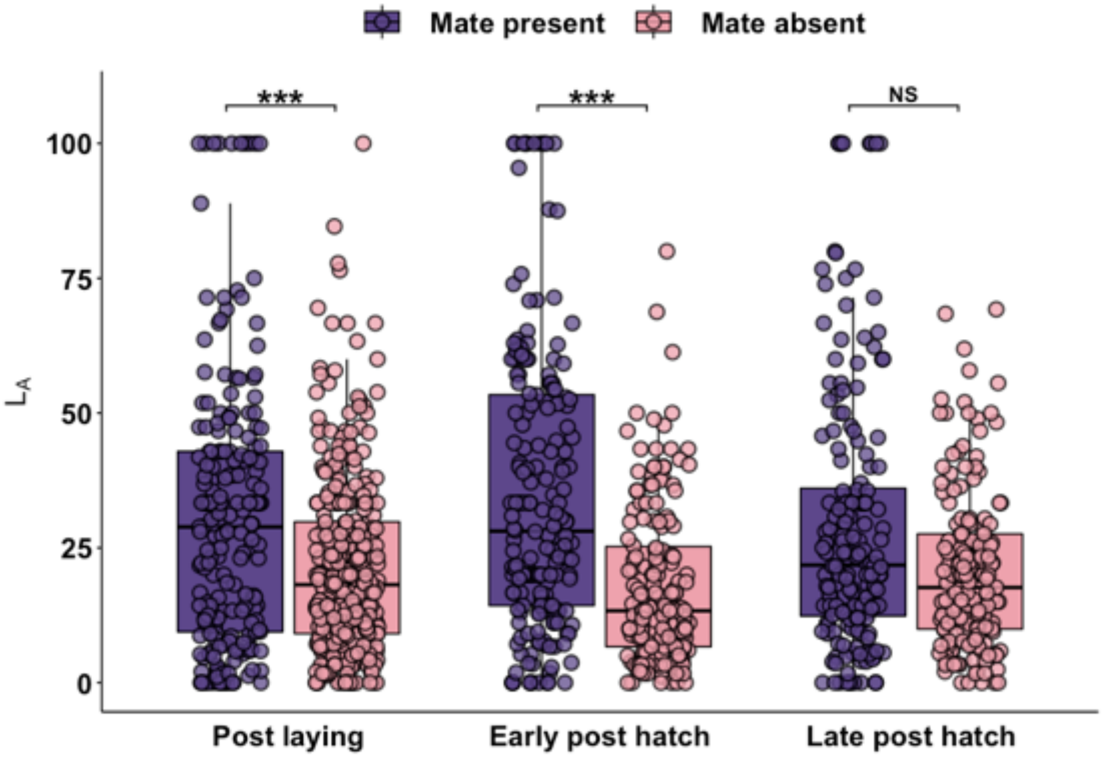


Figure 6.3: L_A for all parental care behaviours examined (brood defence behaviours and visual hemisphere use during care) in paired male *T.temporalis* performing biparental care with a female mate present (purple) and for single male *T.temporalis* performing uniparental care in the absence of their biparental mate post female mate removal (pink) in the post laying and early and late post hatch periods of care. Asterisks indicate significant differences between groups at $p < 0.001$ *** and $p > 0.05$ NS.

6.4 Discussion

Our results show that male biparental *T.temporalis* compensate for the absence or loss of a female mate by increasing the frequency of brood defence attacks during parental care in response to the increased predation pressure on the brood that results in their absence and additionally, that the absence of a biparental female mate was also associated with changes in laterality of parental care behaviours. Specifically, aligned directional biases in parental care behaviours at the population level were reduced when female parents were absent and thus, alignment of lateral biases within a population can vary in the face of changing selective pressures on short time scales. The overall strength of laterality of parental care behaviours exhibited by males at the individual level was reduced in the early and late post hatch periods of care in the absence of a female mate however, this reduction in the strength of laterality may be confounded by individuals exhibiting weaker laterality more generally in the uniparental male care treatment (this chapter) relative to biparental control treatment (chapter 5).

(i) Do males respond to a change in social selective pressures and predation pressures when a female biparental mate is removed?

In the wild, male desertion in *T. temporalis* leads to brood loss within a few days (Mboko 1998), which likely results from the greater radius of territory defended by males and associated ability to detect predators at a further distance from the brood, but the impacts of female desertion or loss in this species are unknown. Our findings show that male *T. temporalis* responded behaviourally to the absence of a biparental female mate by increasing brood defence. This is consistent with findings in other monogamous biparental substrate brooding fish (Lavery and Reebs 1994; van Breukelen and Itzkowitz 2011) and in biparental bird species (Bulla et al. 2019; Mainwaring and Hartley 2020). In this study single males performed a greater number of brood defence attacks than paired biparental males and both male and female biparental parents combined in the first three days post hatching, suggesting that males compensate extensively to maximise offspring survival (Lehtonen et al. 2011). This adjustment could result from an increase in the risk that each individual predator poses to a caring parent when brood defence is not shared with a biparental mate and is likely to carry substantial energetic costs (Bulla et al. 2019), which could have implications for individual fitness but this is currently unknown.

(ii) Do changes in social and predation pressures affect laterality of parental care behaviours?

Strength of laterality at the individual level, L_A

Previous work has reported plasticity in laterality at the individual level in response to enhanced predation pressures, whereby those experiencing stronger predation pressures exhibited stronger lateralisation (Broder and Angeloni 2014; Ferrari et al. 2015; Chivers et al. 2016), as may be the case when one parent is removed. If laterality was a plastic trait, we might expect the strength of laterality (L_A) at the individual level to increase in line with previous findings in single parents (Ferrari et al. 2015; Chivers et al. 2016), but we did not observe this. Overall L_A scores across care behaviours in the uniparental treatment (chapter 6 data) were lower than in the biparental treatment (chapter 5 data) in the post laying period of care when biparental care was performed prior to female mate removals (Figure 6.3). Thus we cannot conclude that the lower L_A in the early post hatch period of care in single males (after female mate removal) relative to paired males was due to the change in social and predation pressures as L_A remained low throughout the experiment. Several factors could account for the lower L_A scores in parental care behaviours prior to mate removals, including population differences in the strength of laterality (Brown et al. 2004; Bisazza and Brown

2011) or individual variation in personality traits associated with laterality such as boldness (Reddon and Hurd 2008, 2009c; Irving and Brown 2013; Chivers et al. 2016). Alternatively the observed reduction in L_A could also be attributed to differences in the environments of experimental tanks, specifically variation in how potential brood predators within these tanks were perceived (Bisazza et al. 1997; Sovrano et al. 2001; McLean and Morrell 2020). Further experimental work is needed to overcome these potentially confounding factors to better understand flexibility in L_A in the face of varying selective pressures.

Direction of laterality at the population level, L_R

Aligned directional biases at the population level (indicated by L_R) have been proposed to result from social pressures to coordinate and align behavioural responses (Ghirlanda and Vallortigara 2004; Vallortigara and Rogers 2005). If this is the case, then the proportion of individuals exhibiting aligned biases should increase and decrease when the costs of not doing so increases and decreases respectively (Chivers et al. 2016). Offspring fitness is directly impacted by the coordination of behavioural responses when biparental care is performed as evidenced by reduced offspring survival when one parent deserts (Keenleyside and Mackereth 1992; Balshine-Earn 1997). Thus, aligned biases may be selected for during care when the benefits of exhibiting such biases outweigh the costs. However, the balance of costs and benefits may vary if the social pressure to align behavioural responses is also variable. For example, if a single parent deserts a brood or dies in a biparental species, then the remaining parent is left to respond to the increased predation pressure that results from brood defence behaviours not been shared between biparental parents. Here, we showed that single males exhibited less of a rightward bias at the population level (L_R closer to zero) in the visual hemisphere used to monitor offspring during parental care in the absence of a mate (uniparental care) relative to when a mate was present (biparental care; Figure 6.2).

Although the strength of laterality exhibited across parental care behaviours at the individual level was generally lower in chapter 6 (the uniparental treatment) relative to chapter 5 (the biparental treatment), this is likely to have little impact on the directional preferences of the sampled population. In both chapter 5 (biparental control treatment) and chapter 6 (uniparental treatment) in the post-lay stage of care prior to female mate removal, all individuals exhibited significant preferences for a particular side, irrespective of directional preference, in each of the parental care behaviours examined (see supplemental methods S6.1; Appendix 6.2, Table S6.2.3). Thus, although L_A was reduced in the biparental treatment (chapter 6) when all parental behaviours were considered, it remained significantly different from a random expectation of no laterality (i.e. individuals continued to exhibit a preferred directional bias despite that preference being weaker). Additionally, the strength of laterality exhibited by males did not differ with the parental care behaviour performed in the presence and absence of a biparental mate suggesting that although L_A was reduced overall when all parental care behaviours were considered, there were no significant differences when each care behaviour was considered independently in paired (mate present) and single males (mate absent).

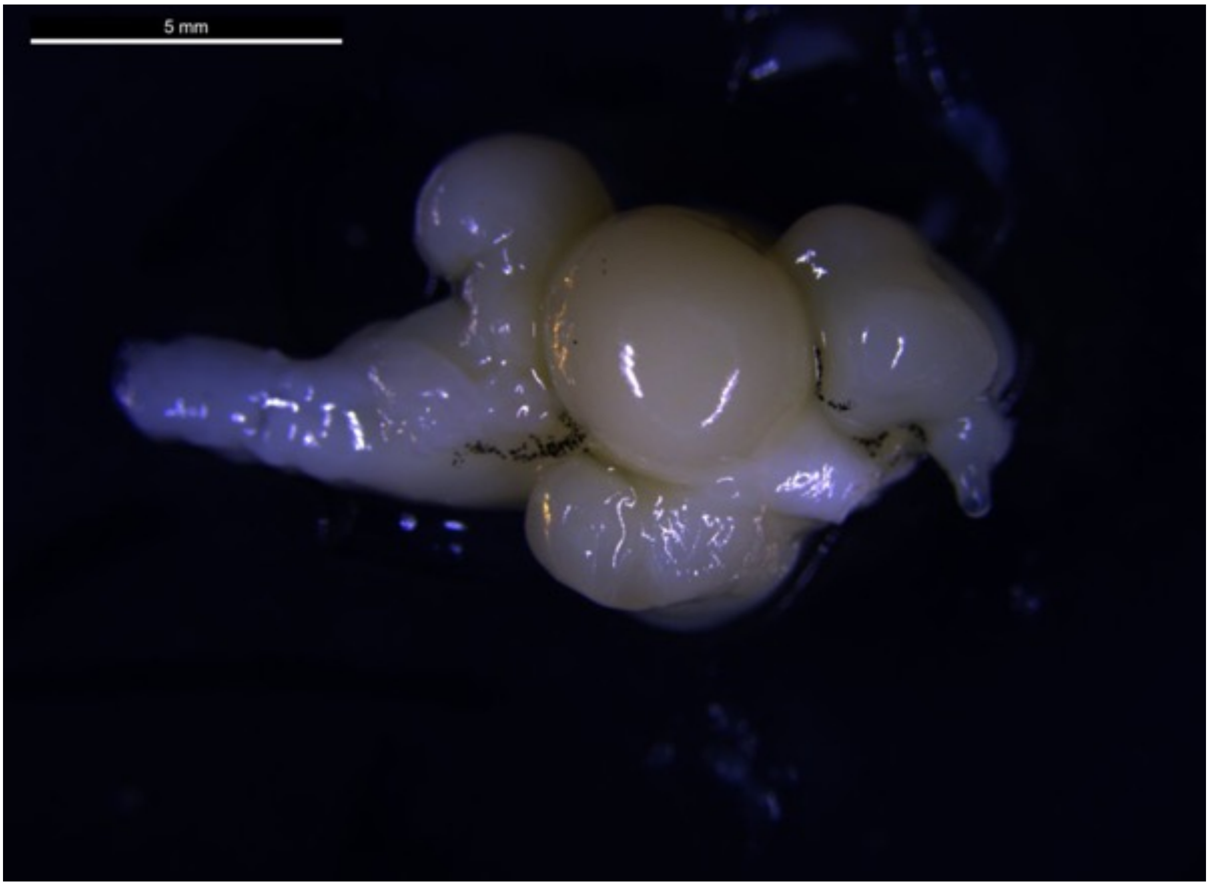
In a uniparental context, the costs of exhibiting aligned directional biases in care behaviours may outweigh the benefits, particularly if these biases make individuals more vulnerable as a result of increased predictably in behaviour (Ghirland and Vallortigara 2004; Vallortigara 2006; Manns 2021). Thus, exhibiting flexibility in the expression of laterality could have implications for individual fitness, especially in environments/contexts where the selective pressures that influence the expression of laterality are variable. Previous work has shown plasticity of laterality as a result of the selective pressures experienced, which are unlikely to stem from a change in cerebral organisation per se. Ferrari et al. (2015) report that 4 days exposure to predator cues evoked stronger lateralisation in fish from environments with high levels of background risk relative to those from low background risk levels, while Chivers et al. (2016) have shown that shoaling fish exposed to elevated predation pressures increased their lateralisation and individuals who aligned their biases with the rest of the shoal were found to have improved escape responses compared to individuals at odds with the shoal.

A reduction in the L_R of parental care behaviours could also result from a reduction in the quality of care provided by a single parent in the absence of a biparental mate. Lehtonen et al. (2011) found that females in the absence of a biparental mate performed extensive compensation in the *quantity* of care (frequency of brood defence attacks) but at the cost of a reduction in the *quality* of care. Exhibiting laterality in parental care behaviours at the population or individual level could enhance performance in care behaviours and thus the quality of care provided, which could in turn impact offspring survival and ultimately fitness. Laterality has been shown to impact performance in a number of fitness related behaviours (Bisazza and Dadda 2005; Magat and Brown 2009; Bibost and Brown 2014; Chivers et al. 2016), including predatory escape performance (Dadda et al. 2010), but whether this extends to parental care behaviour is currently unknown.

We found no differences in the alignment of lateral biases at the population level (L_R) in parental care behaviours related to predators, i.e. L_R did not vary for the eye used to detect a predator prior to attack, the side of a predator that was preferentially attacked and the eye used to monitor potential brood predators during care in males performing uniparental and biparental care (Figure 6.2). This may result from social pressures to align behavioural responses still being evident in a reproductive context when a biparental mate is absent such as during antagonistic interactions (Kuwamura 1992; Mboko and Kohda 1995). In species where individuals have to defend their young from potential brood predators, defend their territories against conspecifics and/or for individuals who face high predation pressures and must defend themselves against predatory threats while also performing parental care, the benefits of exhibiting aligned directional biases in behaviours that are associated with such aggressive or antagonistic interactions may outweigh the costs. The relationship between the expression of laterality, and the underlying selective pressures and associated costs and benefits that are driving the expression of this trait is likely complex. Furthermore, interplay between different selection pressures and the associated costs and benefits in varying environments/contexts may be important in this regard (Ferrari et al. 2015).

We found evidence that *T. temporalis* males compensate for the absence of their biparental mate by increasing the frequency of brood defence attacks, and that the change in social and predation pressures that result from the loss of a partner are associated with flexibility in laterality of parental care behaviours. This flexibility could result from differences in the balance of costs and benefits of being lateralised that are experienced by individuals in specific behavioural contexts, but these costs and benefits are currently unknown. Experiments examining laterality of parental care behaviours, that identify the associated costs and benefits of exhibiting such biases for both sexes in a species where the social and predation pressures associated with care are highly variable, such as in species where biparental, male-only and female-only care all exist within in a single population (Balshine-Earn 1997), would be beneficial in this regard.

Chapter 7: General Discussion



Lateral view of a 1 year old male *Dimidiochromis compressiceps* brain

© user: Tiffany Armstrong | Personal photo

This thesis aimed to investigate the relationship between cerebral lateralisation, reproduction and the performance of reproductive behaviours, particularly parental care. In the first half of this thesis (chapters 2-4), I investigated whether variation in laterality, predominantly between the sexes, was linked to reproduction and/or the performance of reproductive behaviours. I also explored laterality and its associated costs and benefits in different reproductive contexts: in biparental individuals when reproductive and performing parental care and in a non-reproductive non-social state. In the latter half of this thesis (chapters 5 and 6), I explored whether parental care behaviours were lateralised, an area of research that has received very little attention outside of mammalian taxa, and whether laterality of parental care behaviours can vary under different selection pressures thought to drive such biases. This final chapter summarises the key findings of this thesis in a broader context and discusses the importance of examining the relationship between laterality, reproduction and the performance of reproductive behaviours with regard to our understanding of how variation in laterality arises and why it may be maintained.

7.1 The detour test as a methodology to examine cerebral lateralisation at the behavioural level

For over 20 years, the detour test has been widely used to measure turning biases around a barrier, which partially obscures a visual stimulus, as a proxy for preferences in the eye used to view the stimulus and thus hemispheric processing in several animal taxa including fish (Bisazza et al. 1997; 1998a, 1998b; Reddon and Hurd 2008, 2009a), birds (Vallortigara et al.

1999a), reptiles (Csermely et al. 2010) and molluscs (Domenici et al. 2017). In line with other studies in fish (Bisazza et al. 1998a), and consistent with findings in Poeciliid fish more specifically (Bisazza et al. 1997; 1998b), I demonstrate that both male and female guppies exhibit consistent directional biases at the individual level across three behavioural contexts examined using a detour test (chapter 2). While population level biases in the detour test with an empty environment and a novel object as a stimulus have not been reported for guppies, some studies have reported population level laterality when an individual or group of opposite sex conspecifics is viewed, while others studies report no such relationship (Bisazza et al. 1997, 1998b). In chapter 2, I found no evidence of aligned directional biases in laterality in any of the three behavioural contexts examined and propose that the absence of any biases may result from assessing laterality in a novel experimental tank or alternatively as a result of differences in the way visual stimuli are perceived as a result of sexual motivation (Bisazza et al. 1997, 1998b; Kaarthigeyan and Dharmaretnam 2005), rather than failure to detect such biases since an adequate sample size with an equal sex distribution was assessed.

A recent study has questioned the reliability of the detour test: Roche et al. (2020) report that laterality measured using the detour test was variable within individuals under repeated testing in four species of fish. However, recent studies have shown contrasting results that suggest lateral biases are consistent under repeated testing in various fish species including guppies (*Poecilia reticulata*; Mclean and Morrell 2020, chapter 2) and mosquitofish (*Gambusia holbrooki*; Vinogradov et al. 2021). One factor that could account for the discrepancies in the findings between the afore mentioned studies and those of Roche et al (2020) is a failure to consider sex, which is surprising given that sex differences in the repeatability of several behavioural traits are widely documented (Bell et al. 2009), as are sex differences in the strength of cerebral lateralisation in fish (Bisazza et al. 1998b; Reddon and Hurd 2008, 2009; Irving and Brown 2013; Byrnes et al. 2016).

While I am confident that the detour test represents a sound methodology to examine cerebral lateralisation at the behavioural level in fish, there is substantial variation in the available literature in relation to the experimental design and protocol used to examine laterality using this methodology. This includes the design of (Irving and Brown 2013; Vila Pouca et al. 2018, and acclimation period in (Bisazza et al. 2001b; Sundin and Jutfelt 2018), the detour test arena, the duration of time between consecutive detours (Roche et al. 2013; Sundin and Jutfelt 2018) and the method of encouragement used if individuals do not independently approach the runway (Vossen et al. 2016; Sundin and Jutfelt 2016; McLean and Morrell 2020). Some studies have also suggested that the direction and strength of lateralisation are context specific (Bisazza et al. 1997; Sovrano 2004). To ensure consistency in this thesis, I used a standardised arena and experimental protocol when assessing cerebral lateralisation at the behavioural level using a detour test, as described in the relevant chapters. The only discrepancy between experiments was the size of the detour tank and associated components (barrier detoured around and dip nets), to ensure they were appropriate in size for the species being assessed. Careful consideration was also taken to ensure the context used in each experiment was appropriate to address the outlined research question(s), which is a consideration that should remain at the forefront of future research in this field.

While measures of footedness/pawedness/appendage use, equivalent to handedness in humans, are standard measures of laterality in mammalian and insect taxa (Warren 1980;

MacNeilage 2014; Frasnelli et al 2012; Niven and Frasnelli 2018), very few alternatives to the detour test exist for measuring laterality in fish, and none are used with any regularity (Sovrano et al. 2001; Dadda et al. 2012). While Roche et al (2020) critiqued the methodology, and suggested that alternatives to the detour test are needed, no suggestions were provided by the authors. A standardised and validated methodology of the detour test to assess behavioural laterality, namely using a consistent experimental design and protocol, needs to be established and widely implemented to enhance confidence in drawing sound conclusions and making accurate and reliable comparisons in the wider literature more generally.

7.2 Variation in laterality as a function of sex, reproduction and parental care

In chapters 2, 3 and 4, I investigated sex differences in laterality and the potential for any differences between the sexes to be linked to reproduction and/or the performance of reproductive behaviours. I also examined the potential costs and benefits that lateralised individuals may experience in different reproductive states (chapter 4). Variation in laterality is well documented throughout the animal kingdom, both within and between species and populations (Wiper et al. 2017; Bisazza et al. 1997; Takeuchi and Hori. 2008), with sex representing an increasingly recognised but understudied source of variation in laterality (Alonso et al. 1991; Andrew and Brennan 1984; Reddon and Hurd 2008; Irving and Brown 2013; Ward et al. 1990; Wells 2003; Reddon and Hurd 2008, 2009a). Despite substantial variation between the sexes in the performance of social behaviours (Shepard et al. 2009; Archard and Braithwaite 2011; Kulik et al. 2015; Lucon-Xiccato et al. 2016b), the influence of social behaviours on laterality has only recently been considered, since it was proposed that situations where asymmetrical organisms must coordinate their behaviour with that of other asymmetrical organisms, e.g. during social interactions or while living groups, represents a selection pressure that could favour the evolution of aligned directional biases in laterality at the population level (Ghirlanda and Vallortigara 2004; Vallortigara 2006). Investigations of laterality in social contexts have considered group living (Chivers et al. 2016), aggressive interactions (Bisazza and de Santi 2003; Benelli et al. 2015), social interactions (Fuss et al. 2019) and more recently reproduction and reproductive social behaviours (Templeton et al. 2012; Forsatkar et al. 2015; Romano et al. 2016; Benelli et al. 2017a, 2017b; Keener et al. 2018; Zoidis and Lomac-MacNair 2017; Schnell et al. 2019). However, most studies have largely considered whether reproductive behaviours are lateralised, with demonstrations often being restricted to a single sex due to sex-specific roles in reproductive behaviours (see Table 1.1 for an overview and references within).

In chapter 2 (Mclean and Morrell 2020), I investigated whether there were sex differences in the pattern (direction, strength and consistency) of laterality exhibited across three behavioural contexts in a female live-bearing species with a clear division of roles in reproduction and parental care, *Poecilia reticulata*. While I found no differences between the sexes in the direction or strength of laterality exhibited in three different behavioural contexts, I found that the strength of laterality exhibited by males across behavioural contexts was more consistent than in females, while there were no differences in the consistency of directional biases between the sexes. I propose that the observed sex-difference is driven by variation in additional factors associated with reproduction. Two factors that could be influential in this regard are laterality as the subject of sexual selection and variation in sexual motivation but further work is needed to fully explore these potential mechanisms. Two additional experiments could provide fruitful in this regard: i) exploring whether laterality is a sexually selected trait and ii) assessing the influence of gestation and reproductive state on laterality.

Guppies are a model species to explore whether lateralisation is a sexually selected trait, since laterality can be easily observed at the behavioural level and females perform mate choice, which is strongly associated with male colouration, specifically carotenoid (orange) pigmentation (Brooks and Endler 2001; Houde 2019). Future studies could therefore benefit from considering associations between laterality and sexual colouration in this species. Secondly, studies considering the influence of gestation and related sexual motivation on lateral biases are needed. Sexual motivation varies with reproductive state (Magurrran and Seghers 1994a; Macbeth and Luine 2010) and studies have shown that sexual motivation influences lateral biases (Bisazza et al. 1997, 1998b). An interesting experiment would be to investigate lateralisation in female live-bearers at different stages of gestation. Studies in this regard would need to consider the behavioural context in which laterality was examined since gestation could influence how individuals respond. For example, studies have shown that live-bearing is associated with enhanced cognition in spatial learning, which is important for foraging success and offspring survival (Magurran and Seghers 1994b; Pawluski et al. 2006), hence it is possible that females could respond differently in contexts related to spatial learning, such as exploratory behaviours or with novel objects, compared to sexual contexts with behaviours related to mating opportunities as a result of their reproductive state and associated sexual motivation.

In Chapter 3 (McLean and Morrell 2021) I further explored variation in laterality by investigating whether there were sex differences in laterality in a species with uniparental male care, and assessing whether any variation was linked to reproduction and the performance of reproductive behaviours. I provide further evidence of sex-specific variation in laterality but provide the first evidence that this variation is linked to reproduction and parental care. In threespine sticklebacks, a species with uniparental male care and greater cognitive costs of reproduction in the caring (male) than non-caring (female) sex (Bell and Foster 1994), males were more strongly lateralised than females but only when individuals were in a reproductive state during the breeding season. I propose that laterality could be selected for in the caring sex, due to the selective advantages of laterality being beneficial for caring parents (Vallortigara and Rogers 2005; Vallortigara 2006), specifically an enhanced ability to perform simultaneous information processing or ‘multi-tasking’. Simultaneous information processing would likely benefit caring parents who must perform parental care behaviours and other behaviours critical for survival simultaneously, which could in turn help to reduce the cognitive demand associated with care. There is evidence that lateralised individuals exhibit an enhanced ability to multi-task, relative to their non-lateralised counterparts, when performing ecological behaviours (foraging) and sexual/reproductive behaviours (avoiding unsolicited male mating attempts) simultaneously (Dadda and Bisazza 2006b). However, further work is needed to determine the ability of lateralised caring parents to perform an additional task simultaneously while performing caring behaviours. Studies investigating visual hemisphere biases at the behavioural level and resultant hemispheric processing in the performance of behaviours, particularly predator avoidance or foraging behaviours, which are critical for parental survival, could prove fruitful in this regard.

It is possible that other advantages of cerebral lateralisation, either independently or in combination, could drive variation in laterality between the caring and non-caring sex (Chapter 3). Hemisphere dominance decreases the initiation of conflicting responses in different brain hemispheres when stimuli are visualised with different eyes (Andrew 1991; Vallortigara 2000, 2006). There is evidence of hemisphere dominance, specifically using the left eye/right brain hemisphere when viewing/processing familiar stimuli, in chicks and fish (Andrew 1991; Miklosi et al. 1997) and right eye/left brain hemisphere when viewing/processing stimuli relating to negative or unfamiliar stimuli in fish (Bisazza et al. 1997, 1998). Thus it is possible that caring parents could benefit from processing information relating to offspring and predators in different brain hemispheres as a result of hemisphere dominance, which could ultimately improve efficiency in producing appropriate responses to positive/familiar and negative/unfamiliar stimuli during care. Indeed in chapter 5 and 6, biparental *Telmatochromis temporalis* exhibited different hemisphere biases during care in the performance of tasks related to predators and offspring.

Alternatively, cerebral lateralisation is also associated with an enhanced neural capacity that is thought to result from having one of two possible brain hemispheres available to perform additional functions, reducing the need to repeat the same function in both hemispheres and the expensive neural tissue and circuitry associated with doing so (Levy 1977; Rogers 2002; Vallortigara 2006). This increased neural capacity has resulted in lateralised individuals exhibiting an enhanced performance in a number of behaviours including cognitive performance (learning; Bibost and Brown 2014 and numerical discrimination; Dadda et al. 2015) and spatial tasks (Prior et al. 2004; Sovrano et al. 2005). Thus, cerebral lateralisation could allow caring parents an enhanced performance in spatial tasks resulting from an enhanced neural capacity, which could be particularly advantageous for species who need to navigate a nest or breeding site from landmarks, including threespine sticklebacks (chapter 3) and *Telmatochromis temporalis* (chapters 5 and 6) or locate food sources to feed young.

In Chapter 3, I also report the first evidence that reproduction and the performance of reproductive behaviours drive variation in laterality within a single sex (McLean and Morrell 2021). Specifically, males with experience of courting and parenting were more strongly lateralised than males absent in this experience and although males were lateralised during reproduction while exhibiting nuptial colouration, laterality was reduced outside of the breeding season when individuals were in a non-reproductive state. Gene regulation and hormone expression have been shown to vary substantially throughout reproduction, with some patterns of gene regulation and hormone expression being unique to specific reproductive stages or associated with the performance of specific behaviours (Mayer and Borg 1995; Pàll et al. 2002; Garland et al. 2016; Bukhari et al. 2019). Furthermore, the expression of laterality has been associated with both hormone (Schaafsma and Groothuis 2011) and gene expression (Lee et al. 2017) in fish. I propose that differences in hormone levels and gene expression patterns in different contexts when specific behaviours are performed are likely the underlying causal mechanisms for the expression of, and observed variation in, laterality during reproduction but this area of research requires further investigation. Although the performance of reproductive behaviours is linked to stronger lateralisation, chapter 3 was unable to disentangle the effects of mating and parenting on cerebral lateralisation. Studies comparing laterality in virgin males vs. males with courting experience, males with courting experience vs. males with courting and mating experience, and males with courting and mating experience vs. males with courting, mating and parenting experience, would be beneficial in identifying whether the performance of courtship, mating or parenting behaviours independently or conjointly are the underlying drivers of this variation.

Stronger laterality in a reproductive state or while individuals are performing care as observed in chapter 3 could be linked to lateralisation of reproductive behaviours. Asymmetry in the male gonopodium is correlated with sidedness of mating preference and the number of neuromasts in the lateral line in a live-bearing fish, *Jenynsia lineata* (Torres-Dowdall et al. 2020), suggesting that lateralisation of functionally linked traits may be integrated. In chapter 3, lateral biases when viewing a shelter/habitat in the detour test could be functionally linked with other reproductive behaviours, especially in male threespine sticklebacks (caring sex), who defend territories with nest sites from conspecifics (Tinbergen 1952). It is possible that behaviours relating to courtship such as displays, mating behaviours including approaching a mate, or parental care behaviours including visual hemisphere biases in monitoring and defending offspring, are also lateralised in this species. Chapters 5 and 6 of this thesis provide evidence that brood defence and visual hemisphere use during parental care are lateralised in a biparental fish species, *T. temporalis*, but whether such asymmetries are synchronised with asymmetries in other related behaviours is currently unknown.

Chapter 4 explored the relationship between laterality, its associated costs and benefits and reproduction and parental care in a biparental cichlid fish, *T. temporalis*. Specifically, laterality in exploratory behaviour and performance in two tasks previously used to assess the costs and benefits of laterality respectively: a group choice task when conspecific groups are seen in different visual hemispheres simultaneously (Dadda et al. 2009) and foraging ability in the presence (dual task) and absence (single task control) of a predator (Dadda and Bisazza 2006a). These behaviours were assessed while individuals were reproductive and actively performing parental care in the first seven days after egg hatching and in a non-reproductive state. While *T. temporalis* individuals exhibited laterality in both a reproductive and non-reproductive state, there was no variation between the sexes. Additionally, there was no evidence that lateralised individuals were better able to multi-task, an identified benefit of cerebral lateralisation, irrespective of reproductive state but lateralised individuals were quicker to undertake the task, which involved entering an area that required multiple stimuli to be processed. The increase in emergence time exhibited by lateralised individuals could be attributed to another benefit of laterality, an enhanced ability to cope with divided attention, resulting from the partitioning of information relating to foraging and predators in different brain hemispheres, allowing separate and parallel processing, which increases the overall efficiency of information processing (Rogers 2000, 2002; Vallortigara and Rogers 2005).

Lateralised individuals also suffered a cost in the task that required communication and cooperation between brain hemispheres (group choice task). Strong inherent side biases meant that lateralised individuals were more likely to choose the stimulus group presented in their ‘preferred’ visual hemisphere when stimulus groups were presented in opposing visual hemispheres in two trials. This cost will likely have damaging implications in behavioural contexts when individuals have to make decisions based on a quick assessment of the surrounding environment or where stimuli are seen in different visual hemispheres simultaneously (Dadda et al. 2009). This could be especially detrimental in behaviours that are tightly associated with fitness. For example, individuals performing brood defence behaviours during parental care may exhibit a reduced efficiency in recognising the greatest predatory threat to their brood when brood predators are presented in different visual hemispheres simultaneously as a result of strong inherent side biases, which could have damaging implications for offspring survival and individual fitness.

Chapters 2-4 suggest that reproduction and the performance of reproductive behaviours, specifically parental care, are key but previously unidentified drivers of variation in laterality both between the sexes and within a single sex. In chapter 3 I found that sex differences in laterality were associated with reproduction, while in chapter 2 and 4 I find evidence of variation between the sexes in the patterns of laterality exhibited in a female live-bearing species, but no variation in laterality between the sexes in a biparental species respectively. In chapter 3 I also found that variation within a single sex was linked to the performance of reproductive behaviours. In Chapter 2 males and females exhibited different patterns of laterality, while in chapter 3 males and females exhibited different patterns of laterality in different reproductive states. Had only a single sex or individuals in a non-reproductive state been considered then this variation could have been masked. Thus, chapters 2-4 highlight the importance of considering sex, the reproductive biology of the species in question and the context in which laterality is examined when assessing cerebral lateralisation, since these three factors may interact to influence the resultant pattern of laterality. Investigations of laterality are largely dominated by single sex studies, or studies where differences between sexes have not been examined (Bianki and Filippova 2003; Reddon and Hurd 2008), despite several studies highlighting the importance of examining sex differences in laterality (Camp et al. 1984; Bisazza et al. 1998b; Reddon 2008, 2009a).

As a result, the true extent of sex-specific variation in laterality in the literature is likely largely underrepresented (Reddon and Hurd 2008).

The observed variation in laterality, between the sexes and within a sex, associated with reproduction and the performance of reproductive behaviours suggests that the costs and benefits of laterality that individuals experience are likely highly variable in different contexts or when different behaviours are performed (Chivers et al. 2017; Corballis 2006, 2008). A trade-off in the costs and benefits that individuals experience in unique contexts, environmental conditions or when performing specific behaviours are likely responsible for the resultant patterns of, and observed variation in, laterality (Broder and Angeloni 2014; Ferrari et al. 2015; Wiper 2017) but currently these costs and benefits within a reproductive context are unknown. As a result, how laterality will influence performance in reproduction and reproductive behaviours is also poorly understood. Identifying patterns of laterality in different behavioural contexts, the costs and benefits experienced within each context and how the resultant variation could impact performance in such behaviours is essential to i) understand the driving forces underlying variation in laterality and ii) provide explanations for why the observed variation is maintained, i.e. what is the selective advantage. In order to achieve this a comprehensive approach is required with the costs and benefits of laterality being considered in various contexts, for a range of fitness related behaviours in a variety of animal taxa (Chivers et al. 2017). Reproductive behaviours, which chapters 2-4 identify as a key but previously unexplored source of variation in laterality are of specific interest since there is recent evidence that lateralisation in courtship leads to higher mating success in the red flour beetle, *Tribolium castaneum* (Boukouvala et al. 2019), the rice weevil, *Sitophilus oryzae* and the confused flour beetle, *Tribolium confusum* (Benelli et al. 2017a). Implications of laterality in a reproductive context should be considered using a life-history framework since such biases could impact both parental and offspring fitness. One reproductive behaviour that is critical for offspring survival in several species and often highly variable between the sexes is parental care, which chapter 3 highlighted, along with courting and mating, as a key driver of variation in laterality. However to date, little is known about lateralisation of parental care behaviours with the available evidence limited to mother-infant interactions in mammals (Karenina et al. 2017).

7.3 Laterality in a fitness related reproductive behaviour: parental care

While there is evidence of laterality in some reproductive behaviours, including courtship (Hews et al. 2004; Ventolini et al. 2005; Templeton et al. 2012), aggression (Forsatkar et al 2015), visual inspection of mates (Templeton et al. 2012; Schnell et al. 2019) and approach behaviour (Benelli et al. 2017a; Keener et al. 2018; Schnell et al. 2019), demonstrations in parental care behaviours have been limited to mother-offspring interactions in a range of mammal species (reviewed in Karenina et al. 2017). In chapter 5, I provide the first evidence that parental care behaviours are lateralised at both the individual and population level in a biparental fish species, in both male and female parents. In chapter 6, I provide further evidence that laterality may be a plastic trait, whereby individuals exhibited flexibility in lateral biases in parental care behaviours within a single reproductive event when faced with varying social and predation pressures. Population level lateralisation is thought to arise when the fitness of one asymmetric individual depends on what other asymmetrical individuals do, such as in group living situations or when social interactions are performed (Rogers 2000;

Ghirlanda and Vallortigara 2004). There is some evidence to support this theory from examinations of group living (Chivers et al. 2016), comparisons of laterality in shoaling and non-shoaling species (Bisazza et al. 2010) and social interactions in a social species (Reddon and Balshine 2010), but this thesis provides the first evidence of aligned lateral biases in social behaviours within a reproductive context. Such biases in parental care behaviours must provide a selective advantage for caring parents, but this advantage is currently unknown. An appropriate next step would be to examine whether lateral biases in parental care behaviours improves behavioural performance of the parents during care, which in turn could influence offspring fitness. An interesting experiment would be to compare the success of brood defence attacks with regard to offspring survival using selected lines bred for varying degrees of, and directional biases within, laterality.

In chapter 4, biparental *T. temporalis* individuals exhibit aligned directional biases in exploratory behaviour, while in chapter 5 the same species exhibited aligned directional biases in parental care behaviours. There is evidence of morphological asymmetries being associated or synchronised with laterality in the behaviours with which they are functionally linked (Heuts 1999; Matsui et al. 2013; Řežucha and Reichard 2015), but whether this extends to behaviours that are functionally linked within a given context, such as during reproduction or foraging is unknown. If behaviours that are functionally linked are lateralised, opposing biases may be selected for allowing for processing of familiar/positive and unfamiliar/negative stimuli within a given context in opposing brain hemispheres. Current evidence of lateralisation of emotion in animals suggests that unfamiliar/negative and familiar/positive emotions are processed in the right and left brain hemispheres respectively (Andrew and Rogers 2002; Vallortigara and Rogers 2005), which is consistent with right brain hemisphere processing during exploratory behaviour (chapter 4) and left brain processing of parental care behaviours related to offspring (chapter 5) reported in this thesis.

Lateral biases in behaviours may also be linked to personality-like behavioural traits but the current evidence is equivocal. Personality traits, consistent individual differences across time and/or contexts are widely studied across animal taxa, including a range of fish species (Conrad et al. 2011). These traits have a variety of ecological and evolutionary consequences since they influence the behaviours and cognitive abilities of an individual (Reddon and Hurd 2009c; Carere and Locurto 2011), which contribute to an individual's fitness (Smith and Blumstein 2008; Moiron et al. 2019). Propensity to take risks (boldness), exploration, aggression, activity and sociability represent the most widely studied personality traits in fish (Conrad et al. 2011), with assessments of boldness being the most prominent (Budaev and Brown 2011; Brown et al. 2007a). Boldness is strongly linked with fear responses, which are lateralised in vertebrate taxa (Brown and Bibost 2014). Thus, it is possible that boldness may be a lateralised trait or may influence the expression of laterality in individuals. We report that lateralised individuals were quicker to emerge from a holding area into a novel environment that contained multiple stimuli (Chapter 4), a common examination of boldness in fish (Budaev and Brown 2011). While we propose that lateralised individuals emerged more quickly due to an enhanced ability to process multiple stimuli more efficiently than non-lateralised individuals, it is possible that the increased willingness of lateralised individuals to enter a novel environment resulted from a relationship between individual differences in boldness and laterality, whereby lateralised individuals were also the boldest. This is consistent with findings in other fish species where boldness has been linked to stronger laterality when exploring a novel environment (*Archocentrus nigrofasciatus*: Reddon and

Hurd 2009c) and in contexts where predation pressures are enhanced (*Brachyraphis episcopi*: Brown et al. 2005, 2007a).

Other personality traits such as aggression and sociability could be particularly important with regard to fitness related reproductive behaviours since personality characteristics have been shown to influence parental behaviour (Budaev et al. 1999). Sex roles in parental behaviours are often specific but can be variable (Itzkowitz et al. 2005). If personality is linked with individual differences in behaviour then it is possible that personality traits that are tightly associated with reproduction, such as aggression, could select for different patterns of cerebral lateralisation particularly between the sexes. In convict cichlids, *Archocentrus nigrofasciatus*, aggression has been linked with stronger laterality in males (Reddon and Hurd 2008), but whether this resulted from sex specific roles during parental behaviours is unknown. More recently, research methodologies have shifted focus from considering a single isolated personality trait to considering a suite of traits in a cohesive manner known as behavioural syndromes, correlations between behaviours that are consistent across time and contexts (Sih et al. 2004; Moretz et al. 2007; Gabriel and Black 2010). In some fish species, correlations between boldness, aggression and sociability have been reported (*Poecilia reticulata*; Irving and Brown 2013), but studies considering behavioural syndromes and laterality in the context of fitness related behaviours, specifically reproduction, are needed to determine whether animal personalities play any role in the maintenance of variability in lateralised traits.

Evidence of changes in the patterns of laterality expressed within a single reproductive cycle both by a single sex (chapter 3) or by individuals experiencing varying selective pressures (chapter 6) suggest that laterality may represent a plastic trait and thus be more flexible than previously thought. This is in line with recent evidence of changes in laterality under different selective pressures on short time frames (Broder and Angeloni 2014; Ferrari et al. 2015; Chivers et al. 2016). Plasticity in behavioural laterality in different contexts in the face of changing environmental cues or selection pressures, could allow individuals to balance the costs and benefits experienced from exhibiting consistent directional biases either as individuals or from aligning biases with the wider population (Ferrari et al. 2015; Chivers et al. 2016), which could in turn allow individuals to maximise their fitness, particularly during key life events or stages such as during reproduction.

Flexibility in laterality on short time frames is unlikely to represent changes in cerebral organisation per say; instead these biases likely represent variation in the costs and benefits of exhibiting laterality within a given context and changes in the hormone and gene pathways that are likely responsible for the expression of laterality at the behavioural level (Schaafsma and Groothuis 2011; Broder and Angeloni 2014; Lee et al. 2017). Additionally, this flexibility could explain why laterality may not be repeatable within a single context. Roche et al (2020) report that behavioural laterality was not repeatable when individuals were repeatedly tested within a given context however, this study failed to consider three key factors identified in this thesis that could be influential with regard to the plasticity of laterality: sex, reproductive state or stage and the selective pressures experienced by individuals. Additional studies are needed that examine the consistency of lateral biases under repeated testing within a single context for males and females in both a reproductive and non-reproductive state whereby the selective pressures experienced are relatively stable to examine i) how repeatable laterality is and ii) to what extent laterality is a plastic trait, since plasticity in laterality could provide new insights that that may further our understanding of variation in laterality and provide additional explanations for why this variation may persist.

7.4 Conclusions

In conclusion, reproduction and the performance of reproductive behaviours are key, but previously unidentified source of variation in laterality and parental care behaviours represent a fitness related reproductive behaviour that is also lateralised. Both lateral biases in reproductive behaviours and variation resulting from reproduction and the performance of reproductive behaviours can impact behavioural performance within this domain and other behavioural domains that could have consequential impacts for individual fitness of both parents and their offspring. Together these findings highlight the role of sex, reproduction and reproductive behaviours in our understanding of variation in laterality, particularly why it has evolved and why it persists.

References

Alonso, J., Castellano, M.A. and Rodriguez, M., 1991. Behavioral lateralization in rats: prenatal stress effects on sex differences. *Brain research*, *539*(1), pp.45-50.

Amoroso, E.C., 1968. The evolution of viviparity. *Proceedings of the Royal Society of Medicine*, *61*(11P2), pp.1188-1200.

Amundsen, T., 2003. Fishes as models in studies of sexual selection and parental care. *Journal of Fish Biology*, *63*, 17-52.

Andrew, R.J. and Brennan, A., 1984. Sex differences in lateralization in the domestic chick. *Neuropsychologia*, *22*(4), pp.503-509.

Andrew, R.J. and Rogers, L.J., 2002. The nature of lateralization in tetrapods. *Comparative vertebrate lateralization*, *94*, p.125.

Andrew, R.J., 1991. The nature of behavioural lateralization in the chick. *Neural and behavioural plasticity: The use of the domestic chick as a model*, pp.536-554.

Andrew, R.J., 2002. The earliest origins and subsequent evolution of lateralization. *Comparative vertebrate lateralization*, pp.70-93.

Andrew, R.J., Tommasi, L. and Ford, N., 2000. Motor control by vision and the evolution of cerebral lateralization. *Brain and Language*, *73*(2), pp.220-235.

Archard, G.A. and Braithwaite, V.A., 2011. Variation in aggressive behaviour in the poeciliid fish Brachyrhaphis episcopi: population and sex differences. *Behavioural processes*, *86*(1), pp.52-57.

Ariyomo, T.O. and Watt, P.J., 2013. Aggression and sex differences in lateralization in the zebrafish. *Animal Behaviour*, *86*(3), pp.617-622.

Arnott, G., Ashton, C. and Elwood, R.W., 2011. Lateralization of lateral displays in convict cichlids. *Biology letters*, *7*(5), pp.683-685.

Atchison, D.A. and Smith, G., 2000. *Optics of the human eye* (Vol. 2). Oxford: Butterworth-Heinemann.

Balshine-Earn, S., 1997. The benefits of uniparental versus biparental mouth brooding in Galilee St. Peter's fish. *Journal of fish biology*, *50*(2), pp.371-381.

Balshine, S., Wong, M.Y. and Reddon, A.R., 2017. Social motivation and conflict resolution tactics as potential building blocks of sociality in cichlid fishes. *Behavioural processes*, *141*, pp.152-160.

Barber, I., Nairn, D. and Huntingford, F.A., 2001. Nests as ornaments: revealing construction by male sticklebacks. *Behavioral Ecology*, *12*(4), pp.390-396.

Basile, M., Boivin, S., Boutin, A., Blois-Heulin, C., Hausberger, M. and Lemasson, A., 2009. Socially dependent auditory laterality in domestic horses (Equus caballus). *Animal cognition*, *12*(4), pp.611-619.

Bastock, M. 1967. *Courtship: a zoological study* (Vol. 4). Transaction Publishers, Piscataway.

Bateman, A.J., 1948. Intra-sexual selection in Drosophila. *Heredity*, *2*(3), pp.349-368.

Bates, D., Maechler, M., Bolker, B. and Walker, S., 2015. Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software* 67(1), pp.1-48.

Bell, A.M., Hankison, S.J. and Laskowski, K.L., 2009. The repeatability of behaviour: a meta-analysis. *Animal behaviour*, *77*(4), pp.771-783.

Bell, M.A. and Foster, S.A. eds., 1994. *The evolutionary biology of the threespine stickleback*. Oxford University Press.

Benelli, G., Romano, D., Kavallieratos, N., Conte, G., Stefanini, C., Mele, M., Athanassiou, C. and Canale, A., 2017b. Multiple behavioural asymmetries impact male mating success in the khapra beetle, Trogoderma granarium. *Journal of Pest Science*, *90*(3), pp.901-909.

Benelli, G., Romano, D., Messing, R.H. and Canale, A., 2015. First report of behavioural lateralisation in mosquitoes: right-biased kicking behaviour against males in females of the Asian tiger mosquito, Aedes albopictus. *Parasitology research*, *114*(4), pp.1613-1617.

Benelli, G., Romano, D., Stefanini, C., Kavallieratos, N.G., Athanassiou, C.G. and Canale, A., 2017a. Asymmetry of mating behaviour affects copulation success in two stored-product beetles. *Journal of Pest Science*, *90*(2), pp.547-556.

Benjamini, Y. and Hochberg, Y., 1995. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal statistical society: series B (Methodological)*, *57*(1), pp.289-300.

Bianki, V.L. and Filippova, E.B., 2003. *Sex differences in lateralization in the animal brain*. CRC Press.

Bibost, A.L. and Brown, C., 2013. Laterality influences schooling position in rainbowfish, Melanotaenia spp. *PLoS One*, *8*(11), p.e80907.

Bibost, A.L. and Brown, C., 2014. Laterality influences cognitive performance in rainbowfish Melanotaenia duboulayi. *Animal cognition*, *17*(5), pp.1045-1051.

Bibost, A.L., Kydd, E. and Brown, C., 2013. The effect of sex and early environment on the lateralization of the rainbowfish Melanotaenia duboulayi. In *Behavioral lateralization in vertebrates* (pp. 9-24). Springer, Berlin, Heidelberg.

Biddle, F.G., Coffaro, C.M., Ziehr, J.E. and Eales, B.A., 1993. Genetic variation in paw preference (handedness) in the mouse. *Genome*, *36*(5), pp.935-943.

Bisazza, A. and Brown, C., 2011. Lateralization of cognitive functions in fish. In *Fish cognition and behavior* (pp. 298-324). Wiley.

Bisazza, A. and Dadda, M., 2005. Enhanced schooling performance in lateralised fishes. *Proceedings of the Royal Society B: Biological Sciences*, *272*(1573), pp.1677-1681.

Bisazza, A. and de Santi, A., 2003. Lateralization of aggression in fish. *Behavioural brain research*, *141*(2), pp.131-136.

Bisazza, A. and Vallortigara, G., 1997. Rotational swimming preferences in mosquitofish: evidence for brain lateralization?. *Physiology & behavior*, *62*(6), pp.1405-1407.

Bisazza, A., 1993. Male competition, female mate choice and sexual size dimorphism in poeciliid fishes. *Marine & Freshwater Behaviour & Phy*, *23*(1-4), pp.257-286.

Bisazza, A., Facchin, L. and Vallortigara, G., 2000. Heritability of lateralization in fish: concordance of right–left asymmetry between parents and offspring. *Neuropsychologia*, *38*(7), pp.907-912.

Bisazza, A., Facchin, L., Pignatti, R. and Vallortigara, G., 1998b. Lateralization of detour behaviour in poeciliid fish: the effect of species, gender and sexual motivation. *Behavioural brain research*, *91*(1-2), pp.157-164.

Bisazza, A., Lippolis, G. and Vallortigara, G., 2001a. Lateralization of ventral fins use during object exploration in the blue gourami (Trichogaster trichopterus). *Physiology & behavior*, *72*(4), pp.575-578.

Bisazza, A., Pignatti, R. and Vallortigara, G., 1997. Laterality in detour behaviour: interspecific variation in poeciliid fish. *Animal behaviour*, *54*(5), pp.1273-1281.

Bisazza, A., Rogers, L.J. and Vallortigara, G., 1998a. The origins of cerebral asymmetry: a review of evidence of behavioural and brain lateralization in fishes, reptiles and amphibians. *Neuroscience & Biobehavioral Reviews*, *22*(3), pp.411-426.

Bisazza, A., Sovrano, V.A. and Vallortigara, G., 2001b. Consistency among different tasks of left–right asymmetries in lines of fish originally selected for opposite direction of lateralization in a detour task. *Neuropsychologia*, *39*(10), pp.1077-1085.

Boorman, C.J. and Shimeld, S.M., 2002. The evolution of left–right asymmetry in chordates. *Bioessays*, *24*(11), pp.1004-1011.

Boukouvala, M.C., Romano, D., Kavallieratos, N.G., Athanassiou, C.G., Stefanini, C., Canale, A. and Benelli, G., 2019. Asymmetric courtship boosts male mating success in the red flour beetle, Tribolium castaneum (Herbst)(Coleoptera: Tenebrionidae). *Journal of Stored Products Research*, *81*, pp.1-6.

Bradshaw, J.L. and Rogers, L.J., 1993. The evolution of lateral asymmetries, language, tool use & intellect//Review. *Canadian Journal of Experimental Psychology*, *47*(4), p.757.

Bradshaw, J.L., 1989. *Hemispheric specialization and psychological function*. John Wiley & Sons.

Brandão, M.L., de Almeida Fernandes, A.M.T. and Gonçalves-de-Freitas, E., 2019. Male and female cichlid fish show cognitive inhibitory control ability. *Scientific reports*, *9*(1), pp.1-8.

Brewster, J. and Houde, A., 2003. Are female guppies more likely to flee when approached by two males?. *Journal of Fish Biology*, *63*(4), pp.1056-1059.

Broder, E.D. and Angeloni, L.M., 2014. Predator-induced phenotypic plasticity of laterality. *Animal Behaviour*, *98*, pp.125-130.

Brooks, R. and Endler, J.A., 2001. Female guppies agree to differ: phenotypic and genetic variation in mate-choice behavior and the consequences for sexual selection. *Evolution*, *55*(8), pp.1644-1655.

Brown, C. and Bibost, A.L., 2014. Laterality is linked to personality in the black-lined rainbowfish, Melanotaenia nigrans. *Behavioral ecology and sociobiology*, *68*(6), pp.999-1005.

Brown, C. and Braithwaite, V.A., 2005. Effects of predation pressure on the cognitive ability of the poeciliid Brachyraphis episcopi. *Behavioral Ecology*, *16*(2), pp.482-487.

Brown, C. and Irving, E., 2014. Individual personality traits influence group exploration in a feral guppy population. *Behavioral Ecology*, *25*(1), pp.95-101.

Brown, C. and Magat, M., 2011a. Cerebral lateralization determines hand preferences in Australian parrots. *Biology letters*, *7*(4), pp.496-498.

Brown, C. and Magat, M., 2011b. The evolution of lateralised foot use in parrots: a phylogenetic approach. *Behavioral Ecology*, *22*(6), pp.1201-1208.

Brown, C., Gardner, C. and Braithwaite, V.A., 2004. Population variation in lateralised eye use in the poeciliid Brachyraphis episcopi. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(suppl_6), pp.S455-S457.

Brown, C., Jones, F. and Braithwaite, V., 2005. In situ examination of boldness–shyness traits in the tropical poeciliid, Brachyraphis episcopi. *Animal Behaviour*, *70*(5), pp.1003-1009.

Brown, C., Jones, F. and Braithwaite, V.A., 2007a. Correlation between boldness and body mass in natural populations of the poeciliid Brachyrhaphis episcopi. *Journal of Fish Biology*, *71*(6), pp.1590-1601.

Brown, C., Western, J. and Braithwaite, V.A., 2007b. The influence of early experience on, and inheritance of, cerebral lateralization. *Animal Behaviour*, *74*(2), pp.231-238.

Budaev, S. and Brown, C., 2011. Personality traits and behaviour. *Fish cognition and behavior*, pp.135-165.

Budaev, S.V., Zworykin, D.D. and Mochek, A.D., 1999. Individual differences in parental care and behaviour profile in the convict cichlid: a correlation study. *Animal Behaviour*, *58*(1), pp.195-202.

Bukhari, S.A., Saul, M.C., James, N., Bensky, M.K., Stein, L.R., Trapp, R. and Bell, A.M., 2019. Neurogenomic insights into paternal care and its relation to territorial aggression. *Nature communications*, *10*(1), pp.1-11.

Bulla, M., Valcu, M., Rutten, A.L. and Kempenaers, B., 2019. Temporary mate removal during incubation leads to variable compensation in a biparental shorebird. *Frontiers in Ecology and Evolution*, *7*, p.93.

Byrnes, E.E., Pouca, C.V. and Brown, C., 2016. Laterality strength is linked to stress reactivity in Port Jackson sharks (Heterodontus portusjacksoni). *Behavioural brain research*, *305*, pp.239-246.

Camp, D.M., Robinson, T.E. and Becker, J.B., 1984. Sex differences in the effects of early experience on the development of behavioral and brain asymmetries in rats. *Physiology & behavior*, *33*(3), pp.433-439.

Cantalupo, C., Bisazza, A. and Vallortigara, G., 1995. Lateralization of predator-evasion response in a teleost fish (Girardinus falcatus). *Neuropsychologia*, *33*(12), pp.1637-1646.

Carere, C. and Locurto, C., 2011. Interaction between animal personality and animal cognition. *Current Zoology*, *57*(4), pp.491-498.

Cassells, B., Collins, R.L. and Wahlsten, D., 1990. Path analysis of sex difference, forebrain commissure area and brain size in relation to degree of laterality in selectively bred mice. *Brain research*, *529*(1-2), pp.50-56.

Chivers, D.P., McCormick, M.I., Allan, B.J., Mitchell, M.D., Gonçalves, E.J., Bryshun, R. and Ferrari, M.C., 2016. At odds with the group: changes in lateralization and escape performance reveal conformity and conflict in fish schools. *Proceedings of the Royal Society B: Biological Sciences*, *283*(1841), p.20161127.

Chivers, D.P., McCormick, M.I., Warren, D.T., Allan, B.J., Ramasamy, R.A., Arvizu, B.K., Glue, M. and Ferrari, M.C., 2017. Competitive superiority versus predation savvy: the two sides of behavioural lateralization. *Animal Behaviour*, *130*, pp.9-15.

Chotkowski, M.A. and Marsden, J.E., 1999. Round goby and mottled sculpin predation on lake trout eggs and fry: field predictions from laboratory experiments. *Journal of Great Lakes Research*, *25*(1), pp.26-35.

Clayton, N.S. and Krebs, J.R., 1994. Memory for spatial and object-specific cues in food-storing and non-storing birds. *Journal of Comparative Physiology A*, *174*(3), pp.371-379.

Clutton-Brock, T.H., 1991. *The evolution of parental care* (Vol.64). Princeton University Press.

Collin, S.P. and Shand, J., 2003. Retinal sampling and the visual field in fishes. In *Sensory processing in aquatic environments* (pp. 139-169). Springer, New York, NY.

Collins, R.L., Sargent, E.E. and Neumann, P.E., 1993. Genetic and behavioral tests of the McManus hypothesis relating response to selection for lateralization of handedness in mice to degree of heterozygosity. *Behavior genetics*, *23*(4), pp.413-421.

Connell, J.H., 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. *The American Naturalist*, *122*(5), pp.661-696.

Conrad, J.L., Weinersmith, K.L., Brodin, T., Saltz, J.B. and Sih, A., 2011. Behavioural syndromes in fishes: a review with implications for ecology and fisheries management. *Journal of fish biology*, *78*(2), pp.395-435.

Cooper Jr, W.E., Dimopoulos, I. and Pafilis, P., 2015. Sex, age, and population density affect aggressive behaviors in island lizards promoting cannibalism. *Ethology*, *121*(3), pp.260-269.

Corballis, M.C., 2006. Cerebral asymmetry: a question of balance. *Cortex; a journal devoted to the study of the nervous system and behavior*, *42*(1), pp.117-118.

Corballis, M.C., 2008. Of mice and men–and lopsided birds. *Cortex*, *44*(1), pp.3-7.

Corballis, M.C., 2012. Lateralization of the human brain. *Progress in brain research*, *195*, pp.103-121.

Coutelis, J.B., Petzoldt, A.G., Speder, P., Suzanne, M. and Noselli, S., 2008, June. Left–right asymmetry in Drosophila. In *Seminars in cell & developmental biology* (Vol. 19, No. 3, pp. 252-262). Academic Press.

Csermely, D., Bonati, B. and Romani, R., 2010. Lateralisation in a detour test in the common wall lizard (Podarcis muralis). *Laterality*, *15*(5), pp.535-547.

Dadda, M. and Bisazza, A., 2006a. Does brain asymmetry allow efficient performance of simultaneous tasks?. *Animal Behaviour*, *72*(3), pp.523-529.

Dadda, M. and Bisazza, A., 2006b. Lateralised female topminnows can forage and attend to a harassing male simultaneously. *Behavioral Ecology*, *17*(3), pp.358-363.

Dadda, M. and Bisazza, A., 2012. Prenatal light exposure affects development of behavioural lateralization in a livebearing fish. *Behavioural processes*, *91*(1), pp.115-118.

Dadda, M., Agrillo, C., Bisazza, A. and Brown, C., 2015. Laterality enhances numerical skills in the guppy, Poecilia reticulata. *Frontiers in Behavioral Neuroscience*, *9*, p.285.

Dadda, M., Koolhaas, W.H. and Domenici, P., 2010. Behavioural asymmetry affects escape performance in a teleost fish. *Biology letters*, *6*(3), pp.414-417.

Dadda, M., Nepomnyashchikh, V.A., Izvekov, E.I. and Bisazza, A., 2012. Individual-level consistency of different laterality measures in the goldbelly topminnow. *Behavioral neuroscience*, *126*(6), p.845.

Dadda, M., Zandona, E., Agrillo, C. and Bisazza, A., 2009. The costs of hemispheric specialization in a fish. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1677), pp.4399-4407.

De Perera, T.B. and Braithwaite, V.A., 2005. Laterality in a non-visual sensory modality—the lateral line of fish. *Current Biology*, *15*(7), pp.R241-R242.

Deckel, A.W., 1995. Laterality of aggressive responses in Anolis. *Journal of Experimental Zoology*, *272*(3), pp.194-200.

Denenberg, V.H., 1981. Hemispheric laterality in animals and the effects of early experience. *Behavioral and brain sciences*, *4*(1), pp.1-21.

Deng, C. and Rogers, L.J., 1997. Differential contributions of the two visual pathways to functional lateralization in chicks. *Behavioural brain research*, *87*(2), pp.173-182.

Deng, C.H.A.O. and Rogers, L.J., 2002. Factors affecting the development of lateralization in chicks. *Comparative vertebrate lateralization. Cambridge University Press, Cambridge*, pp.206-246.

Domenici, P., Allan, B.J., Watson, S.A., McCormick, M.I. and Munday, P.L., 2014. Shifting from right to left: the combined effect of elevated CO_2 and temperature on behavioural lateralization in a coral reef fish. *PLoS One*, *9*(1), p.e87969.

Domenici, P., Lefrançois, C. and Shingles, A., 2007. Hypoxia and the antipredator behaviours of fishes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *362*(1487), pp.2105-2121.

Domenici, P., Torres, R. and Manríquez, P.H., 2017. Effects of elevated carbon dioxide and temperature on locomotion and the repeatability of lateralization in a keystone marine mollusc. *Journal of Experimental Biology*, *220*(4), pp.667-676.

Ensminger, A.L. and Westneat, D.F., 2012. Individual and Sex Differences in Habituation and Neophobia in House Sparrows (P asser domesticus). *Ethology*, *118*(11), pp.1085-1095.

Facchin, L., Bisazza, A. and Vallortigara, G., 1999. What causes lateralization of detour behavior in fish? Evidence for asymmetries in eye use. *Behavioural brain research*, *103*(2), pp.229-234.

Faraway, J. J. 2016 *Linear models with R*. Chapman and Hall/CRC.

Ferrari, M.C., McCormick, M.I., Allan, B.J., Choi, R.B., Ramasamy, R.A. and Chivers, D.P., 2015. The effects of background risk on behavioural lateralization in a coral reef fish. *Functional Ecology*, *29*(12), pp.1553-1559.

Festa-Bianchet, M., Apollonio, M., Mari, F. and Rasola, G., 1990. Aggression among lekking male fallow deer (Dama dama): territory effects and relationship with copulatory success. *Ethology*, *85*(3), pp.236-246.

Fine, M.L., McELROY, D.A.V.I.D., Rafi, J., King, C.B., Loesser, K.E. and Newton, S., 1996. Lateralization of pectoral stridulation sound production in the channel catfish. *Physiology & behavior*, *60*(3), pp.753-757.

Forsatkar, M.N., Dadda, M. and Nematollahi, M.A., 2015. Lateralization of aggression during reproduction in male Siamese fighting fish. *Ethology*, *121*(11), pp.1039-1047.

Franklin III, W.E. and Lima, S.L., 2001. Laterality in avian vigilance: do sparrows have a favourite eye?. *Animal Behaviour*, *62*(5), pp.879-885.

Franssen, C.L., Bardi, M., Shea, E.A., Hampton, J.E., Adam Franssen, R., Kinsley, C.H. and Lambert, K.G., 2011. Fatherhood alters behavioural and neural responsiveness in a spatial task. *Journal of neuroendocrinology*, *23*(11), pp.1177-1187.

Frasnelli, E. and Vallortigara, G., 2018. Individual-level and population-level lateralization: two sides of the same coin. *Symmetry*, *10*(12), p.739.

Frasnelli, E., Vallortigara, G. and Rogers, L.J., 2012. Left–right asymmetries of behaviour and nervous system in invertebrates. *Neuroscience & Biobehavioral Reviews*, *36*(4), pp.1273-1291.

Fry, F.E.J., 1971. The effect of environmental factors on the physiology of fish. *Fish physiology*, pp.1-98.

Fuss, T., Nöbel, S. and Witte, K., 2019. It's in the eye of the beholder: visual lateralisation in response to the social environment in poeciliids. *Journal of fish biology*, *94*(5), pp.759-771.

Gabriel, P.O. and Black, J.M., 2010. Behavioural syndromes in Steller's jays: the role of time frames in the assessment of behavioural traits. *Animal Behaviour*, *80*(4), pp.689-697.

Garland Jr, T., Zhao, M. and Saltzman, W., 2016. Hormones and the evolution of complex traits: insights from artificial selection on behavior. *Integrative and comparative biology*, *56*(2), pp.207-224.

Ghirlanda, S. and Vallortigara, G., 2004. The evolution of brain lateralization: a game-theoretical analysis of population structure. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(1541), pp.853-857.

Gilbert, S.F. and Barresi, M.J., 2016. Early amphibian development. *Developmental biology. 11th ed. Sunderland: Sinauer Associates, Inc*, pp.333-64.

Gómez-Laplaza, L.M. and Gerlai, R., 2011. Can angelfish (Pterophyllum scalare) count? Discrimination between different shoal sizes follows Weber's law. *Animal cognition*, *14*(1), pp.1-9.

Gonzalez-Voyer, A., Winberg, S. and Kolm, N., 2009. Social fishes and single mothers: brain evolution in African cichlids. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1654), pp.161-167.

Goodwin, N.B., Dulvy, N.K. and Reynolds, J.D., 2002. Life-history correlates of the evolution of live bearing in fishes. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *357*(1419), pp.259-267.

Grande, C. and Patel, N.H., 2009. Nodal signalling is involved in left–right asymmetry in snails. *Nature*, *457*(7232), pp.1007-1011.

Gross, M.R. and Sargent, R.C., 1985. The evolution of male and female parental care in fishes. *American Zoologist*, *25*(3), pp.807-822.

Gross, M.R., 2005. The evolution of parental care. *The Quarterly review of biology*, *80*(1), pp.37-45.

Güntürkün, O., 1997. Avian visual lateralization: a review. *Neuroreport: An International Journal for the Rapid Communication of Research in Neuroscience*.

Güntürkün, O., Diekamp, B., Manns, M., Nottelmann, F., Prior, H., Schwarz, A. and Skiba, M., 2000. Asymmetry pays: visual lateralization improves discrimination success in pigeons. *Current Biology*, *10*(17), pp.1079-1081.

Gutiérrez-Ibáñez, C., Reddon, A.R., Kreuzer, M.B., Wylie, D.R. and Hurd, P.L., 2011. Variation in asymmetry of the habenular nucleus correlates with behavioural asymmetry in a cichlid fish. *Behavioural brain research*, *221*(1), pp.189-196.

Hager, M.C. and Helfman, G.S., 1991. Safety in numbers: shoal size choice by minnows under predatory threat. *Behavioral Ecology and Sociobiology*, *29*(4), pp.271-276.

Harris, L.J., 2000. On the evolution of handedness: a speculative analysis of Darwin's views and a review of early studies of handedness in “the nearest allies of man”. *Brain and language*, *73*(2), pp.132-188.

Harrison, X.A., 2014. Using observation-level random effects to model overdispersion in count data in ecology and evolution. *PeerJ*, *2*, p.e616.

Heg, D. and Bachar, Z., 2006. Cooperative breeding in the Lake Tanganyika cichlid Julidochromis ornatus. *Environmental Biology of Fishes*, *76*(2), pp.265-281.

Heuts, B.A., 1999. Lateralization of trunk muscle volume, and lateralization of swimming turns of fish responding to external stimuli. *Behavioural Processes*, *47*(2), pp.113-124.

Hews, D.K. and Worthington, R.A., 2001. Fighting from the right side of the brain: left visual field preference during aggression in free-ranging male tree lizards (Urosaurus ornatus). *Brain, Behavior and Evolution*, *58*(6), pp.356-361.

Hews, D.K., Castellano, M. and Hara, E., 2004. Aggression in females is also lateralised: left-eye bias during aggressive courtship rejection in lizards. *Animal Behaviour*, *68*(5), pp.1201-1207.

Hick, K., Reddon, A.R., O’Connor, C.M. and Balshine, S., 2014. Strategic and tactical fighting decisions in cichlid fishes with divergent social systems. *Behaviour*, *151*(1), pp.47-71.

Hiermes, M., Rick, I.P., Mehlis, M. and Bakker, T.C., 2016. The dynamics of color signals in male threespine sticklebacks Gasterosteus aculeatus. *Current zoology*, *62*(1), pp.23-31.

Hixon, M.A. and Menge, B.A., 1991. Species diversity: prey refuges modify the interactive effects of predation and competition. *Theoretical Population Biology*, *39*(2), pp.178-200.

Hopkins, W.D. and De Lathouwers, M., 2006. Left nipple preferences in infant Pan paniscus and P. troglodytes. *International journal of primatology*, *27*(6), pp.1653-1662.

Hopkins, W.D., 1999. Heritability of hand preference in chimpanzees (Pan troglodytes): evidence from a partial interspecies cross-fostering study. *Journal of Comparative Psychology*, *113*(3), p.307.

Hopkins, W.D., Dahl, J.F. and Pilcher, D., 2001. Genetic influence on the expression of hand preferences in chimpanzees (Pan troglodytes): Evidence in support of the right-shift theory and developmental instability. *Psychological Science*, *12*(4), pp.299-303.

Hori, M., 1993. Frequency-dependent natural selection in the handedness of scale-eating cichlid fish. *Science*, *260*(5105), pp.216-219.

Houde, A., 2019. *Sex, color, and mate choice in guppies*. Princeton University Press.

Houde, E.D., 1997. Patterns and consequences of selective processes in teleost early life histories. In *Early life history and recruitment in fish populations* (pp. 173-196). Springer, Dordrecht.

Hughes, R.N., 1968. Behaviour of male and female rats with free choice of two environments differing in novelty. *Animal Behaviour*, *16*(1), pp.92-96.

Irving, E. and Brown, C., 2013. Examining the link between personality and laterality in a feral guppy Poecilia reticulata population. *Journal of fish biology*, *83*(2), pp.311-325.

Itzkowitz, M., Santangelo, N., Cleveland, A., Bockelman, A. and Richter, M., 2005. Is the selection of sex-typical parental roles based on an assessment process? A test in the monogamous convict cichlid fish. *Animal Behaviour*, *69*(1), pp.95-105.

Jefferies, R.P.S 1979. Calcichordates. In *Encylopedia of Earth Sciences, Vol. VII: The Enclyopedia of Paleontology,* ed. R.W. Fairbridge & D. Jablonski, pp 161-167. Strondsburg, PA: Dowden, Hutchinson & Ross Inc.

Jefferies, R.P.S. and Lewis, D.N., 1978. The English Silurian fossil Placocystites forbesianus and the ancestry of the vertebrates. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, pp.205-323.

Johannesen, A., Dunn, A.M. and Morrell, L.J., 2012. Olfactory cue use by threespined sticklebacks foraging in turbid water: prey detection or prey location?. *Animal Behaviour*, *84*(1), pp.151-158.

Jozet-Alves, C., Romagny, S., Bellanger, C. and Dickel, L., 2012b. Cerebral correlates of visual lateralization in Sepia. *Behavioural brain research*, *234*(1), pp.20-25.

Jozet-Alves, C., Viblanc, V.A., Romagny, S., Dacher, M., Healy, S.D. and Dickel, L., 2012a. Visual lateralization is task and age dependent in cuttlefish, Sepia officinalis. *Animal Behaviour*, *83*(6), pp.1313-1318.

Juan, T., Géminard, C., Coutelis, J.B., Cerezo, D., Polès, S., Noselli, S. and Fürthauer, M., 2018. Myosin1D is an evolutionarily conserved regulator of animal left–right asymmetry. *Nature communications*, *9*(1), pp.1-12.

Kaarthigeyan, J. and Dharmaretnam, M., 2005. Relative levels of motivation and asymmetries of viewing and detour task in guppies (Poecilia reticulata). *Behavioural brain research*, *159*(1), pp.37-41.

Kabadayi, C., Bobrowicz, K. and Osvath, M., 2018. The detour paradigm in animal cognition. *Animal cognition*, *21*(1), pp.21-35.

Kalinka, A.T. and Tomancak, P., 2012. The evolution of early animal embryos: conservation or divergence?. *Trends in ecology & evolution*, *27*(7), pp.385-393.

Kamimura, Y., 2006. Right-handed penises of the earwig Labidura riparia (Insecta, Dermaptera, Labiduridae): evolutionary relationships between structural and behavioral asymmetries. *Journal of Morphology*, *267*(11), pp.1381-1389.

Kamimura, Y., Mitsumoto, H. and Lee, C.Y., 2014. Duplicated female receptacle organs for traumatic insemination in the tropical bed bug Cimex hemipterus: adaptive variation or malformation?. *PloS one*, *9*(2), p.e89265.

Karenina, K. and Giljov, A., 2018. Mother and offspring lateralised social behavior across mammalian species. *Progress in brain research*, *238*, pp.115-141.

Karenina, K., Giljov, A., Ingram, J., Rowntree, V.J. and Malashichev, Y., 2017. Lateralization of mother–infant interactions in a diverse range of mammal species. *Nature Ecology & Evolution*, *1*(2), pp.1-4.

Katoh, R., Munehara, H. and Kohda, M., 2005. Alternative male mating tactics of the substrate brooding cichlid Telmatochromis temporalis in Lake Tanganyika. *Zoological Science*, *22*(5), pp.555-561.

Keener, W., Webber, M.A., Szczepaniak, I.D., Markowitz, T.M. and Orbach, D.N., 2018. The sex life of harbor porpoises (Phocoena phocoena): lateralised and aerial behavior. *Aquatic Mammals*, *44*(6), p.620.

Keenleyside, M.H. and Mackereth, R.W., 1992. Effects of loss of male parent on brood survival in a biparental cichlid fish. *Environmental Biology of Fishes*, *34*(2), pp.207-212.

Kieffer, J.D. and Colgan, P.W., 1992. The role of learning in fish behaviour. *Reviews in Fish Biology and Fisheries*, *2*(2), pp.125-143.

Koboroff, A., Kaplan, G. and Rogers, L.J., 2008. Hemispheric specialization in Australian magpies (Gymnorhina tibicen) shown as eye preferences during response to a predator. *Brain Research Bulletin*, *76*(3), pp.304-306.

Kokko, H. and Jennions, M.D., 2008. Parental investment, sexual selection and sex ratios. *Journal of evolutionary biology*, *21*(4), pp.919-948.

Kotrschal, A., Räsänen, K., Kristjansson, B.K., Senn, M. and Kolm, N., 2012. Extreme sexual brain size dimorphism in sticklebacks: a consequence of the cognitive challenges of sex and parenting?. *PLoS One*, *7*(1), p.e30055.

Krause, J., Loader, S.P., McDermott, J. and Ruxton, G.D., 1998. Refuge use by fish as a function of body length–related metabolic expenditure and predation risks. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *265*(1413), pp.2373-2379.

Kulik, L., Amici, F., Langos, D. and Widdig, A., 2015. Sex differences in the development of social relationships in rhesus macaques (Macaca mulatta). *International journal of primatology*, *36*(2), pp.353-376.

Kurvers, R.H., Krause, S., Viblanc, P.E., Herbert-Read, J.E., Zaslansky, P., Domenici, P., Marras, S., Steffensen, J.F., Svendsen, M.B., Wilson, A.D. and Couillaud, P., 2017. The evolution of lateralization in group hunting sailfish. *Current Biology*, *27*(4), pp.521-526.

Kuwamura, T., 1986. Parental care and mating systems of cichlid fishes in Lake Tanganyika: a preliminary field survey. *Journal of Ethology*, *4*(2), pp.129-146.

Kuwamura, T., 1992. Overlapping territories of Pseudosimochromis curvifrons males and other herbivorous cichlid fishes in Lake Tanganyika. *Ecological Research*, *7*(1), pp.43-53.

Kuwamura, T., 1997. The evolution of parental care and mating systems among Tanganyikan cichlids. *Fish communities in lake Tanganyika*.

Kvarnemo, C. and Ahnesjo, I., 1996. The dynamics of operational sex ratios and competition for mates. *Trends in Ecology & Evolution*, *11*(10), pp.404-408.

Kynard, B.E., 1978. Breeding behavior of a lacustrine population of threespine sticklebacks (Gasterosteus aculeatus L.). *Behaviour*, *67*(3-4), pp.178-206.

Lang, M. and Orgogozo, V., 2012. Distinct copulation positions in Drosophila pachea males with symmetric or asymmetric external genitalia. *Contributions to Zoology*, *81*(2), pp.87-94.

Lavery, R.J. and Reebs, S.G., 1994. Effect of mate removal on current and subsequent parental care in the convict cichlid (Pisces: Cichlidae). *Ethology*, *97*(4), pp.265-277.

Leary, R.F. and Allendorf, F.W., 1989. Fluctuating asymmetry as an indicator of stress: implications for conservation biology. *Trends in Ecology & Evolution*, *4*(7), pp.214-217.

Lee, H.J., Kusche, H. and Meyer, A., 2012. Handed foraging behavior in scale-eating cichlid fish: its potential role in shaping morphological asymmetry. *PLoS One*, *7*(9), p.e44670.

Lee, H.J., Schneider, R.F., Manousaki, T., Kang, J.H., Lein, E., Franchini, P. and Meyer, A., 2017. Lateralised feeding behavior is associated with asymmetrical neuroanatomy and lateralised gene expressions in the brain in scale-eating cichlid fish. *Genome biology and evolution*, *9*(11), pp.3122-3136.

Lehman, R.A., 1981. Lateralised asymmetry of behavior in animals at the population and individual level. *Behavioral and Brain Sciences*, *4*(1), pp.28-28.

Lehtonen, T.K., Wong, B.B., Svensson, P.A. and Meyer, A., 2011. Adjustment of brood care behaviour in the absence of a mate in two species of Nicaraguan crater lake cichlids. *Behavioral Ecology and Sociobiology*, *65*(4), pp.613-619.

Lenth R.V., 2016. Least-Squares Means: The R Package lsmeans. *Journal of Statistical Software,* 69(1), pp.1-33.

Levy, J., 1969. Possible basis for the evolution of lateral specialization of the human brain. *Nature*, *224*(5219), pp.614-615.

Levy, J., 1977. The mammalian brain and the adaptive advantage of cerebral asymmetry. *Annals of the New York Academy of Sciences*, *299*(1), pp.264-272.

Lippolis, G., Bisazza, A., Rogers, L.J. and Vallortigara, G., 2002. Lateralisation of predator avoidance responses in three species of toads. *Laterality: Asymmetries of Body, Brain and Cognition*, *7*(2), pp.163-183.

Lippolis, G., Westman, W., McAllan, B. and Rogers, L., 2005. Lateralisation of escape responses in the stripe-faced dunnart, Sminthopsis macroura (Dasyuridae: Marsupialia). *Laterality: Asymmetries of Body, Brain, and Cognition*, *10*(5), pp.457-470.

Loiselle, B.A., Ryder, T.B., Duraes, R., Tori, W., Blake, J.G. and Parker, P.G., 2007. Kin selection does not explain male aggregation at leks of 4 manakin species. *Behavioral Ecology*, *18*(2), pp.287-291.

López-Sepulcre, A., Gordon, S.P., Paterson, I.G., Bentzen, P. and Reznick, D.N., 2013. Beyond lifetime reproductive success: the posthumous reproductive dynamics of male Trinidadian guppies. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1763), p.20131116.

Lucon-Xiccato, T., Chivers, D.P., Mitchell, M.D. and Ferrari, M.C., 2016a. Prenatal exposure to predation affects predator recognition learning via lateralization plasticity. *Behavioral Ecology*, p.arw155.

Lucon-Xiccato, T., Dadda, M. and Bisazza, A., 2016b. Sex differences in discrimination of shoal size in the guppy (Poecilia reticulata). *Ethology*, *122*(6), pp.481-491.

Macbeth, A.H. and Luine, V.N., 2010. Changes in anxiety and cognition due to reproductive experience: a review of data from rodent and human mothers. *Neuroscience & Biobehavioral Reviews*, *34*(3), pp.452-467.

MacNeilage, P.F., 2014. Evolution of the strongest vertebrate rightward action asymmetries: Marine mammal sidedness and human handedness. *Psychological bulletin*, *140*(2), p.587.

Magat, M. and Brown, C., 2009. Laterality enhances cognition in Australian parrots. *Proceedings of the Royal Society B: Biological Sciences*, *276*(1676), pp.4155-4162.

Magurran, A. E. 2005 *Evolutionary ecology: the Trinidadian guppy*. Oxford University Press on Demand.

Magurran, A.E. and Phillip, D.A., 2001. Evolutionary implications of large-scale patterns in the ecology of Trinidadian guppies, Poecilia reticulata. *Biological Journal of the Linnean Society*, *73*(1), pp.1-9.

Magurran, A.E. and Seghers, B.H., 1994a. A cost of sexual harassment in the guppy, Poecilia reticulata. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *258*(1351), pp.89-92.

Magurran, A.E. and Seghers, B.H., 1994b. Sexual conflict as a consequence of ecology: evidence from guppy, Poecilia reticulata, populations in Trinidad. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *255*(1342), pp.31-36.

Mainwaring, M.C. and Hartley, I.R., 2020. Sex-specific patterns of minimal compensation of care during and after short term mate removal in biparental blue tits. *Behavioural processes*, *173*, p.104026.

Manns, M., 2021. Laterality for the next decade: Costs and benefits of neuronal asymmetries–putting lateralization in an evolutionary context. *Laterality*, pp.1-4.

Matsui, S., Takeuchi, Y. and Hori, M., 2013. Relation between morphological antisymmetry and behavioral laterality in a poeciliid fish. *Zoological science*, *30*(8), pp.613-618.

Mayer, I. and Borg, B., 1995. Androgens and behaviour in the threespined stickleback. *Behaviour*, *132*(13-14), pp.1025-1035.

Mboko, S.K. and Kohda, M., 1995. Pale and dark dichromatism related to microhabitats in a herbivorous Tanganyikan cichlid fish, Telmatochromis temporalis. *Journal of Ethology*, *13*(1), pp.77-83.

Mboko, S.K. and Kohda, M., 1999. Piracy mating by large males in a monogamous substrate-breeding cichlid in Lake Tanganyika. *Journal of Ethology*, *17*(1), pp.51-55.

Mboko, S.K., 1998. Studies on the feeding and breeding ecology of the cichlid fish, Telmatochromis temporalis in Lake Tanganyika. *D. Sci. Thesis from Osaka City Univ*.

McCleary, R.A., 1960. Type of response as a factor in interocular transfer in the fish. *Journal of Comparative and Physiological Psych.*

McGrew, W.C. and Marchant, L.F., 1999. Laterality of hand use pays off in foraging success for wild chimpanzees. *Primates*, *40*(3), pp.509-513.

McLean, S. and Morrell, L.J., 2020. Consistency in the strength of laterality in male, but not female, guppies across different behavioural contexts. *Biology letters*, *16*(5), p.20190870.

McLean, S. and Morrell, L.J., 2021. Sex Differences in Laterality Are Associated with Reproduction in Threespine Stickleback. *The American Naturalist*, *197*(6).

Miklosi, A., Andrew, R.J. and Savage, H., 1997. Behavioural lateralisation of the tetrapod type in the zebrafish (Brachydanio rerio). *Physiology & behavior*, *63*(1), pp.127-135.

Milliken, G.W., Stafford, D.K., Dodson, D.L., Pinger, C.D. and Ward, J.P., 1991. Analyses of feeding lateralization in the small-eared bushbaby (Otolemur garnettii): a comparison with the ring-tailed lemur (Lemur catta). *Journal of Comparative Psychology*, *105*(3), p.274.

Moiron, M., Araya-Ajoy, Y.G., Mathot, K.J., Mouchet, A. and Dingemanse, N.J., 2019. Functional relations between body mass and risk-taking behavior in wild great tits. *Behavioral Ecology*, *30*(3), pp.617-623.

Moretz, J.A., Martins, E.P. and Robison, B.D., 2007. Behavioral syndromes and the evolution of correlated behavior in zebrafish. *Behavioral ecology*, *18*(3), pp.556-562.

Mowrey, W.R. and Portman, D.S., 2012. Sex differences in behavioral decision-making and the modulation of shared neural circuits. *Biology of sex differences*, *3*(1), pp.1-14.

Nakagawa, S., Gillespie, D.O.S., Hatchwell, B.J. and Burke, T., 2007. Predictable males and unpredictable females: sex difference in repeatability of parental care in a wild bird population. *Journal of evolutionary biology*, *20*(5), pp.1674-1681.

Nepomnyashchikh, V.A. and Izvekov, E.I., 2006. Variability of the behavioral laterality in Teleostei (Pisces). *Journal of Ichthyology*, *46*(2), pp.S235-S242.

Neville, A.C., 1978. On the general problem of asymmetry. *Behavioral and Brain Sciences*, *1*(2), pp.308-309.

Niven, J.E. and Bell, A.T., 2018. Lessons in Lateralisation from the Insects. *Trends in ecology & evolution*, *33*(7), pp.486-488.

Niven, J.E. and Frasnelli, E., 2018. Insights into the evolution of lateralization from the insects. *Progress in brain research*, *238*, pp.3-31.

Northcutt, R.G., 2012. Evolution of centralized nervous systems: two schools of evolutionary thought. *Proceedings of the National Academy of Sciences*, *109*(Supplement 1), pp.10626-10633.

Nottebohm, F., 1971. Neural lateralization of vocal control in a passerine bird. I. Song. *Journal of Experimental Zoology*, *177*(2), pp.229-261.

Nyland, K.B., Lombardo, M.P. and Thorpe, P.A., 2003. Left-sided directional bias of cloacal contacts during house sparrow copulations. *The Wilson Journal of Ornithology*, *115*(4), pp.470-473.

Páll, M.K., Mayer, I. and Borg, B., 2002. Androgen and behavior in the male threespined stickleback, Gasterosteus aculeatus I.—Changes in 11-ketotestosterone levels during the nesting cycle. *Hormones and Behavior*, *41*(4), pp.377-383.

Paradis, A.R., Pepin, P. and Brown, J.A., 1996. Vulnerability of fish eggs and larvae to predation: review of the influence of the relative size of prey and predator. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*(6), pp.1226-1235.

Pascual, A., Huang, K.L., Neveu, J. and Préat, T., 2004. Brain asymmetry and long-term memory. *Nature*, *427*(6975), pp.605-606.

Pawluski, J.L., Walker, S.K. and Galea, L.A., 2006. Reproductive experience differentially affects spatial reference and working memory performance in the mother. *Hormones and behavior*, *49*(2), pp.143-149.

Payton, M.E., Greenstone, M.H. and Schenker, N., 2003. Overlapping confidence intervals or standard error intervals: what do they mean in terms of statistical significance?. *Journal of Insect Science*, *3*(1).

Plath, M., Makowicz, A.M., Schlupp, I. and Tobler, M., 2007. Sexual harassment in live-bearing fishes (Poeciliidae): comparing courting and noncourting species. *Behavioral Ecology*, *18*(4), pp.680-688.

Prior, H., Wiltschko, R., Stapput, K., Güntürkün, O. and Wiltschko, W., 2004. Visual lateralization and homing in pigeons. *Behavioural brain research*, *154*(2), pp.301-310.

Quaranta, A., Siniscalchi, M. and Vallortigara, G., 2007. Asymmetric tail-wagging responses by dogs to different emotive stimuli. *Current Biology*, *17*(6), pp.R199-R201.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/

Raff, R.A. and Kaufman, T.C., 1991. *Embryos, genes, and evolution: the developmental-genetic basis of evolutionary change*. Indiana University Press.

Reddon, A.R. and Balshine, S., 2010. Lateralization in response to social stimuli in a cooperatively breeding cichlid fish. *Behavioural Processes*, *85*(1), pp.68-71.

Reddon, A.R. and Hurd, P.L., 2008. Aggression, sex and individual differences in cerebral lateralization in a cichlid fish. *Biology letters*, *4*(4), pp.338-340.

Reddon, A.R. and Hurd, P.L., 2009a. Sex differences in the cerebral lateralization of a cichlid fish when detouring to view emotionally conditioned stimuli. *Behavioural processes*, *82*(1), pp.25-29.

Reddon, A.R. and Hurd, P.L., 2009c. Individual differences in cerebral lateralization are associated with shy–bold variation in the convict cichlid. *Animal Behaviour*, *77*(1), pp.189-193.

Reddon, A.R., Gutiérrez-Ibáñez, C., Wylie, D.R. and Hurd, P.L., 2009b. The relationship between growth, brain asymmetry and behavioural lateralization in a cichlid fish. *Behavioural brain research*, *201*(1), pp.223-228.

Regaiolli, B., Spiezio, C. and Hopkins, W.D., 2018. Asymmetries in mother-infant behaviour in Barbary macaques (Macaca sylvanus). *PeerJ*, *6*, p.e4736.

Reichert, B.E., Cattau, C.E., Fletcher Jr, R.J., Kendall, W.L. and Kitchens, W.M., 2012. Extreme weather and experience influence reproduction in an endangered bird. *Ecology*, *93*(12), pp.2580-2589.

Reynolds, J.D., Goodwin, N.B. and Freckleton, R.P., 2002. Evolutionary transitions in parental care and live bearing in vertebrates. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *357*(1419), pp.269-281.

Řežucha, R. and Reichard, M., 2015. Strategic exploitation of fluctuating asymmetry in male Endler's guppy courtship displays is modulated by social environment. *Journal of evolutionary biology*, *28*(2), pp.356-367.

Roche, D.G., Amcoff, M., Morgan, R., Sundin, J., Andreassen, A.H., Finnøen, M.H., Lawrence, M.J., Henderson, E., Norin, T., Speers-Roesch, B. and Brown, C., 2020. Behavioural lateralization in a detour test is not repeatable in fishes. *Animal Behaviour*, *167*, pp.55-64.

Roche, D.G., Binning, S.A., Strong, L.E., Davies, J.N. and Jennions, M.D., 2013. Increased behavioural lateralization in parasitized coral reef fish. *Behavioral Ecology and Sociobiology*, *67*(8), pp.1339-1344.

Rogers, L. and Kaplan, G., 2006. An Eye for a Predator: Lateralization in Birds, with Particular Reference. *Behavioural and morphological asymmetries in vertebrates*, p.47.

Rogers, L., 1996. Behavioral, structural and neurochemical asymmetries in the avian brain: a model system for studying visual development and processing. *Neuroscience & Biobehavioral Reviews*, *20*(3), pp.487-503.

Rogers, L., 2006. Factors influencing development of lateralization. *Cortex*.

Rogers, L.J. and Workman, L., 1993. Footedness in birds. *Animal behaviour*.

Rogers, L.J., 1989. Laterality in animals. *International Journal of Comparative Psychology*, *3*(1).

Rogers, L.J., 2000. Evolution of hemispheric specialization: advantages and disadvantages. *Brain and language*, *73*(2), pp.236-253.

Rogers, L.J., 2002. Advantages and disadvantages of lateralization. *Comparative vertebrate lateralization*, pp.126-153.

Rogers, L.J., 2009. Hand and paw preferences in relation to the lateralised brain. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *364*(1519), pp.943-954.

Rogers, L.J., Vallortigara, G. and Andrew, R.J., 2013. *Divided brains: the biology and behaviour of brain asymmetries*. Cambridge University Press.

Rogers, L.J., Zucca, P. and Vallortigara, G., 2004. Advantages of having a lateralised brain. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *271*(suppl_6), pp.S420-S422.

Romano, D., Donati, E., Canale, A., Messing, R.H., Benelli, G. and Stefanini, C., 2016. Lateralised courtship in a parasitic wasp. *Laterality: Asymmetries of Body, Brain and Cognition*, *21*(3), pp.243-254.

Royle, N.J., Smiseth, P. and Kölliker, M., 2012. The evolution of parental care: summary, conclusions and implications. In *The evolution of parental care* (pp. 327-345). Oxford University Press.

Ruxton, G.D. and Beauchamp, G., 2008. Time for some a priori thinking about post hoc testing. *Behavioral ecology*, *19*(3), pp.690-693.

Samuk, K., Iritani, D. and Schluter, D., 2014. Reversed brain size sexual dimorphism accompanies loss of parental care in white sticklebacks. *Ecology and evolution*, *4*(16), pp.3236-3243.

Sandyk, R., 1991. Relevance of the habenular complex to neuropsychiatry: a review and hypothesis. *International journal of neuroscience*, *61*(3-4), pp.189-219.

Sargent, R.C. and Gross, M.R., 1986. Williams' principle: an explanation of parental care in teleost fishes. In *The behaviour of teleost fishes* (pp. 275-293). Springer, Boston, MA.

Schaafsma, S.M. and Groothuis, T.G., 2011. Sex-specific effects of postnatal testosterone on lateralization in cichlid fish. *Animal Behaviour*, *81*(1), pp.283-288.

Scharf, I., Pamminger, T. and Foitzik, S., 2011. Differential response of ant colonies to intruders: attack strategies correlate with potential threat. *Ethology*, *117*(8), pp.731-739.

Schneider, C.A., Rasband, W.S. and Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature methods*, *9*(7), pp.671-675.

Schnell, A.K., Jozet-Alves, C., Hall, K.C., Radday, L. and Hanlon, R.T., 2019. Fighting and mating success in giant Australian cuttlefish is influenced by behavioural lateralization. *Proceedings of the Royal Society B*, *286*(1898), p.20182507.

Schuett, W. and Dall, S.R., 2009. Sex differences, social context and personality in zebra finches, Taeniopygia guttata. *Animal Behaviour*, *77*(5), pp.1041-1050.

Schwarz, I.M. and Rogers, L.J., 1992. Testosterone: a role in the development of brain asymmetry in the chick. *Neuroscience letters*, *146*(2), pp.167-170.

Shepard, K.N., Michopoulos, V., Toufexis, D.J. and Wilson, M.E., 2009. Genetic, epigenetic and environmental impact on sex differences in social behavior. *Physiology & behavior*, *97*(2), pp.157-170.

Sih, A., Bell, A. and Johnson, J.C., 2004. Behavioral syndromes: an ecological and evolutionary overview. *Trends in ecology & evolution*, *19*(7), pp.372-378.

Smith, B.R. and Blumstein, D.T., 2008. Fitness consequences of personality: a meta-analysis. *Behavioral Ecology*, *19*(2), pp.448-455.

Smith, C. and Wootton, R.J., 1994. The cost of parental care in Haplochromis ‘argens’(Cichlidae). *Environmental Biology of Fishes*, *40*(1), pp.99-104.

Snekser, J.L., Santangelo, N., Nyby, J. and Itzkowitz, M., 2011. Sex differences in biparental care as offspring develop: a field study of convict cichlids (Amatitlania siquia). *Environmental Biology of Fishes*, *91*(1), pp.15-25.

Sokal, R. R., and F. J. Rohlf. 1981. *Biometry: the principles and practice of statistics in biological research*, Freeman, San Francisco.

Soma, M. and Hasegawa, T., 2004. The effect of social facilitation and social dominance on foraging success of budgerigars in an unfamiliar environment. *Behaviour*, pp.1121-1134.

Sovrano, V.A. and Andrew, R.J., 2006. Eye use during viewing a reflection: behavioural lateralisation in zebrafish larvae. *Behavioural brain research*, *167*(2), pp.226-231.

Sovrano, V.A., 2004. Visual lateralization in response to familiar and unfamiliar stimuli in fish. *Behavioural Brain Research*, *152*(2), pp.385-391.

Sovrano, V.A., Bisazza, A. and Vallortigara, G., 2001. Lateralization of response to social stimuli in fishes: A comparison between different methods and species. *Physiology & behavior*, *74*(1-2), pp.237-244.

Sovrano, V.A., Dadda, M. and Bisazza, A., 2005. Lateralised fish perform better than nonlateralised fish in spatial reorientation tasks. *Behavioural brain research*, *163*(1), pp.122-127.

Stamps, J.A., Briffa, M. and Biro, P.A., 2012. Unpredictable animals: individual differences in intraindividual variability (IIV). *Animal Behaviour*, *83*(6), pp.1325-1334.

Stein, L.R., Trapp, R.M. and Bell, A.M., 2016. Do reproduction and parenting influence personality traits? Insights from threespine stickleback. *Animal behaviour*, *112*, pp.247-254.

Stoffel, M.A., Nakagawa, S. and Schielzeth, H., 2017. rptR: Repeatability estimation and variance decomposition by generalised linear mixed-effects models. *Methods in Ecology and Evolution*, *8*(11), pp.1639-1644.

Sundin, J. and Jutfelt, F., 2016. 9–28 d of exposure to elevated p CO2 reduces avoidance of predator odour but had no effect on behavioural lateralization or swimming activity in a temperate wrasse (Ctenolabrus rupestris). *ICES Journal of Marine Science*, *73*(3), pp.620-632.

Sundin, J. and Jutfelt, F., 2018. Effects of elevated carbon dioxide on male and female behavioural lateralization in a temperate goby. *Royal Society open science*, *5*(3), p.171550.

Sutherland, R.J., 1982. The dorsal diencephalic conduction system: a review of the anatomy and functions of the habenular complex. *Neuroscience & Biobehavioral Reviews*, *6*(1), pp.1-13.

Takeuchi, Y. and Hori, M., 2008. Behavioural laterality in the shrimp-eating cichlid fish Neolamprologus fasciatus in Lake Tanganyika. *Animal Behaviour*, *75*(4), pp.1359-1366.

Takeuchi, Y. and Oda, Y., 2017. Lateralised scale-eating behaviour of cichlid is acquired by learning to use the naturally stronger side. *Scientific reports*, *7*(1), pp.1-9.

Takeuchi, Y., Hori, M. and Oda, Y., 2012. Lateralised kinematics of predation behavior in a Lake Tanganyika scale-eating cichlid fish. *PLoS One*, *7*(1), p.e29272.

Templeton, J.J., McCracken, B.G., Sher, M. and Mountjoy, D.J., 2014. An eye for beauty: lateralised visual stimulation of courtship behavior and mate preferences in male zebra finches, Taeniopygia guttata. *Behavioural processes*, *102*, pp.33-39.

Templeton, J.J., Mountjoy, D.J., Pryke, S.R. and Griffith, S.C., 2012. In the eye of the beholder: visual mate choice lateralization in a polymorphic songbird. *Biology letters*, *8*(6), pp.924-927.

Tinbergen, N., 1952. The curious behavior of the stickleback. *Scientific American*, *187*(6), pp.22-27.

Torres-Dowdall, J., Rometsch, S.J., Aguilera, G., Goyenola, G. and Meyer, A., 2020. Asymmetry in genitalia is in sync with lateralised mating behavior but not with the lateralization of other behaviors. *Current Zoology*, *66*(1), pp.71-81.

Vallortigara, G. and Andrew, R.J., 1991. Lateralization of response by chicks to change in a model partner. *Animal Behaviour*, *41*(2), pp.187-194.

Vallortigara, G. and Andrew, R.J., 1994. Differential involvement of right and left hemisphere in individual recognition in the domestic chick. *Behavioural Processes*, *33*(1-2), pp.41-57.

Vallortigara, G. and Rogers, L., 2005. Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behavioral and brain sciences*.

Vallortigara, G., 2000. Comparative neuropsychology of the dual brain: a stroll through animals' left and right perceptual worlds. *Brain and language*, *73*(2), pp.189-219.

Vallortigara, G., 2006. The evolutionary psychology of left and right: costs and benefits of lateralization. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*, *48*(6), pp.418-427.

Vallortigara, G., Regolin, L. and Pagni, P., 1999a. Detour behaviour, imprinting and visual lateralization in the domestic chick. *Cognitive Brain Research*, *7*(3), pp.307-320.

Vallortigara, G., Rogers, L.J. and Bisazza, A., 1999b. Possible evolutionary origins of cognitive brain lateralization. *Brain Research Reviews*, *30*(2), pp.164-175.

Vallortigara, G., Rogers, L.J., Bisazza, A., Lippolis, G. and Robins, A., 1998. Complementary right and left hemifield use for predatory and agonistic behaviour in toads. *Neuroreport*, *9*(14), pp.3341-3344.

van Breukelen, N.A. and Itzkowitz, M., 2011. Mate removal leads to increase in parental defence behaviour in free-ranging convict cichlids. *Animal behaviour*, *82*(5), pp.1023-1026.

Van Iersel, J.J.A., 1953. An analysis of the parental behaviour of the male threespined stickleback (Gasterosteus aculeatus L.). *Behaviour. Supplement*, pp.III-159.

Van Valen, L., 1962. A study of fluctuating asymmetry. *Evolution*, pp.125-142.

Ventolini, N., Ferrero, E.A., Sponza, S., Della Chiesa, A., Zucca, P. and Vallortigara, G., 2005. Laterality in the wild: preferential hemifield use during predatory and sexual behaviour in the black-winged stilt. *Animal Behaviour*, *69*(5), pp.1077-1084.

Vidal, A., Perrot, C., Jasmin, J.N., Lartigau, E., Arnaud, A., Cézilly, F. and Béchet, A., 2018. Lateralization of complex behaviours in wild greater flamingos. *Animal Behaviour*, *144*, pp.67-74.

Vila Pouca, C., Gervais, C., Reed, J. and Brown, C., 2018. Incubation under climate warming affects behavioral lateralisation in Port Jackson sharks. *Symmetry*, *10*(6), p.184.

Vinogradov, I.M., Jennions, M.D., Neeman, T. and Fox, R.J., 2021. Repeatability of lateralisation in mosquitofish Gambusia holbrooki despite evidence for turn alternation in detour tests. *Animal Cognition*, pp.1-11.

Von Hippel, F. ed., 2010. *Tinbergen's legacy in behaviour: sixty years of landmark stickleback papers*. Brill.

Vossen, L.E., Jutfelt, F., Cocco, A., Thörnqvist, P.O. and Winberg, S., 2016. Zebrafish (Danio rerio) behaviour is largely unaffected by elevated pCO2. *Conservation physiology*, *4*(1).

Walker, S.F., 1980. Lateralization of functions in the vertebrate brain: A review. *British Journal of Psychology*, *71*(3), pp.329-367.

Ward, J.P., Milliken, G.W., Dodson, D.L., Stafford, D.K. and Wallace, M., 1990. Handedness as a function of sex and age in a large population of Lemur.. *Journal of Comparative Psychology*, *104*(2), p.167.

Warren, J.M., 1980. Handedness and laterality in humans and other animals. *Physiological psychology*, *8*(3), pp.351-359.

Wells, D.L., 2003. Lateralised behaviour in the domestic dog, Canis familiaris. *Behavioural processes*, *61*(1-2), pp.27-35.

Westin, L., 1990. Orientation mechanisms in migrating European silver eel (Anguilla anguilla): temperature and olfaction. *Marine Biology*, *106*(2), pp.175-179.

Westin, L., 1998. The spawning migration of European silver eel (Anguilla anguilla L.) with particular reference to stocked eel in the Baltic. *Fisheries Research*, *38*(3), pp.257-270.

Wiltschko, W., Traudt, J., Güntürkün, O., Prior, H. and Wiltschko, R., 2002. Lateralization of magnetic compass orientation in a migratory bird. *Nature*, *419*(6906), pp.467-470.

Wiper, M.L., 2017. Evolutionary and mechanistic drivers of laterality: A review and new synthesis. *Laterality: Asymmetries of Body, Brain and Cognition*, *22*(6), pp.740-770.

Wolf, M.E. and Goodale, M.A., 1987. Oral asymmetries during verbal and non-verbal movements of the mouth. *Neuropsychologia*, *25*(2), pp.375-396.

Wourms, J.P. and Lombardi, J., 1992. Reflections on the evolution of piscine viviparity. *American Zoologist*, *32*(2), pp.276-293.

Wourms, J.P., 1977. Reproduction and development in chondrichthyan fishes. *American Zoologist*, *17*(2), pp.379-410.

Zaynagutdinova, E., Karenina, K. and Giljov, A., 2020. Lateralization in monogamous pairs: wild geese prefer to keep their partner in the left hemifield except when disturbed. *Current Zoology*.

Zoidis, A.M. and Lomac-MacNair, K.S., 2017. A note on suckling behavior and laterality in nursing humpback whale calves from underwater observations. *Animals*, *7*(7), p.51.

Appendix 3

Appendix 3.1 supplementary tables

Table S3.1: The effects of sex, stage of reproduction and their interaction on A) L_R and B) L_A. For all models, individual ID was fitted as a random factor and body size as a covariate. The intercept represents females in a reproductive state. Significant p-values are highlighted in bold text.

	estimate	**s.e.**	**df**	***t***	***p***
A) Direction of laterality (L_R)					
Intercept	-3.553	34.400	154	-0.103	0.918
Sex (male)	2.020	8.475	154	0.238	0.812
Stage (non-reproductive)	-4.115	9.136	154	-0.450	0.653
Body size (cm)	2.673	7.146	154	0.374	0.709
Sex (male) x Stage (non-reproductive)	-3.060	11.893	154	-0.257	0.797
B) Strength of laterality (L_A)					
Intercept	59.388	20.198	154	2.940	**0.003**
Sex (male)	9.665	4.976	154	1.942	0.053
Stage (non-reproductive)	2.754	5.364	154	0.513	0.608
Body size (cm)	-7.046	4.196	154	-1.679	0.095
Sex (male) x Stage (non-reproductive)	-16.376	6.983	154	-2.345	**0.020**

Appendix 5

Appendix 5.1: Supplementary information for chapter 5

How the null expectation of no laterality was calculated for L_R and L_A is detailed below using R syntax.

Random expectation of no laterality for L_R across all stages of parental care for brood defence behaviours and visual hemifield use during care (Table 5.2A)

Brood defence behaviours:

Firstly the mean number of attacks performed by individuals across all three stages of parental care was calculated.

```
mean(data$attacks) #Mean = 19
```

A number of right (r) and left (l) behavioural biases was simulated for an individual that does not have any laterality when 19 brood defence attacks were performed.

Note: directional biases are drawn from a binomial distribution (rbinom).

```
r <- rbinom(1000,19,0.5)
l <- 19-r
```

A relative laterality index, L_R, was calculated based on following equation

[(right-left)/(right + left)*100].

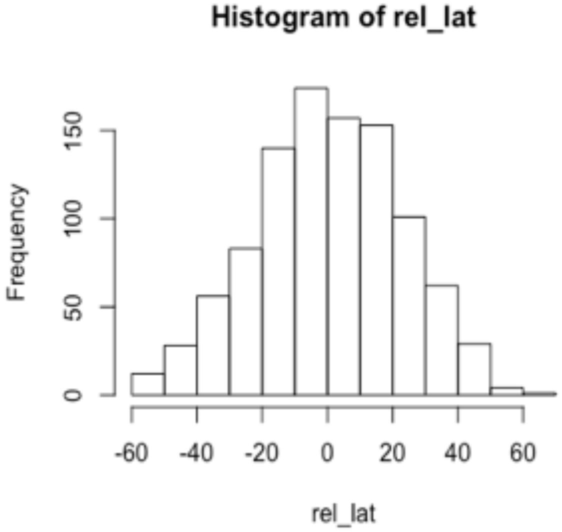
```
rel_lat <- ((r-l)/19)*100
```

A mean L_R was generated across 1000 independent simulation runs

```
mean(rel_lat)
```

The distribution of simulated L_R scores visualises the null expectation of no laterality

```
hist(rel_lat)
```



The mean relative laterality index is approximately 0.4 (across 1000 independent simulation runs).Therefore, ~0 is the null expectation of no laterality for all three components of brood defence behaviour (eye used to detect a brood predator, side of a brood predator that is preferentially attacked and the eye used to view the nest site/brood following a brood defence attack).

Visual hemifield use during care:

Firstly the number of observations of visual hemifield use during care was determined. 1x observation obtained at 30s intervals for 30m duration; n=60 observations.

```
[(30*60)/30]
```

A number of right (r) and left (l) behavioural biases is simulated for an individual that does not have any laterality when 60 observations of visual hemifield use are obtained.

```
r<-rbinom(1000,60,0.5)
l <- 60-r
```

Relative laterality indices were calculated based on following equation

[(right-left)/(right + left)*100].

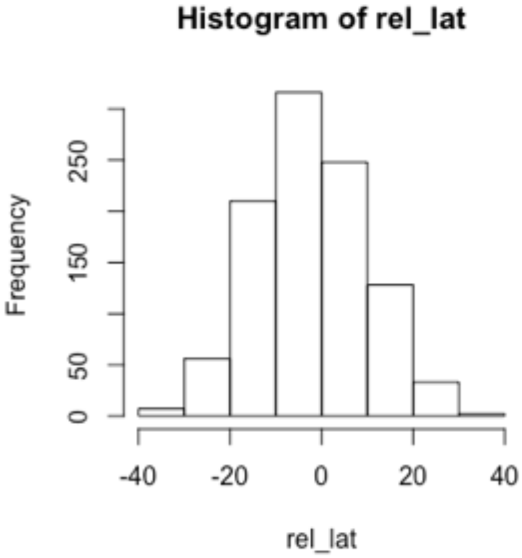
```
rel_lat <- ((l-r)/60)*100
```

A mean L_R is generated across 1000 independent simulation runs.

```
mean(rel_lat)
```

The distribution of simulated L_R scores visualises the null expectation of no laterality.

```
hist(rel_lat)
```



The mean relative laterality index is approximately -0.23 (across 1000 independent simulation runs). Therefore, ~0 is the null expectation of no laterality for both components of visual hemifield use during care (visual hemifield used to monitor brood predators and visual hemifield used to monitor a brood).

Random expectation of no laterality for L_A across all stages of parental care for brood defence behaviours and visual hemifield use during care (Table 5.2B)

Brood defence behaviours:

Firstly the mean number of attacks performed by individuals across all three stages of parental care was calculated.

```
mean(data$attacks) #Mean = 19
```

A number of right (r) and left (l) behavioural biases is simulated for an individual that does not have any laterality when 19 brood defence attacks are performed.

```
r <- rbinom(1000,19,0.5)
l <- 19-r
```

Relative and absolute laterality indices were calculated based on following equation [(right-left)/(right + left)*100].

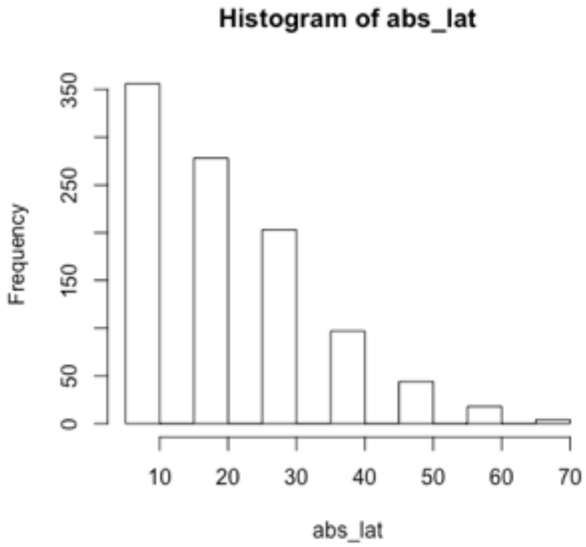
```
rel_lat <- ((r-l)/19)*100
abs_lat <- abs(rel_lat)
```

A mean L_A is generated across 1000 independent simulation runs.

```
mean(abs_lat)
```

The distribution of simulated L_A scores visualises the null expectation of no laterality.

```
hist(abs_lat)
```



The mean absolute laterality index is approximately 19 (across 1000 independent simulation runs). Therefore, ~19 is the null expectation of no laterality for L_A for all three components of brood defence behaviour (eye used to detect a brood predator, side of a brood predator that is preferentially attacked and the eye used to view the nest site/brood following a brood defence attack).

Visual hemifield use during care:

Firstly the number of observations of visual hemifield use during care were determined. 1x observation obtained at 30s intervals for 30m duration; n=60 observations.

```
[(30*60)/30]
```

A number of right (r) and left (l) behavioural biases were simulated for an individual that does not have any laterality when 60 observations of visual hemifield use are obtained.

```
r<-rbinom(1000,60,0.5)
l <- 60-r
```

Relative and absolute laterality indices were calculated based on following equation [(right-left)/(right + left)*100].

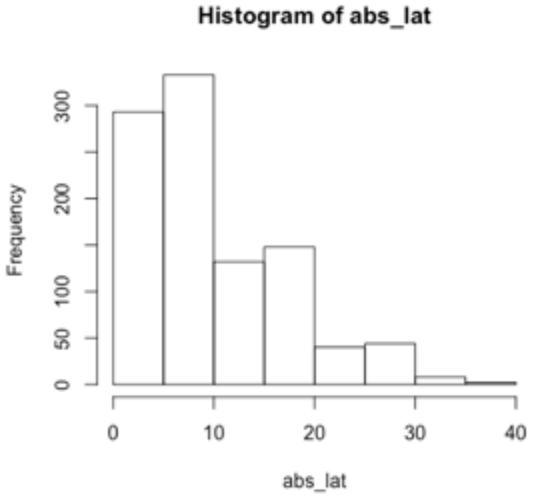
```
rel_lat <- ((l-r)/60)*100
abs_lat <- abs(rel_lat)
```

A mean L_A was generated across 1000 independent simulation runs.

```
mean(abs_lat)
```

The distribution of simulated L_A scores visualises the null expectation of no laterality.

```
hist(abs_lat)
```



The mean absolute laterality index is approximately 10 (across 1000 independent simulation runs).Therefore, ~10 is the null expectation of no laterality, not 0, for L_A in both components of visual hemifield use during care (visual hemifield used to monitor brood predators and visual hemifield used to monitor a brood).

Random expectation of no laterality, L_A, across all parental care behaviours for each sex during each stage of parental care (Table 5.4)

Brood defence behaviours & visual hemifield use:

Firstly a mean number of attacks was calculated for each sex at each stage of parental care Note: data here represents a subset of data that contains a single sex at a specific stage of parental care e.g. males at the post laying stage of care.

```
mean(data$attacks)
```

mean no. of brood defence attacks by males at the post laying stage of care = 19

mean no. of brood defence attacks by females at the post laying stage of care = 24

mean no. of brood defence attacks by males at the early post hatch stage of care = 22

mean no. of brood defence attacks by females at the early post hatch stage of care = 15

mean no. of brood defence attacks by males at the late post hatch stage of care = 23

mean no. of brood defence attacks by females at the late post hatch stage of care = 21

A number of right (r) and left (l) directional biases in brood defence behaviour are simulated for an individual that does not have any laterality using the mean number of attacks performed by each sex and for each stage.

For males at the post laying stage of care:

```
r <- rbinom(1000,19,0.5)
l <- 19-r
```

Relative and absolute laterality indices were calculated based on following equation [(right-left)/(right + left)*100].

```
rel_lat <- ((r-l)/19)*100
abs_lat <- abs(rel_lat)
```

A mean L_A was generated across 1000 independent simulation runs.

```
mean(abs_lat)
```

The mean absolute laterality index is approximately 18 (across 1000 independent simulation runs).

A number of right (r) and left (l) biases in visual hemifield use during care was then simulated for an individual that does not have any laterality when 60 observations of visual hemifield use are obtained for each sex at each stage of parental care.

For males at the post laying stage of care:

```
r <- rbinom(1000,60,0.5)
l <- 60-r
```

Relative and absolute laterality indices were calculated based on following equation [(right-left)/(right + left)*100].

```
rel_lat <- ((r-l)/60)*100
abs_lat <- abs(rel_lat)
```

A mean L_A was generated across 1000 independent simulation runs.

```
mean(abs_lat)
```

The mean absolute laterality index is approximately 10 (across 1000 independent simulation runs).

To generate a mean L_A across all five components of parental care examined for each sex at each stage of parental care, a mean generated from the mean L_A of brood defence behaviours and mean L_A of visual hemifield use was then calculated.

```
mean((10+18)/2)
```

The mean absolute laterality index across all components of parental care examined is approximately 14 (across 1000 independent simulation runs).Therefore, ~14 is the null expectation of no laterality, not 0, for L_A across all components of parental care behaviour for males in the post egg laying period of care.

Below is the calculations of a null expectation of no laterality , L_A, across all parental care behaviours for each sex during each stage of parental care examined:

For females in the post egg laying period of care (mean no. of attacks =24):

Brood defence behaviour:

```
r <- rbinom(1000,24,0.5)

l <- 24-r

rel_lat <- ((r-l)/24)*100

abs_lat <- abs(rel_lat)

mean(abs_lat);   mean LA = 16
```

visual hemifield use during care:

```
r <- rbinom(1000,60,0.5)

l <- 60-r

rel_lat <- ((r-l)/60)*100

abs_lat <- abs(rel_lat)

mean(abs_lat); mean LA = 10

mean((10+16)/2)
```

The mean absolute laterality index across all components of parental care examined is approximately 13 (across 1000 independent simulation runs).Therefore, ~13 is the null expectation of no laterality, not 0, for L_A across all components of parental care behaviour for females in the post egg laying period of care.

For males in the early post hatch period of care (mean no. of attacks =22):

Brood defence behaviour:

```
r <- rbinom(1000,22,0.5)

l <- 22-r

rel_lat <- ((r-l)/22)*100

abs_lat <- abs(rel_lat)

mean(abs_lat);   mean L_A = 17
```

visual hemifield use during care:

```
r <- rbinom(1000,60,0.5)

l <- 60-r

rel_lat <- ((r-l)/60)*100

abs_lat <- abs(rel_lat)

mean(abs_lat); mean L_A = 10

mean((10+17)/2)
```

The mean absolute laterality index across all components of parental care examined is approximately 13.5 (across 1000 independent simulation runs).Therefore, ~13.5 is the null expectation of no laterality, not 0, for L_A across all components of parental care behaviour for males in the early post hatch period of care.

For females in the early post hatch period of care (mean no. of attacks =15):

Brood defence behaviour:

```
r <- rbinom(1000,15,0.5)

l <- 15-r

rel_lat <- ((r-l)/15)*100

abs_lat <- abs(rel_lat)

mean(abs_lat);   mean L_A = 20
```

visual hemifield use during care:

```
r <- rbinom(1000,60,0.5)

l <- 60-r

rel_lat <- ((r-l)/60)*100

abs_lat <- abs(rel_lat)

mean(abs_lat); mean L_A = 10

mean((10+20)/2)
```

The mean absolute laterality index across all components of parental care examined is approximately 15 (across 1000 independent simulation runs).Therefore, ~15 is the null expectation of no laterality, not 0, for L_A across all components of parental care behaviour for females in the early post hatch period of care.

For males in the late post hatch period of care (mean no. of attacks = 23):

Brood defence behaviour:

```
r <- rbinom(1000,23,0.5)

l <- 23-r

rel_lat <- ((r-l)/23)*100

abs_lat <- abs(rel_lat)

mean(abs_lat);   mean LA = 16
```

visual hemifield use during care:

```
r <- rbinom(1000,60,0.5)

l <- 60-r

rel_lat <- ((r-l)/60)*100

abs_lat <- abs(rel_lat)

mean(abs_lat); mean LA = 10

mean((10+16)/2)
```

The mean absolute laterality index across all components of parental care examined is approximately 13 (across 1000 independent simulation runs).Therefore, ~13 is the null expectation of no laterality, not 0, for L_A across all components of parental care behaviour for males in the late post hatch period of care.

For females in the late post hatch period of care (mean no. of attacks = 21):

Brood defence behaviour:

```
r <- rbinom(1000,21,0.5)

l <- 21-r

rel_lat <- ((r-l)/21)*100

abs_lat <- abs(rel_lat)

mean(abs_lat);   mean LA = 17
```

visual hemifield use during care:

```
r <- rbinom(1000,60,0.5)

l <- 60-r

rel_lat <- ((r-l)/60)*100

abs_lat <- abs(rel_lat)

mean(abs_lat); mean LA = 10

mean((10+17)/2)
```

The mean absolute laterality index across all components of parental care examined is approximately 13.5 (across 1000 independent simulation runs).Therefore, ~13.5 is the null expectation of no laterality, not 0, for L_A across all components of parental care behaviour for females in the late post hatch period of care.

Appendix 6

Appendix 6.1: supplementary methodology

Methodology to assess the strength of laterality in parental care behaviours at the individual level

To examine whether individuals exhibited significant individual level laterality in parental care behaviours in the biparental control treatment (chapter 5) and uniparental treatment (chapter 6) prior to female mate removals in the post-lay period of care, a mean L_A was calculated for each individual for each parental care behaviour. One-sample t-tests were used to examine whether the mean L_A for each parental care behaviour differed from a null expectation of no laterality (see below for the calculation of null expectation of no laterality), in the biparental and uniparental treatments. The false discovery rate method was used to correct for multiple testing and the adjusted p-values are reported here (Benjamini and Hochberg 1995).

Calculation of a null expectation of no laterality, L_A in parental care behaviours

How the null expectation of no laterality (L_A) was calculated during the post-lay period in the biparental treatment (chapter 5) and uniparental treatment (chapter 6) is detailed below using R syntax.

Random expectation of no laterality for L_A in brood defence behaviours for chapter 5 and chapter 6:

Firstly the mean number of attacks performed by individuals in the post lay stage in chapter 5 and chapter 6 were calculated.

Chapter 5:

```
mean(data.five$attacks) #Mean = 20
```

Chapter 6:

```
mean(data.six$attacks) #Mean = 28
```

A number of right (r) and left (l) behavioural biases was simulated for an individual that does not have any laterality when 20 [chapter 5] and 28 [chapter 6] brood defence attacks were performed.

Note: directional biases are drawn from a binomial distribution (rbinom).

Chapter 5:

```
r <- rbinom(1000,20,0.5)

l <- 20-r
```

Chapter 6:

```
r <- rbinom(1000,28,0.5)

l <- 28-r
```

A relative laterality index, L_R, was calculated based on following equation

[(right-left)/(right + left)*100]. An absolute laterality index (L_A) was then calculated as $|L_R|$.

Chapter 5:

```
rel_lat <- ((r-l)/20)*100

abs_lat <- abs(rel_lat)
```

Chapter 6:

```
rel_lat <- ((r-l)/28)*100

abs_lat <- abs(rel_lat)
```

A mean L_A was generated across 1000 independent simulation runs

Chapter 5:

```
mean(abs_lat) # 17
```

Chapter 6:

```
mean(abs_lat) # 14
```

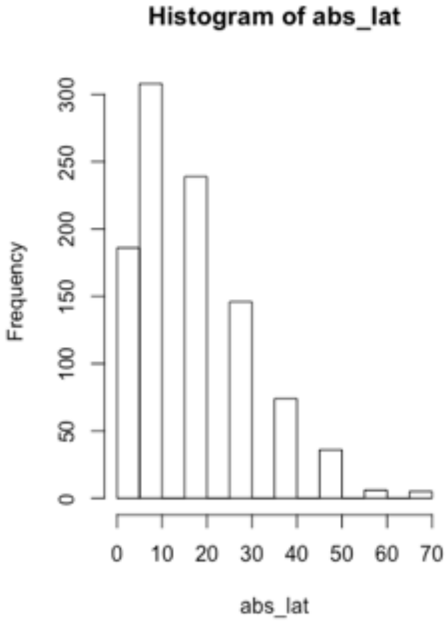
The distribution of simulated L_A scores visualises the null expectation of no laterality

Chapter 5:

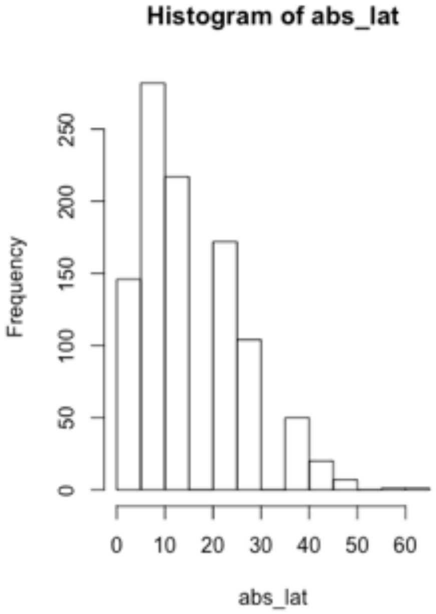
```
hist(abs_lat)
```

Chapter 6:

```
hist(abs_lat)
```



Chapter 5: the mean L_A is approximately 17 (across 1000 independent simulation runs).Therefore, ~17 is the null expectation of no laterality for all three components of brood defence behaviour (eye used to detect a brood predator, side of a brood predator that is preferentially attacked and the eye used to view the nest site/brood following a brood defence attack).



Chapter 6: the mean L_A is approximately 14 (across 1000 independent simulation runs).Therefore, ~14 is the null expectation of no laterality for all three components of brood defence behaviour (eye used to detect a brood predator, side of a brood predator that is preferentially attacked and the eye used to view the nest site/brood following a brood defence attack).

Random expectation of no laterality for L_A of visual hemisphere use during care for chapter *5 and* chapter *6:*

Firstly the number of observations of visual hemifield use during care was determined. 1x observation obtained at 30s intervals for 30m duration; n=60 observations for both chapter 5 and 6.

```
[(30*60)/30]
```

A number of right (r) and left (l) behavioural biases is simulated for an individual that does not have any laterality when 60 observations of visual hemifield use are obtained.

```
r<-rbinom(1000,60,0.5)
l <- 60-r
```

Relative and absolute laterality indices were calculated based on following equation [(right-left)/(right + left)*100].

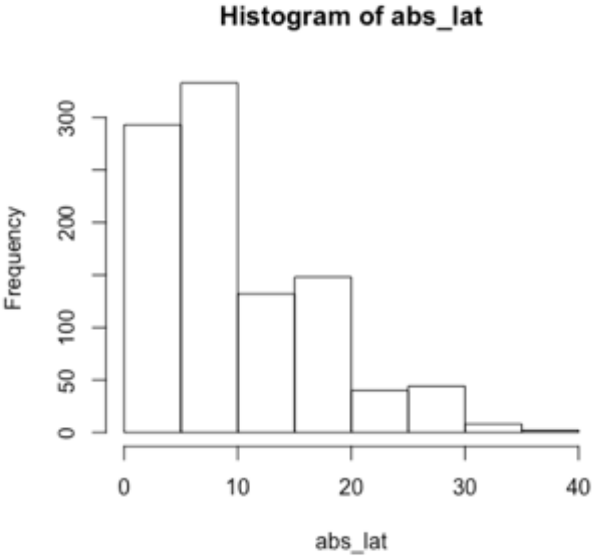
```
rel_lat <- ((l-r)/60)*100

abs_lat <- abs(rel_lat)
```

A mean L_A is generated across 1000 independent simulation runs.

```
mean(abs_lat)
```

The distribution of simulated L_A scores visualises the null expectation of no laterality.

```
hist(abs_lat)
```



The mean absolute laterality index, L_A is approximately 10 (across 1000 independent simulation runs).Therefore, ~10 is the null expectation of no laterality, not 0, for L_A in both components of visual hemifield use during care (visual hemifield used to monitor brood predators and visual hemifield used to monitor a brood).

Appendix 6.2: supplementary tables

Table S6.2.1: Results of linear mixed effects models examining factors affecting the number of brood defence attacks performed in the post lay period of care when biparental care is performed in chapter 5 and chapter 6. The minimum adequate model containing all main effects, regardless of significance, is reported here. Significant p-values are highlighted in bold. The intercept represents female *T. temporalis* from chapter 6.

	estimate	**s.e.**	**df**	**t**	**p**
Intercept	27.869	6.228	4.875	4.475	**0.006**
Sex (male)	2.753	4.256	33.978	0.647	0.522
Experiment (experiment 5)	-9.408	5.272	35.715	-1.784	0.082

Table S6.2.2: Results of linear mixed effects models examining factors affecting the A) L_R and B) L_A of parental care behaviours in the post lay period of care when biparental care was performed in chapter 5 and chapter 6. The minimum adequate model containing all main effects, regardless of significance, is reported here. Significant p-values are highlighted in bold. The intercept represents females from chapter 6 detecting brood predators during care.

	estimate	**s.e.**	**df**	**t**	**p**
A) L_R as response variable					
Intercept	2.105	3.788	151.810	0.556	0.579
Sex					
male	-2.217	2.702	525.802	-0.820	0.412
Experiment					
experiment 5	2.283	3.502	40.815	0.652	0.518
Parental care behaviour					
Attack brood predator	-11.421	4.256	503.660	-2.683	**0.007**
Return to nest site	0.713	4.256	503.660	0.168	0.867
monitor offspring	6.716	4.228	504.400	1.589	0.112
monitor brood predators	-14.212	4.228	504.400	-3.361	**< 0.001**
B) L_A as response variable					
Intercept	20.329	2.983	14.181	6.918	**< 0.001**
Sex					
male	1.103	2.285	37.421	0.483	0.632
Experiment					
experiment 5	9.625	2.381	45.636	4.043	**<0.001**
Parental care behaviour					
attack brood predator	5.027	2.641	476.095	1.904	0.057
return to nest site	2.002	2.641	476.095	0.758	0.448
monitor offspring	-1.710	2.625	479.027	-0.652	0.515
monitor brood predators	-3.006	2.625	479.027	-1.145	0.252

Table S6.2.3: Results of one-sample t-tests examining if the mean L_A in A) biparental control treatment (chapter 5) and B) uniparental treatment (chapter 6) differed from random choices for each of the components of brood defence behaviour and visual hemisphere use during care that were examined. P-values are corrected for multiple testing using the false discovery rate method (Benjamini and Hochberg 1995) and highlighted in bold if significant.

	A) Biparental care			**B) Uniparental care**		
Parental Care Behaviour	**df**	**t**	**p**	**df**	**t**	**p**
Brood defence attacks						
Predator detected	18	2.903	**0.009**	34	3.303	**0.005**
Predator attacked	18	2.995	**0.008**	34	4.536	**< 0.001**
View nest post attack	18	3.363	**0.004**	34	3.869	**< 0.001**
Visual hemisphere use						
Monitor predators	20	6.095	**< 0.001**	33	4.379	**< 0.001**
Monitor nest site	20	4.587	**< 0.001**	33	6.166	**< 0.001**