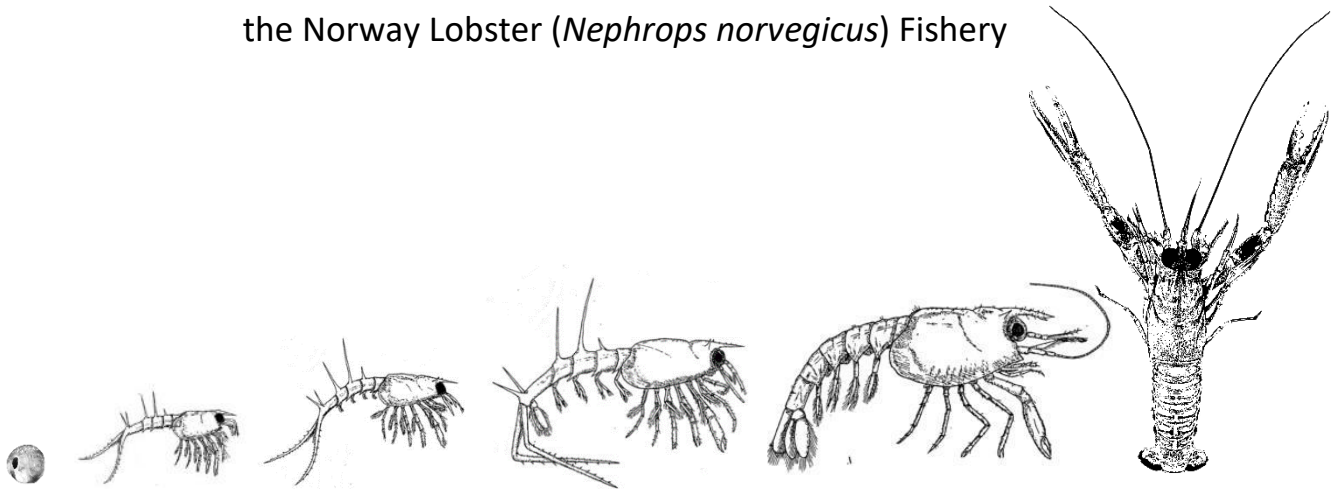
THE UNIVERSITY OF HULL

Optimising Commercial Practices and Developing Aquaculture Techniques for the Norway Lobster (*Nephrops norvegicus*) Fishery



being a Thesis submitted for the Degree of PhD (Doctor of Philosophy)

in the University of Hull

by

Daniel Cowing (BSc, MSc)

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Supervisors:

Dr Magnus Johnson (University of Hull)

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Candidate Declaration

I declare that the work recorded in this thesis is my own, unless otherwise stated and that it is of my own composition. No part of this thesis has been submitted for any other degree.

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Glossary*

Anaesthesia	Full loss of sensory perception, the state after sedation.
Agonistic	Aggressive behaviour.
Berried / Ovigerous	Carrying eggs / embryos.
Broodstock / hens	Group of sexually mature individuals used for breeding or to supply larvae. Only ovigerous females are referred to within this thesis, which are for used for their supply of embryos and subsequent larval release.
Biramous	Dividing into two branches.
Carapace Length	Length measurement from the rear of the eye socket to the rear of the carapace.
Circadian	A biological process occurring on a twenty-four hour cycle.
Coagulative	To congeal or form a clot.
Conicals / cylindrico-conical tanks	A circular larval rearing tank with a sloped bottom.
Conspecifics	Individual of the same species.
Creel	A type of basket or pot used to capture or hold Nephrops, lobsters, crabs and prawns.
Cytotoxic	Killing bacteria or cells.
Ecdysis	The process of moulting or shredding the exoskeleton.
Embryo(s)	Part of the development of the larva before hatching.
Enrichment	To add nutrients to *Artemia* that have been lost during development.
Haemolymph	Circulatory fluid in arthropods, analogous to blood.
Homeostasis	Physiological equilibrium.
Inhalation	Ventilated through the gills.
Inter-individual sequence	How the behaviour of one individual impacts upon the behaviour of another.
Intermoult	The time period between ecdysis.
Interspecific	Occurring between different species.
Intraspecific	Occurring within the same species.
Juveniles	The post larval stages between metamorphosis at zoea stage 4 and sexual maturity.
Mechanoreceptors	Sensory organ for mechanical stimulation.
Meral Spread	A posture where the chelae are raised and spread laterally.
Metamorphosis	The process of transformation from larval form to resemble that of an adult.
Olfactory sensilla	Sensory organ for chemical and pheromones etc.
Ontogenetic	Development of an animal through distinct phases.
Parenteral	Administration by the means of an injection.
Phagocytic	Ingesting foreign particles, bacteria or cells.
Phototaxic / phototaxis	Movement towards (positive) or away (negative) from light.
Prezoea	A hatched larva before it shreds it embryonic cuticle.

Recirculating Aquaculture System (RAS)	Water from the rearing tanks is filtered and reused.
Recumbency	To lie down, can be on the lateral or dorsal sides when referred to Nephrops.
Rheotactic	Orientation towards a water current.
Sedation	Loss of sensory perception, preliminary state of anaesthesia.
Spermatophores	A protein capsule containing spermatozoa.
Thigmotactic	Positive (towards) or negative (away) movement from a physical object.
Velvet box	A storage box used in fisheries merchants to store and transport live velvet crabs.

***The author has freely explained some of the terms used within the thesis, and so they should not be used as textbook definitions.**

Publications

Cowing, D.M., Powell, A., Johnson, M.L., 2015. Evaluation of different concentration doses of eugenol on the behaviour of *Nephrops norvegicus*. Aquaculture 442, 78-85.

The NEPHROPS Manual. 2015. Patent application number GB1509666.

Powell, A., Cowing, D.M., Eriksson, S.P. and Johnson, M.L., 2017. Stress response according to transport procedures in Norway lobster, *Nephrops norvegicus*. Crustacean Research 46, 17-24.

Powell, A., Barrento, S., Cowing, D.M., In Prep. Management and handling of commercial crustaceans. In: Lovrich, G., Thiel, M., In prep. Fisheries and Aquaculture: The Natural History of Crustacea (Volume 9).

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Abstract

The *Nephrops norvegicus* (referred to as *Nephrops*) fishery is regarded as one of the most important fisheries around the UK, yet data suggest that landings are declining. The role of hatcheries for other crustacean species has proved useful for stock enhancement and aquaculture. *Nephrops* larviculture has been relatively unsuccessful, with low survival to post larval stages. This thesis comprises four studies aimed at developing key stages that are important for optimising fishing related practices and the development of aquaculture for this species. In **chapter 3**, the social behaviour of adult *Nephrops* is examined in conjunction with physiological parameters. The findings indicate that the behavioural interactions have a physiological cost that is associated with the sex and size of the individual and the opponent. The findings are useful for developing fishing practices and broodstock holding and highlight the complexity of correlating physiological parameters with social stress. In **chapter 4**, a method for sedating and anaesthetising Norway lobster is investigated and successfully identified. Key behaviours are related to sedation stages as well as to several tested dosages. These techniques could be used to lower stress in Norway lobster during transport and handling. In **chapter 5**, several diets and feeding frequencies are examined for their potential in increasing larval survival and growth. The highest survival and growth were found in the enriched *Artemia* diets, with a continuous feeding frequency. Larvae fed on wild zooplankton had lower survival and delayed growth, especially for stage 1 larvae. The addition of probiotics yielded promising results in terms of better survival and less variation when compared to an enriched *Artemia* diet alone. Survival of larvae was increased to ~40% which is an improvement on previous findings and suggests further investigation for optimising larviculture techniques. In **chapter 6**, the behaviour of post larvae around an adult burrow and an alternative habitat is investigated. The post larvae showed a preference for entering the burrow when it was unoccupied, but would show vigilant behaviour when it was occupied. The post larvae would also find shelter in alternative habitats such as cobbles, which could be a promising application in future release protocols for this species. The results from the thesis demonstrate that *Nephrops* aquaculture for stock enhancement can currently take place, and that techniques for holding and transporting broodstock can be improved.

1. General Introduction

Nephrops norvegicus (Linnaeus, 1758) (referred to as *Nephrops* from here on) is a benthic decapod crustacean residing at depths of between 20 – 800 m and inhabiting areas of suitable muddy substrate, in which they build burrows (Farmer, 1975). *Nephrops* are of significant value to the UK, worth around £100.5 million (in 2016) and of total catch of approximately 30,700 tonnes, the majority of which are caught in Scotland (20,000 tonnes) (MMO, 2016). Around half the UK catch is exported to Spain, France and Italy using live transport methods (Ungfors et al., 2013). Trawl-caught *Nephrops* account for a large proportion of the total *Nephrops* catch, but survival rates are low, limiting supply for the profitable live market (Leocádio et al., 2012). Creel-caught *Nephrops* are a small proportion of the fishery, are usually obtained in inshore waters and represent a more sustainable fishery with higher survival of both caught and discarded individuals (Ziegler and Valentinsson, 2008). The rationale for the development of the thesis was to assist in offering the *Nephrops* creel fishing sector options for expansion whilst creating economic and social benefits. However, simply increasing the fishing effort is not necessarily beneficial for the fishery and the lack of economic incentives in the past has limited the development of the *Nephrops* creel fishery. An initiative such as stock enhancement is seen as a positive development in giving fishers more control of their fishery. Historically, stock enhancement activities are typically led in part by the fishers themselves and in theory receive the benefits of increased recruitment to there fishing grounds from which they gather the broodstock (see glossary for definition). Aquaculture and stock enhancement activities have been used for *Homarus* spp. as a method to boost public awareness, potentially increase recruitment to the fishery and enable fishers to "fish smarter". Both Burton (2003) and Olst et al. (1980) provide a comprehensive review of release strategies and on-growing equipment used for stock enhancement and the aquaculture of lobsters. Seeding areas with *Nephrops* could allow fishers to have greater control over their local habitat at a time when new challenges are appearing such as the new landing obligation (discard ban) and an uncertain future caused by the withdrawal from the European Union (EU). This thesis aims to develop the techniques needed for hatchery rearing and aquaculture of *Nephrops*. Different aquaculture objectives and systems are described in table 1.1 that could be applied to *Nephrops*. The potential for stock enhancement, sea ranching or the establishment of new grounds with hatchery reared larvae or trawl caught

discards could be explored within the fishery, increasing the options available to fishers for a more sustainable, economically viable fishery. However, Kitada (2018) indicates that the average recapture rates (% ± SD) of European lobster in European stock enhancement programmes are at 4.2 ± 3.6 % and that the programmes are not economically viable from an aquaculture perspective. An increase of ~15% in recapture rates along with a ~50% reduction in production costs are identified as aspects that would facilitate the economic viability of lobster stock enhancement (Kitada, 2018). The viability of using such techniques with *Nephrops* remains to be answered, and therefore the viability of *Nephrops* aquaculture is not verified.

In addition, there are major questions whether crustacean stock enhancement activities has major impacts upon the local ecology, the ecosystem as well as whether they are necessary or counterproductive. A set of logical arguments are detailed below;

- **The implication of additional recruitment above that of "normal" survival levels of released hatchery developed individuals into resident populations**; mortality of *Nephrops* larvae (zoea 1-3) in wild populations is estimated to be at approximately 50%, but as high as 97% (Briggs, 2002; Chapman, 1980; Nichols et al., 1987; Smith, 1987). Could this "increase" of stock have possible influences upon the fisheries dynamics independent on whether or not the juveniles (see glossary) survive after release.
- **The ecological implications of releasing naive individuals.** The addition of incoming stock can have impacts of both inter- and intra-specific populations. For example, it is accepted that size-density effects *Nephrops* (Johnson et al., 2013), and therefore will the release of extra individuals to an area cause lower growth for that spatially retained population, or even limit the recruitment to the fishery as suggested for within *Homarus* spp. release programmes (Ellis et al., 2015). Could the carrying capacity be pushed beyond its threshold and therefore be non-viable?
- **Is the larviculture process artificially selecting specific genetic traits** that favour development in the hatchery environment, and therefore lowering the genetic diversity within the population? The understanding of the gene flow between several *Nephrops* sub-populations would indicate the spatial scale of which genetic

differences occur and the scale of which larvae can contribute to other sub-populations.

- **Would there be potential conflict over the ownership of released individuals?** Presuming the release activities will be carried out by the stakeholders that are supportive of the hatchery and should therefore be the beneficiary of the increased stock. Current research activities by NUI Galway indicates that ~20% of tagged individuals were recaptured after four months from release (Power, pers comm). Therefore, it indicates *Nephrops* have relatively high retention rates upon suitable grounds. Consequently, the fishers who remain fishing on the release grounds, should in theory benefit. However further investigation into displacement of released *Nephrops*, larval spatial dispersal, and the governance over the rights to appropriately identified hatchery bred individuals should take place.

There is limited contemporary literature focusing on the larviculture of *Nephrops* and the associated biology, physiology and behaviour, which hinders *Nephrops* aquaculture and hatchery development.

Table 1.1 Comparison of different aquaculture enhancement systems. Taken from Lorenzen et al. (2010).

	Sea ranching	Stock Enhancement	Re-stocking	Supplementation	Re-introduction
Aim of enhancement	Increase fisheries catch	Increase fisheries catch while conserving / increasing naturally occurring stock	Rebuild depleted wild stock to higher abundance	Reduce extinction risk and conserve genetic diversity in small populations	Re-establish populations in historical range
Wild population status	Absent of insignificant	Numerically large. Possibly depleted relative to carrying capacity	Numerically large or small. Depleted relative to carrying capacity	Numerically small. Possibly declining. At risk of extinction	Locally extinct
Aquaculture management	Production orientation. Partial domestication. Conditioning for release. Possible induced sterility.	Integrated programmes: as for re-stocking. Separated programmes: as for sea ranching	Conservation orientated. Minimize domestication. Conditioning for release	Conservation orientated. Minimize domestication. Conditioning for release	Conservation orientated. Minimize domestication. Conditioning for release
Genetic management	Maintain genetic diversity. Selection for high return	Integrated programmes: as for re-stocking. Separated programmes: as for sea ranching: also selection to promote separation	Preserve all wild population genetic characteristics	Preserve all wild population genetic characteristics. Maximize effective population size in hatchery	Assemble diversity of adaptions or use stocks adapted to similar habitats
Population management	Stocking and harvesting to create desired population structure	Integrated programmes: Restricted stocking and harvesting to increase catch while conserving naturally recruiting stock Separated programmes: as for sea ranching: also measures to promote separation	High stocking density over short period; temporarily restricted harvesting or moratorium	Moderate stocking density relative to wild population. No / restricted harvesting	Low stocking density but sufficient for establishment. Minimal harvesting

1.1. The Fishery and General Behaviour

Nephrops have a geographical range that is defined by both the temperature and the habitat in which they live. They are currently fished in the North Eastern Atlantic, the North Sea and the Adriatic Sea. The fishery is divided into 34 functional units, each with separate fishing grounds, and formed into groupings of several units for stock assessment purposes and fisheries management (Ungfors et al., 2013). Stock assessment is primarily based on the size composition of the catches and abundance. Abundance is estimated by using burrow density information captured by underwater television (UWTV) surveys. A conversion factor is applied to the burrow count to estimate the absolute abundance. ICES use reference points, which is calculated from stock abundance and fishing data, to advise and manage the fishing effort and quota. Advice is usually based on two approaches: the Maximum Sustainable Yield (MSY) approach, meaning that the fishing effort is at a level that lands the highest yield which is not detrimental to the long-term recruitment of the population. This is achieved by maintaining a spawning stock biomass above an action point termed MSY $B_{trigger}$. The second approach is a precautionary approach, where the level of fishing effort or quota is set at a conservative level so it does not impact on the level of recruitment. Fishing induced mortality that is currently (2018) above the MSY (F_{MSY}) trigger point are shown in several stocks around the UK (Moray Firth, Farn Deeps, Forth of Clyde and Sound of Jura, Bristol Channel and the Aran grounds), however the stock abundance estimates of all except one fishing population are above the MSY $B_{trigger}$ (Aran grounds), see Appendix 2 for a comparison of the fishing grounds around the UK. Around 31 thousand tonnes of *Nephrops* were landed in the UK and abroad by UK vessels in 2016, which is a 29% reduction compared to the landings recorded in 2012 (MMO, 2016).

The majority of *Nephrops* landed are caught by trawl, the majority of which are sold as frozen tail meat, whereas the creel fishery targets the live trade, typically demanding a higher price per tonne. The creel fishing method is less invasive and stressful for the individuals, which enables them to be kept alive during capture and throughout the onward supply chain (Leocádio et al., 2012). In some cases, such as in Portugal, the *Nephrops* creel fishery is more economically viable than the trawl fishery, which relies on catching additional species (Leocádio et al., 2012).

Adults are caught at a minimum landing size of 20-40mm carapace length (CL), dependent upon location and the fisheries management unit. The size at onset of maturity (SOM) differs with geographical location, with smaller mature females in the northern locations, *i.e.* in the Atlantic and around the UK, when compared Mediterranean populations (Powell and Eriksson, 2013). To assess the maturity status, males are examined for spermatophores and the females for the development and stage of the ovaries (Ungfors et al., 2013). The ovary cycle is either annual or biennial and has been described variously as having either five or nine stages. The five-staged description is typically used and a comprehensive review is provided in Powell and Eriksson, 2013. The stage of the ovary may be determined by colour and volume as indicated in Figure 1.1 (Farmer, 1974a; Smith, 1987; Powell and Eriksson, 2013).

Farmer (1974) described the external sexual development for both males and females and indicated that males reach sexual maturity at a carapace length of 26mm and females at a length of 20mm.

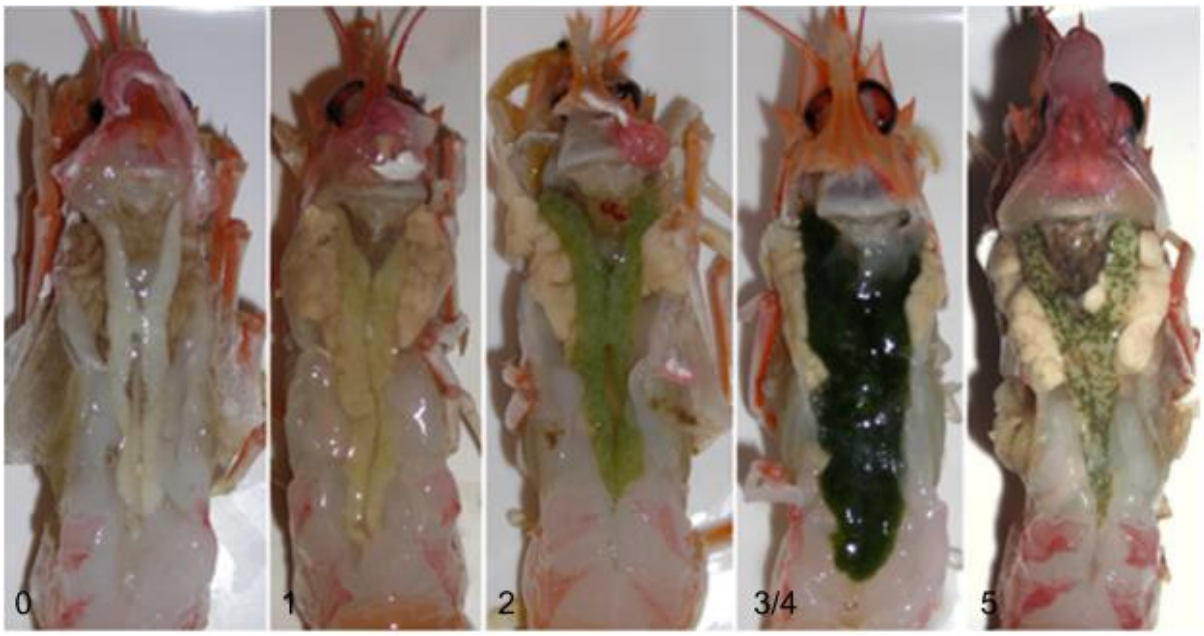

Figure 1.1 The five-stage ovary development, determined by colour in mature *Nephrops.* Taken from Powell & Eriksson (2013).

The adults construct burrows within the sediment, which provide them with increased protection from predators. The suitable habitat for burrow construction has been investigated by Campbell et al. (2009) for which the sediment needs to be cohesive for the excavated tunnels to remain in the structural form. The most abundant burrow populations

are in habitats with particle sizes that are classed as silt and clay with mud (muddy sand), as seen in Figure 1.2 (Campbell et al., 2009).

The burrow density upon suitable habitat influences the rate of growth and size of the sub-population. The high-density populations are smaller in size than low density populations, and this could be attributed to the increased competition for resources such as food and shelter (Tuck et al., 1997).

Nephrops exhibit certain circadian rhythmic behaviours. During specific low light level ranges, the adults are more active and undergo burrow emergence behaviours in which they forage for food and compete for new shelters (Rice and Chapman, 1971; Farmer, 1975). Being out of the burrow significantly increases the chance of being caught from fishing trawls, entrapment in creels and predation. There are several predators for *Nephrops,* with cod (*Gadus morha*) having the highest proportion of stomachs (80%) containing *Nephrops* (Thomas, 1965; Bell et al., 2013). However recent investigations indicate that the number of stomachs that contain *Nephrops* has been steadily declining due to the reduction of larger predators (such as cod) and represents only a relatively small proportion of the total mortality when compared to fishing induced mortality (Farmer, 1975; Johnson et al., 2013).

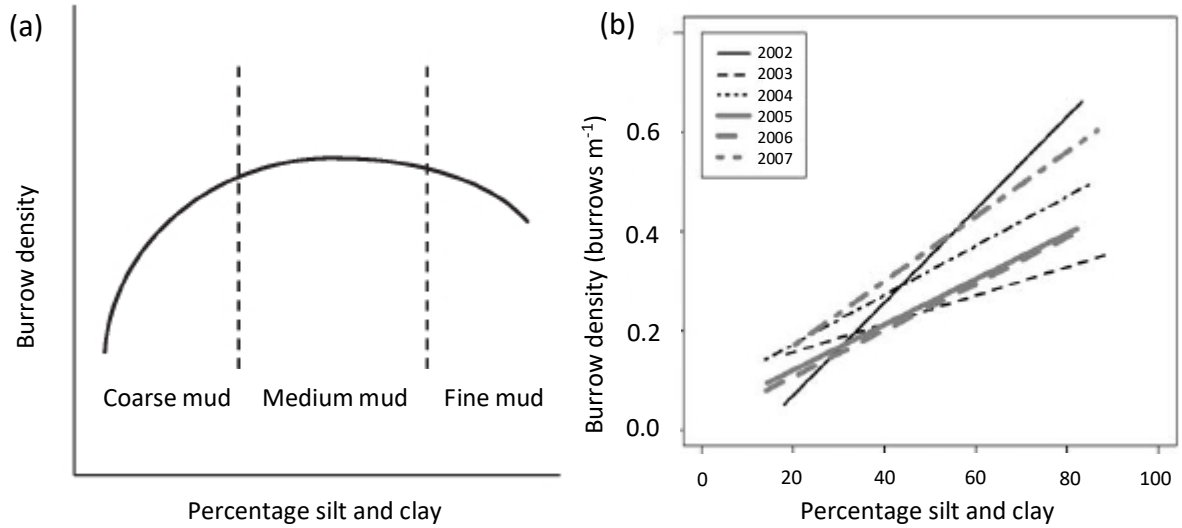

Figure 1.2. (a) The relationship of burrow density and sediment characteristics. (b) Burrow density in correlation to percentage silt and clay in the Fladen fishing grounds. Taken from Campbell et al. (2009).

1.2. *Nephrops* Intraspecific Behaviour

In common with the majority of other crustaceans, *Nephrops* exhibit intraspecific agonistic behaviours where they compete for territory, dominance, food and mating opportunities (Chapman & Rice 1971; Farmer 1975; Katoh et al. 2008). Fighting behaviour is usually observed when one individual enters the burrow of another similar sized individual (Chapman and Rice, 1971; Farmer, 1975). In addition, holding several individuals in close proximity, such as in a creel or tank, will also incur fighting behaviour. Most interactions take place when the *Nephrops* are most active. This occurs during sunset and sunrise (in shallower depths) when most adults will emerge from their burrow. Females tend to remain within the burrows for longer periods and return to the same burrow, whereas males exhibit a greater proportion of exploratory behaviour, and occupy several different burrows (Chapman and Rice, 1971; Mouat, 2002). Furthermore, smaller individuals tend to remain further within the burrow when compared to larger individuals (>50mm CL) which remain in the entrance (Chapman and Rice, 1971). This may indicate that there are sex and size related behavioural differences.

Changes in the behavioural patterns are shown when the females are at the onset of mating and release sex pheromones towards the males (Mouat, 2002; Katoh, 2011). By using the olfactory assessment of another individual to assess their condition and identity, *Nephrops* develop lasting hierarchical community structures (Katoh et al., 2008). Chemical signals or “information currents” about the sender originate from the urinary pores (nephropores) and are propelled from the gill chambers and by scaphognathites (Atema and Voigt, 1995; Breithaupt and Thiel, 2010). Behavioural analysis indicates that interactions produce a ritualized sequence of displays (Chapman and Rice, 1971; Katoh et al., 2008). As with the majority of decapod crustaceans, the display includes a sizing-up of the opponent through meral spread (tactile stimuli). The individuals then increase or reduce their aggressive displays dependent upon several factors including, but not limited to, size, sex and past experience (Figure 1.3).

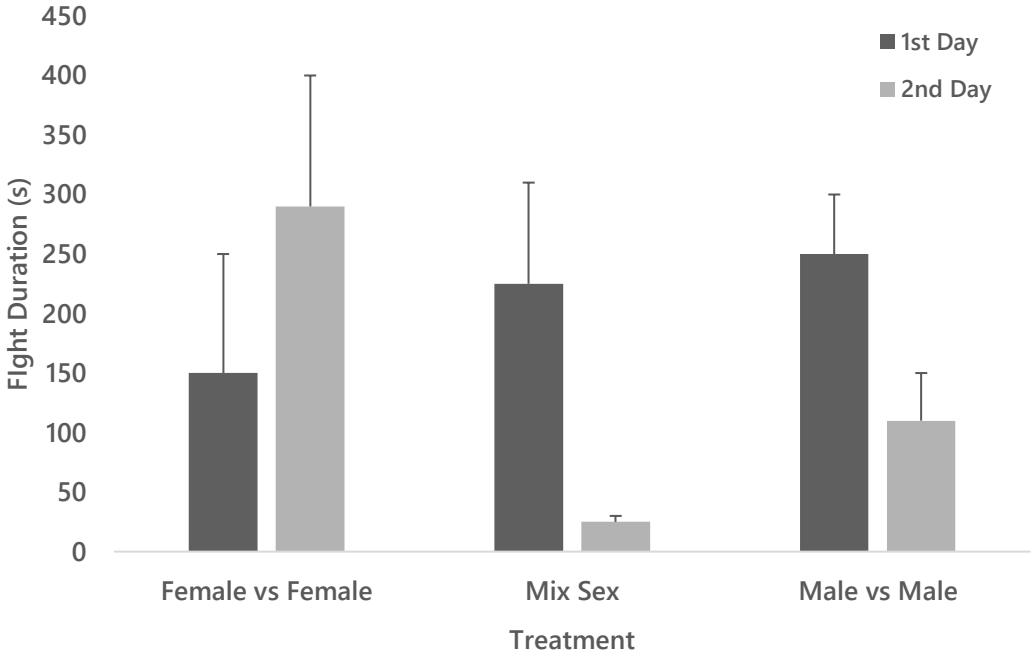

Figure 1.3. Fight duration (seconds) between different sexed opponents over two consecutive days. Taken from Katoh (2011).

If the aggression display is increased from both individuals, the chelipeds are used to push, smack and grab the opponent until one is established as dominant over the other (Katoh et al., 2008). Social stress caused by such interactions has shown to impact upon the physiology within individuals of different crustacean species. For example, glucose and lactate levels increase due to increased and prolonged activity in the common European hermit crab (*Pagurus bernhardus)*, the heartbeat rate increases upon chemical detection of another conspecific and during agonistic encounters in *Homarus* sp. and aerobic respiration is elevated during encounters between swimming crabs (Huntingford et al., 1995; Briffa and Elwood, 2001; 2002; Hernández-Falcón et al., 2005; Briffa and Sneddon, 2007; Moore, 2007; Dissanayake et al., 2009). It is therefore interesting to assess the outcomes for the two opponents in terms of their morphometric and physiological aspects.

1.3. Physiological Stress

Nephrops can be exposed to a number of stressors that will cause changes to their physiology and behaviour (Figure 1.4). The habitat specific stressors affecting *Nephrops* are described in detail by Eriksson et al. (2012) and include but are not limited to hypoxia, ammonia, metals

and hydrogen sulphide. The increase in environmental stress can cause infections and diseases, of which *Hematodinium* has received the most attention due to its effect on both mortality in the population and the marketability of the landed product (Stentiford and Neil, 2011; Albalat et al., 2012). Assessing stress in crustaceans is important to reduce the potential for mortality, particularly for fisheries related operations (Fotedar and Evans, 2011). Assessing the biochemical changes allows the observer to see the individuals' physiological adjustments when exposed to a stressor and how they re-establish internal homeostasis (Fotedar and Evans, 2011; Stoner, 2012). Using haemolymph components has been a way to quantify stress, and the most commonly used parameters are discussed.

Haemolymph glucose levels typically increase when high levels of energy are required. Glycogen is the primary source of energy for crustaceans, especially for continued and intense periods of exercise (Jussila et al., 1999; Stoner, 2012). Glycogenolysis is initiated in the target tissues following the release of CHH (the crustacean hyperglycemic hormone) from the X-organ-sinus gland complex. Haemolymph glucose levels however decrease when individuals are exposed to the air and increasing temperatures (Ridgway et al., 2006b). Glucose levels can fluctuate depending upon the time of data collection from the original time of the mobilisation of the glucose. Glucose levels may be high when first mobilised but then fluctuate depending upon the rate of metabolism and subsequent levels of stress (Kallen et al., 1990; Bergmann et al., 2001). Concentrations may also be influenced by the nutritional state, moult stage and tank conditions (Chang, 1995; Bergmann et al., 2001; Galindo et al., 2009).

Haemolymph lactate levels increase when individuals are exposed to the air, handling and a range of other stressors related to fishing practices (Ridgway, 2005; Stoner, 2012). The increase in haemolymph lactate concentration is due to anaerobic metabolism and excessive locomotory activity (Bergmann et al., 2001; Lorenzon et al., 2007, 2008). These levels remain high after the initial stressor is removed, due to the inability, of crustaceans, to excrete or mobilise lactate rapidly (Lorenzon et al., 2007). Haemolymph glucose and lactate levels have been shown to increase under elevated photophase periods and irradiance, suggesting that these parameters could be used to quantify stress induced by a wide range of stressors (Farhadi and Jensen, 2015).

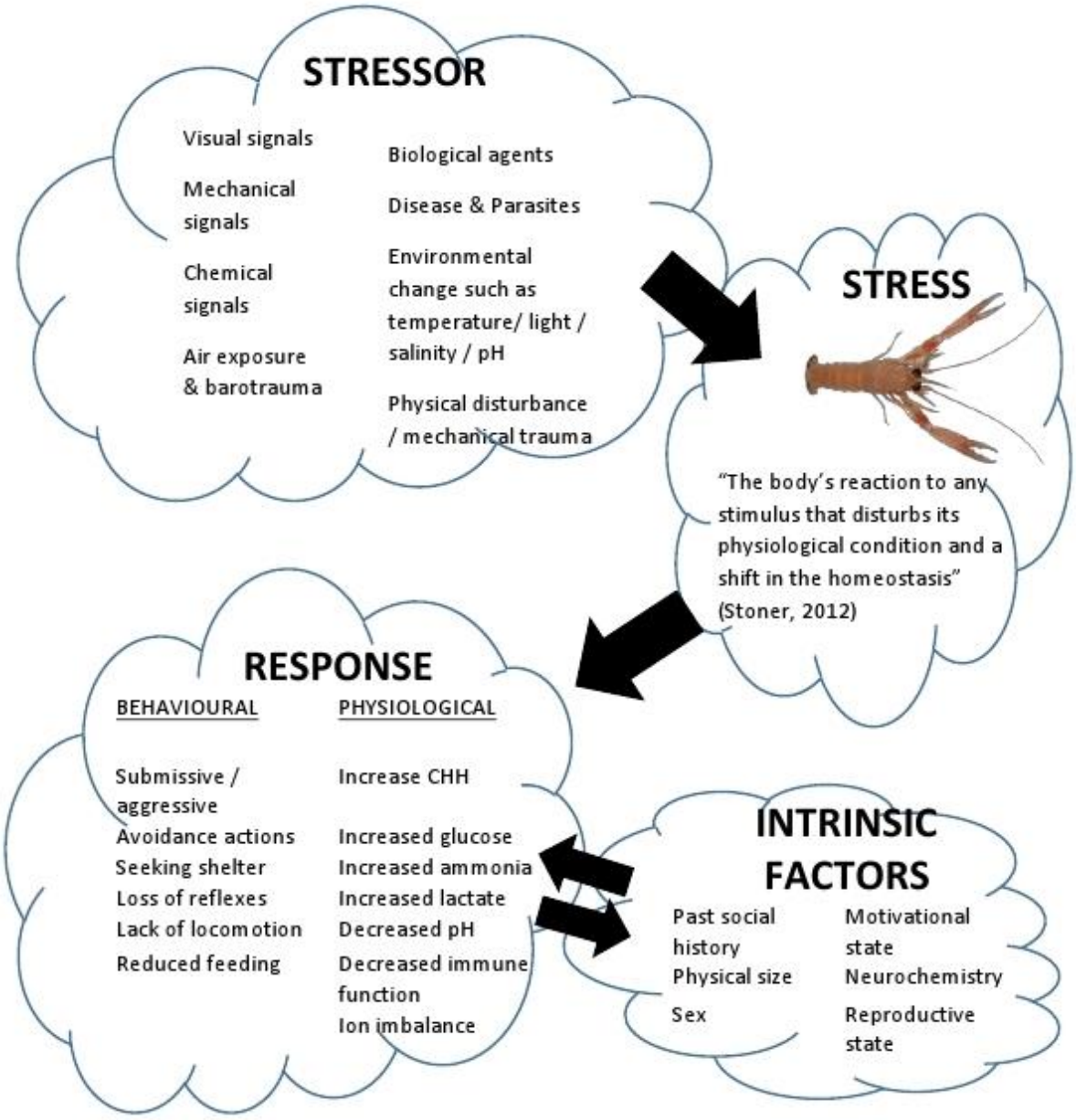

Figure 1.4. Model indicating the relationship between stressors, stress and responses that can be influenced by intrinsic factors. Adapted from Stoner (2012) and Moore (2007).

Haemolymph ammonia concentration increases with physical disturbances and manipulation (Hunter and Uglow, 1993). Again the increase in haemolymph concentration is attributed to anaerobic metabolism, through nitrogen catabolism, and insufficient excretion through the gills (Bergmann et al., 2001). The concentration can lead to lethal levels and cause mortality if not eliminated, and is usually the cause of mortality for some crustacean species when emersed for long periods of time during fishing practices (Durand et al., 1999).

Proteins are released to maintain the haemolymph acid-base levels when metabolic and respiratory acidosis takes place, to repair membranes and when there is a need for higher energy demand (Barclay et al., 1983; Dall and Smith, 1987; Ozbay and Riley, 2002; Lorenzon et al., 2007). It has also been used to assess the nutritional and general physiological state (Leavitt and Bayer, 1977). There are several types of haemolymph proteins, a large proportion (90-95%) of which is hemocyanin (Hc), whose function is to transport oxygen (Bolton et al., 2009). Decreasing protein levels are an indicator of various environmental stressors, such as increased ammonia and temperature (Fotedar and Evans, 2011). Low protein levels have also shown to be a predictor of developing diseases, such as shell disease (Fotedar and Evans, 2011). The need for more simple and rapid methods to quantify protein levels has resulted in the use of a refractometer (Leavitt and Bayer, 1977; Ozbay and Riley, 2002).

Assessing the immune response is another method to quantify stress in crustaceans (Fotedar and Evans, 2011). Haemocyte counts are thought to reflect immune function and its reduced efficiency during an infection. There are three distinct haemocyte types identified; the hyaline, semi granular and the granular cells (Jussila et al., 1997; Eriksson et al., 2012). Counting the distinct groups (differential haemocyte counts, DHC) allows inferences to be made about the mode of action against an infection or stressor as each type of haemocyte has a separate functional activity associated. More commonly, investigators use the total haemocyte count (THC) for assessment as it reduces the time and expertise required to perform such analysis. Low dissolved oxygen levels can cause significant declines in THC (Cheng et al., 2003), whereas physical exercise, disturbance and air exposure may cause the THC to increase (Jussila et al., 1999; Gomez-Jimenez et al., 2000). However, Ridgway et al. (2006) observed a decline in THC specifically in *Nephrops* with increased air exposure (emersion). Therefore, caution in the interpretation of the THC numbers is required as it is likely to be species specific and alter due to a range of stressors, and also the methodology (Fotedar and Evans, 2011). In general within crustaceans a lower or declining THC indicates a reduction in their condition (Persson et al., 1987; Jussila et al., 1997).

It is clear from the literature, that care should be taken with regards to baseline levels, as the normal ranges are typically wide, and therefore should be assessed on a case by case basis (Taylor et al., 1997). Invertebrates respond differently to the same stressor and have different

physiological tolerances (Gomez-Jimenez et al., 2000). Therefore, the ranges described for other crustaceans may not apply to *Nephrops*.

1.4 Physiology and Behaviour

Integrating behavioural traits with physiological processes is limited within scientific literature, especially for decapod crustaceans. Contemporary research in Neotropical cichlid fish (*Cichlasoma dimerus*), which display hierarchal dominance systems established through agonistic interactions similar to that of *Nephrops*, has linked behavioural displays with physiological attributes (Ramallo et al., 2015). The internal physiology will directly influence and be influenced by interspecific and intraspecific interactions. For example, dominant individuals will differ in their internal physiology when compared to submissive individuals, with the former tending to have higher levels of aggressive compounds (such as hormones in cichlid fish; Ramallo et al., 2015). Serotonin is the major compound associated with aggression in decapod crustaceans, although its influence upon the behaviour displayed may be different between species (Peeke et al., 2000). The difference between dominant and submissive individuals is also evident in crustaceans. For example, the heart rates of dominant lobsters were higher when compared to subordinate individuals (Hernández-Falcón et al., 2005). Other factors such as differences in the moult cycle can influence the tendency to avoid aggressive behaviours from conspecifics (Cromarty et al., 2000). Other physiological metabolites such as those described in chapter 1.3 to assess stress parameters have received little attention. This may be because the variables mentioned may not indicate any differences during or after agonistic encounters as shown in swimming crabs (*Necora puber*; Huntingford et al., 1995). However, one such study has investigated the cost of agonistic signalling in hermit crabs (*Paguridae* sp.) with glucose and lactate levels. The results indicate that lactate levels were a determining factor for the length of the fight period for the attackers, and the glucose levels were lower in the subordinate individuals (Briffa and Elwood, 2002). Therefore the consequences of agonistic encounters can impact upon the general fitness of the individual and subsequent survival with increased metabolic debt and reduction in energy stores (Huntingford et al., 1995; Sneddon et al., 1999).

Such research has a direct relation to fisheries and aquaculture operations. Research efforts have included the behavioural and physiological influences of trawl capture on *Nephrops*, but very little on the impact of creel caught capture. Bergmann et al. (2001) suggested higher

levels of glucose and lactate could be due to the agonistic encounters within the creel, and this could be further increased as the individuals within the creel are in a limited space with no means of escape. This also has implications for broodstock health, live transportation practices and managing holding facilities. There is a gap of knowledge in combining *Nephrops* behaviour with physiological metabolites attributed to stress. Understanding how these relate and the mechanisms behind social interactions would be beneficial for both academic and commercial reasons.

1.5. Sedatives

The commercial aquaculture sector uses various methods to reduce stress whilst transporting broodstock (Table 1.2) and one such method is sedation (Ross and Ross, 2008; Harmon, 2009). Sedatives or general anaesthesia can be described as a 'loss of sensation' and assumes that full recovery will occur (Ross and Ross, 2008). Use of sedation or anaesthesia has shown to reduce stress in several species of fish and crustaceans (Coyle et al., 2005; Saydmohammed and Pal, 2009) and that post-transport mortality can be potentially reduced (Pramod et al., 2010; Barrento et al., 2011).

Sedation techniques and procedures for use with crustaceans are not well developed compared to those used for commercial fish species. This is because most operations do not require the use of sedation or anaesthesia, and use other methods such as cooling and distraction (allowing the individual to grasp onto an object and therefore immobilising them) (Ross and Ross, 2008). Investigations that have used sedation on crustaceans found that the results are varied, and concluded that research should be completed for each species prior to sedative use (Coyle et al., 2005). Several studies have commonly used inhalation and parenteral methods to administer sedatives in crustaceans (Ross and Ross, 2008). The most commonly used anaesthetic in aquaculture is MS222. However, the variation in effectiveness with the anaesthetic MS222 is shown in *Crangon septemspinosa*, with 0.5 g/L inducing full anaesthesia; however for the crayfish *Astacus astacus* 1 g/L induced only light sedation (Ross and Ross, 2008). The likely reason for such varied results is due to different nerve cell transmitters being between species (Ross and Ross, 2008). Therefore, a more reliable and cheaper alternative should be investigated for use specifically with *Nephrops*. The most common anaesthetic products that have recently shown promising results in other crustaceans are clove oil, eugenol and AQUI-S (Coyle et al., 2005; Waterstrat and Pinkham,

2005; Vartak and Singh, 2006; Ross and Ross, 2008; Saydmohammed and Pal, 2009;Akbari et al., 2010; Barrento et al., 2011; Huntsberger, 2012; Parodi et al., 2012). Most studies have performed sedative investigations on prawns (*Macrobrachium rosenbergii*, *Litopenaeus vannamei*, *Fenneropenaeus indicus*), crab (*Cancer pagurus*, *Cancer irroratus*), lobster (*Homarus americanus*) and crayfish (*Astacus leptodactylus*). There is a lack of published evidence that any sedative investigation has been performed on *Nephrops*, particularly in relation to the reduction of stress during transport and handling, which are common in aquaculture and fishing practices.

Table 1.2. Methods to reduce stress during transport, handling and holding. Taken from Ross & Ross (2008).

Suggested action	Reason	Application	Qualifications
Reduce duration of exposure to stressor	Stress response is usually proportional to duration of exposure	General	Some effects may result in long recovery times
Work at lower temperatures	Stress-induced mortality increases with water temperature	General	Not always practicable in field conditions
Prevent simultaneous stresses	Stressors may be additive or synergistic	General	Could allow time between exposures to stressors, if in a sequence.
Use dilute salt solutions	Medium becomes more isotonic, reducing osmotic losses and mortality	Transportation	Use with stenohaline freshwater animals may be limited
Withdraw food: 2–3 days in cold water 12–24 hours in tropicals	Reduces oxygen requirements and fouling of medium	General Handling Transportation	Requires additional management input
Reduce stocking density or numbers handled per batch	Reduces interaction and abrasion	General Handling Transportation	May conflict with intensification of aquaculture
Provide adequate, more 'natural', environment	Providing environmental 'comfort' (see preceding paragraphs)	General	Not always possible in some culture systems
Use mild anaesthesia or sedation	Facilitates handling, *temporarily* reduces oxygen consumption, reduces carbon dioxide and ammonia output	Handling Transportation	Anaesthetics can act as stressors

1.6. Reproduction

The reproductive cycle and fecundity of *Nephrops* are influenced by both the geographic latitude and seawater temperature. For this thesis the author has concentrated on the local geographical range around the UK, in which *Nephrops* has an annual reproductive cycle in contrast to biennial cycle found in more northern populations (Sterk and Redant, 1989). The development of the ovaries is described in chapter 1.1. (Farmer, 1975; Smith, 1987; Sterk and Redant, 1989).

During the end of spring and throughout the summer months the males copulate with newly moulted females. The females accommodate the spermatophores taken from the males in the thelycum. The males produce spermatophores in the vasa deferentia region, and are stored in the ejaculatory region until copulation with the females. During copulation, the male

turns the female onto her dorsal side and thrusts the 1st pair of pleopods into the thelycum and sliding the appendices masculinise along the 1st pereopods (Farmer, 1974a).

The oocytes (unfertilized eggs) are fertilized when passed over the thelycum where the spermatophores are stored and extruded onto the abdomen through the gonophore (Farmer, 1974a; Streiff et al., 2004). Farmer (1975) however concluded that this was unlikely and that fertilization is more likely to be internal. Multiple paternity does exist in *Nephrops* and therefore has direct relevance for aquaculture of this species and the future potential for selecting desirable traits (Streiff et al., 2004). The females extrude the eggs by lying on their back and the eggs are passed from the coxopodites towards the abdomen (Farmer, 1975). The eggs are extruded during the later months of summer and into autumn. The eggs are held in place with setae on the pleopods, and remain attached and incubated until the later stages of development. The embryos hatch approximately 6 to 10 months later (Farmer, 1974a; Sardà, 1995).

During incubation, the females remain within their burrows for a large proportion of time and this is apparent from the lower proportion of trawl landings being female during this period. The reason for remaining in the burrow is possibly to reduce the risk of predation (Chapman and Rice, 1971; Farmer, 1975; Sardà, 1995). During this time ovigerous females brood the eggs with increased pleopod activity and the dead embryos are removed through grooming (Eriksson et al., 2006; Pochelon, 2011).

Around the UK, the embryos develop over winter and into early spring. Hatching of the embryos occurs over three to four days during mid-spring through to the end of summer, with the a peak during May and June (Farmer, 1975; Smith, 1987). This coincides with the rise in seawater temperature, which speeds up the development of the embryos and ovaries. After the hatching of the embryos, the females (non-ovigerous) ovaries develop into the final stages before copulation (Farmer, 1974a).

1.7. Procurement and Husbandry of Broodstock

The importance of procuring good quality broodstock and transporting them appropriately was first highlighted in Figueiredo and Vilela (1972) when the broodstock were transported over large distances of 30km and hatch rates were lower than expected. The lower hatch rates may be due to increased stress during transport and fishing procedures. Stress caused by

capture, handling and aerial exposure increases mortality in over 30% of the stock once received at the holding facilities (Coyle, Durborow, & Tidwell, 2004; Ridgway et al., 2006; Lund et al., 2009). Fishing may also cause loss of limbs, damage, internal bleeding, and physiological stress (Juanes and Smith, 1995; Stoner, 2012). For example, exposure to surface light level intensities could cause retinal damage, although how this impacts upon survival is not clear (Loew, 1976; Gaten et al., 2013). The number of eggs remaining on trawl caught broodstock will be diminished due to the escape response behaviour of rapid tail flipping induced by the fishing mechanism, and mechanical abrasion of the net and other organisms on the abdomen (Balasundaram and Pandian, 1982; Newland and Chapman, 1985; Briggs, 2002). Newland and Chapman (1985) indicate that the tail-flip in ovigerous females is controlled by the uropods and telson, holding the abdomen straight, and that the overall responsiveness and swimming endurance is lower when compared to males and non-ovigerous females. It is known that when ovigerous females are stressed, the brood can be prematurely released (Eriksson et al., 2006). Therefore, the preference for less stressed, non-damaged and local broodstock would be preferable for most aquaculture activities. One would therefore assume that hand caught broodstock would be preferable, with fully controlled parameters. However, this may not be feasible as it is time consuming and costly. Therefore, sourcing broodstock with minimal prior stress from creel capture (in preference to trawling) would be an acceptable alternative, and indeed Leocádio et al. (2012) illustrates that creel caught *Nephrops* are in better condition and larger in size than trawl caught *Nephrops*.

The time of the season has an influence upon survival, with higher mortalities during the summer months. It has been shown that the fishing practices involving trawling, in the summer months at high air and water temperatures, with the associated handling and storage, causes an increase in *Nephrops* glucose and lactate levels (Lund et al., 2009; Albalat et al., 2010). It would therefore be preferable from an aquaculture perspective to catch and store live *Nephrops* during the winter months (referred to as “overwintering”) ready for the onset of the larval season in the spring. However, catch rates of females during the summer and into autumn period also infers that gathering broodstock during this time period would be practical, due to the abundant supply and possible lower costs when compared to in the winter (Figure 1.5).

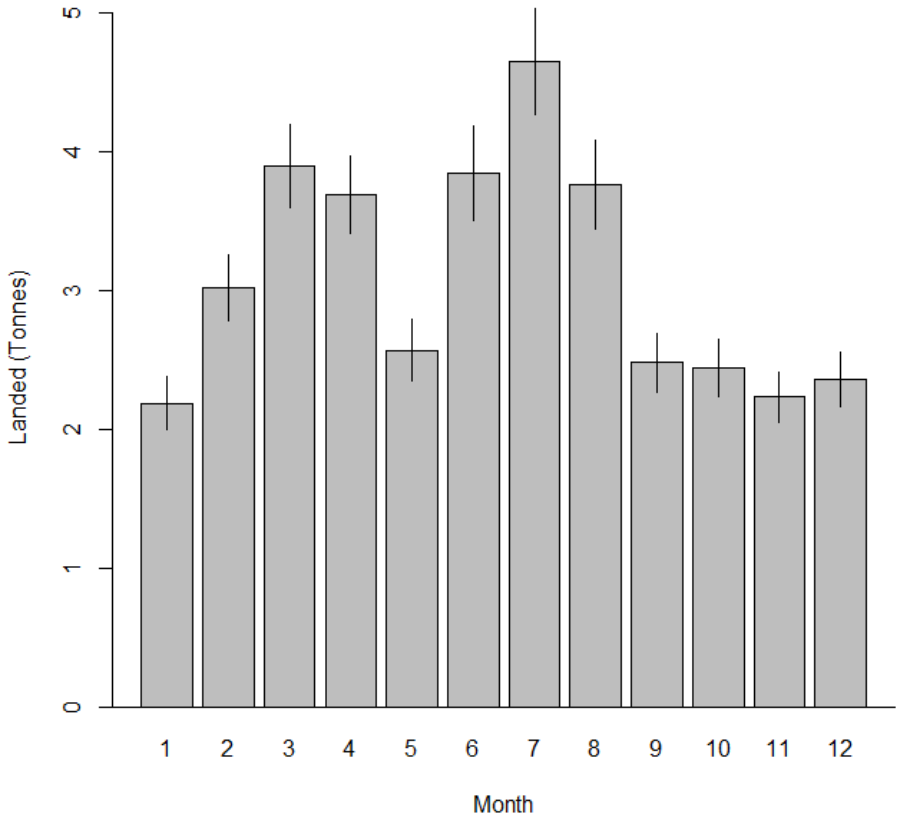

Figure 1.5. Average landings (tonnes) of creel caught *Nephrops* from the years 2011-15 by month (1-12 represents January-December).

There are few accounts of using overwintered *Nephrops* broodstock. The health of the broodstock, egg development and subsequently larval production will be altered by different holding conditions (Pandian 1970; Perkins 1971; Sibert et al. 2004; Calado et al. 2008; Pochelon et al. 2009). Thompson and Ayers (1989) caught ovigerous females during September and held the broodstock over winter. There was low larval survival and in total only 0.1% successfully reached zoea stage 3 (details of larval development are in chapter 1.9). The authors indicate that the low survival was possibly due to fluctuating and less than optimal temperatures during embryo development. Dickey-Collas et al. (2000) improved larval survival up to 21% with overwintering broodstock. The broodstock were held individually in an upwelling, temperature and salinity-controlled system. The water was also filtered, and UV sterilized. Egg loss due to fungal infections is one reason given by Farmer (1975) for low survival, and therefore high water-quality is seen as a priority for *Nephrops* broodstock and larvae. The effects of storage and captivity on *Nephrops* broodstock and their eggs over time is not understood. Poor overall health of the broodstock may have

consequences for embryo development and larval survival. American lobster (*Homarus americanus*) have been shown to have an increased incidence of shell disease with long term storage and poor diets (Tlusty et al., 2008). Additionally, larvae hatched from captive broodstock are found to be shorter in length, less active, and have lower total fatty acid content (mg /g) when compared to wild caught broodstock (Smith et al., 2003). Captivity therefore could have an impact on the health of the broodstock, the biochemical composition of the egg and developing embryos, and impact upon subsequent larval survival and growth (Palacios et al., 1998).

1.8. Embryo Development

Embryo development has been defined with 9 stages defined by the egg mass, colour and notable features of the embryo (Table 1.3; (Dunthorn, 1967; Rosa et al., 2003), and these have been used in this thesis. Other ways to define the developmental stage include the size and the amount of yolk evident in the egg capsule (Figueiredo and Vilela, 1972; Farmer, 1975). The egg structure has 3 membranes that have been described in detail by Smith (1987). The outer membrane is thicker than the other membranes, with the inner membrane forming part of the prezoeal exoskeleton. The prezoea within the egg capsule remains curled into a spherical shape. Newly extruded eggs are dark green in colour with the eye pigment only seen by stage 4. By stage 7 the egg is of an olive colouration with chromatophores present on the limbs and telson of the embryo. During the final egg development stages (8-9) the egg becomes an orange colour with complete prezoea development (Dunthorn, 1967). The change in colour as the embryo develops is due to the release of astaxanthin, and the incorporation of the yolk (which is green) (Goodwin, 1951). The egg volume (mm^3) and water content (%ww) increase with development with lipids and fatty acids used as the main energy source for the development of the embryos (Smith, 1987; Rosa et al., 2003). The energy supplied by the yolk proteins are released through the action of numerous hydrolytic enzymes. This enables growth and development of the embryo through to larval hatching (Subramoniam, 2011). Once hatched the larval cuticle (former inner membrane) is split at the dorsal side of the carapace and the first larval stage is released (Smith, 1987).

Table 1.3. Embryo stage scale used to document egg development. Taken from Powell and Eriksson (2013).

Stage	Embryo mass colour	Description	Proximate analysis	
			Increase	Decrease
1	Dark green	Yolk segmented. No embryo	Wet weight, water, ash, protein	Dry weight, lipid, carbohydrate, energy
2	Dark green	First appearance of embryo		
3	Dark green	Optic lobes and appendages present		
4	Dark green	Eye pigments present as thin crescentic shaped areas. Triangular shaped ocellus		
5	Dark green/greyish green	Well-formed embryo occupying about 1/2 egg capsule. Eye pigment comma-shaped. Ocellus sometimes present		
6	Greyish green/olive	Chromatophores present on limbs		
7	Olive	Embryo occupying about 2/3 capsule. Chromatophores on limbs and telson		
8	Light orange/brownish orange	Yolk almost all absorbed. Eye pigment oval. Chromatophores on carapace		
9	Pale orange/ brownish orange	Formation of complete larva within capsule prior to hatching		

Embryo development is rapid from stage 1 to 2, and 7 to hatching, but is relatively slow during stages 2 to 6 (Smith, 1987). Overall the rate of development can be increased with temperature as described by Dunthorn (1967) and shown in Figure 1.6. A temperature increase from 8°C to 15°C can reduce the incubation period from approximately 300 to 170 days. To date there has been little success in developing embryos in the laboratory sufficient to produce large quantities of viable larvae, *i.e.* larvae that reach postlarval stages (Wear, 1974; Powell and Eriksson, 2013). The survival of crustacean larvae is reduced when the embryo incubation temperatures are at or beyond their natural thermal limits (Smith et al., 2002; Brillon et al., 2005).

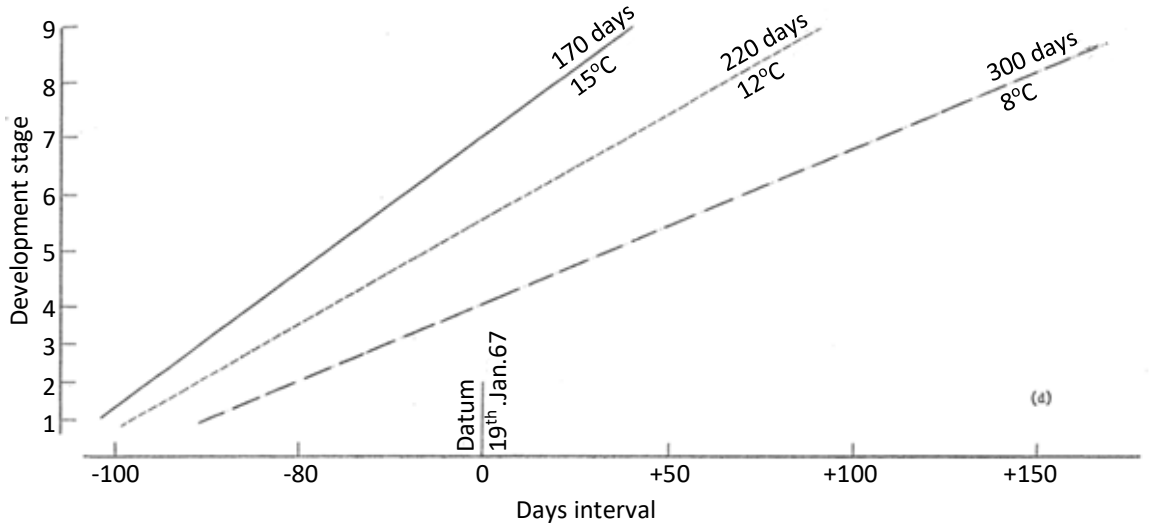

Figure 1.6. Incubation periods compared between three temperatures for embryo development. Taken from (Dunthorn, 1967).

1.9. Larvae

The prezoea emerge from the egg (eclosion) usually with the onset of darkness, with the adult female agitating and fanning the pleopods to detach the eggs and create a current for the larvae to move away (Smith, 1987). Once free of the egg membranes the prezoea larvae emerge and can flex their lower appendages and have an opaque colouration (personal observation). They have limited swimming ability due to the larval cuticle, but quickly (in less than five minutes) resemble zoea stage 1, as also found by Smith (1987). *Nephrops* have 3 planktonic larval stages with the fourth stage described as the first post larval stage (see Figure 1.7) (Jorgensen, 1923; Smith, 1987). The three zoeal stages were first described by Sars (1884) and will be simply referred to here as Z1, Z2, Z3 and PL for zoea stage 1, stage 2, stage 3 and post larvae respectively.

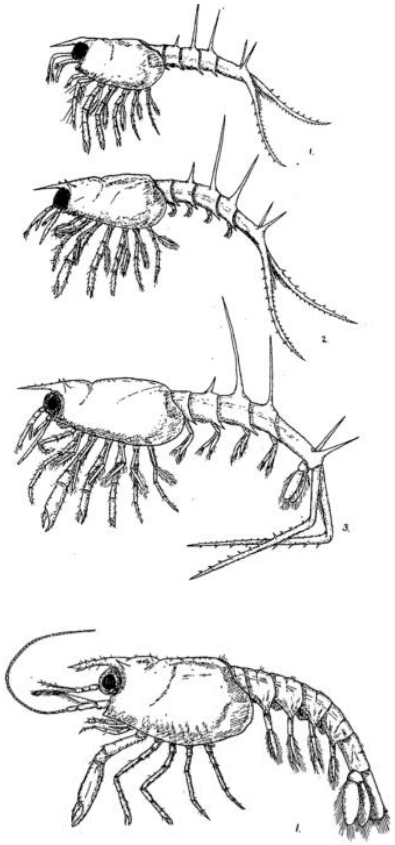

Figure 1.7. Showing the three *Nephrops* planktonic zoeal larval stages and first post larval stage (taken from Jorgensen, 1923).

Larval vertical distribution is related to several environmental variables, including light and pressure (Smith, 1987). Larvae remain at a depth of around 6 to 38m, having a nocturnal vertical migration of 16 to 5m depth (Smith, 1987). Data collected from wild populations show that Z1 larvae actively swim from hatching towards the surface waters (Farmer, 1975; Smith, 1987). In captivity the larvae show a similar positive phototaxic response. Z1 larvae have an approximate size of 5.5-7mm and use the thoracic exopodites in a beating motion for means of locomotion (Neil et al., 1976). Z2 larvae remain at a lower depth and is the most likely cause for the lower numbers caught using plankton nets (Hills, 1975). The pleopods develop from buds seen in Z1 larvae and become biramous, and the larvae are of 7.5-10mm in total length (Powell and Eriksson, 2013). From zoeal stage 3 (Z3), the larvae become negatively phototaxic and migrate towards the benthos. Substrate and habitat selecting behaviour continue through to later post larval (PL) stages 1-2. The larvae grow to 10-12mm in length with further development of three pairs of dorsal lateral teeth, the tail has spines, fully developed

pleopods and uropod (Powell and Eriksson, 2013). Metamorphosis and the PL stage is described further in 1.10. The development cycle is shown in Figure 1.8.

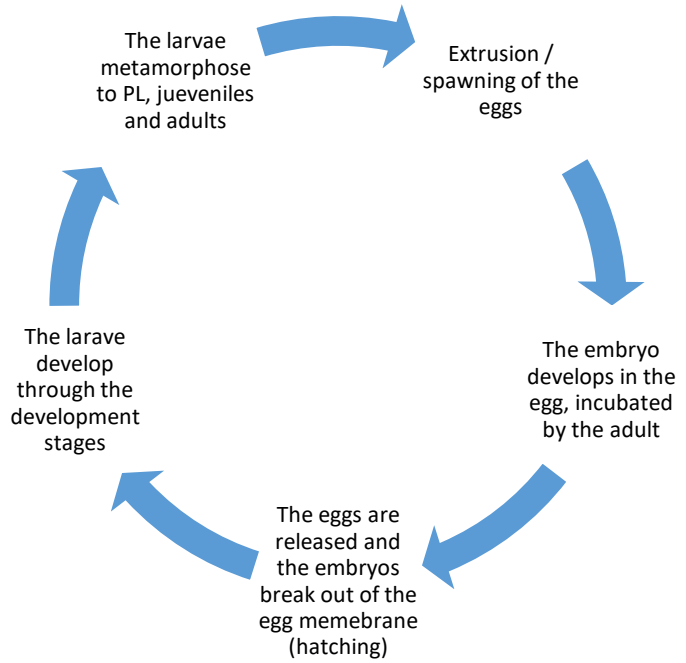

Figure 1.8. The development cycle of *Nephrops*.

Rearing lobsters began as early as the 19th century with over 700,000 *H. gammarus* (European lobster) larvae released in Scotland (Nicosia and Lavalli, 1999). Protocols were established by 1983 with a small-scale system devised in North Wales (Burton, 2003). It was and still is apparent that culturing either *Nephrops* or *Homarus* sp. in a temperate climate and ongrowing to an equivalent landing size incurs a huge amount of risk and is seemingly economically unviable (Wickins and Lee, 2002). Therefore, the main focus of research has been on rearing for experimental and stock enhancement purposes, which could possibly lead to industrial take up such as commercial ranching, enhancement or re-stocking (Table 1.1).

Previous attempts to mass culture *Nephrops* larvae have proved difficult, with low survival to PL stages in the majority of the experiments (Figueiredo and Vilela, 1972; Hills, 1975; Dickey-Collas et al., 2000; Rotllant et al., 2001; Pochelon et al., 2009). The first account in the scientific literature of rearing *Nephrops* was in 1971 by Figueiredo and Vilela (1972), who used eggs taken from the adult and reared them in multiple sized containers. The results indicated that survival was low when taking eggs from the females and that there were problems with bacterial and fungal outbreaks. Survival was improved by taking eggs at the later stages of

development (de Figueiredo, 1979). Hills (1974) instead caught the planktonic larvae and brought them into the laboratory. This resulted in higher survival, especially with larvae captured at the later stages of development *i.e.* Z3, but many larvae were damaged during the capture process (Smith, 1987; Powell and Eriksson, 2013). Large advances in knowledge for larval development were acquired by Anger & Puschel (1986) and Smith (1987) who used static containers of various sizes and kept the larvae at low density and feed with *Artemia* to excess, however survival to the PL stages was still low.

One reason for the low survival may be due to unsuitable rearing tanks. Large communal tanks / hoppers which hold a large number of larvae (*i.e.* mass rearing) have been trialled with limited success (de Figueiredo, 1979; Smith, 1987). One main reason for this may be cannibalism. The high incidence of cannibalism is shared with most crustaceans, especially when developing into the later stages (Z3-PL) (Smith, 1987; Sardà, 1995). Survival was improved with individual housing for each larva, but required a large amount of effort and time by hatchery personnel and therefore non-viable for mass production and aquaculture (Hills, 1975; Smith, 1987).

Stressed or damaged larvae are more susceptible to diseases (Wickins and Lee, 2002). The cuticle has anti-microbial properties, but if penetrated can allow foreign material to enter. The haemocytes and plasma proteins can mount coagulative, phagocytic and cytotoxic responses (Wickins and Lee, 2002), but these may not be adequate to prevent infection. From an aquaculture perspective, disease prevention rather than treatment is the best action to take through better water management, filtration and husbandry.

The majority of mortality throughout the rearing process occurs during ecdysis, where there are significant changes in the behaviour, internal physiology and biochemistry (Chang, 1995). The larvae shed the outer exoskeleton and a new soft exoskeleton is formed. During this time, the larvae are vulnerable to cannibalism and difficulties in the process can cause the larvae to become entrapped within the exoskeleton. Deficiencies in diet and inadequate rearing environments can delay the onset of ecdysis (Anger et al., 1985).

Eriksson and Baden (1997) increased survival of the larvae to post larval stage 1 (PL1) up to 2.6 ± 0.3% with the incorporation of cylindro-conical tanks for the mass culture of early staged larvae (Z1-Z2). This proved to be successful in other later experiments, and it is still used

(Dickey-Collas et al., 2000; Rotllant et al., 2001, 2004; Pochelon et al., 2009). Cylindro-conical tanks (a.k.a. Paxton hopper/ Kreisel like designs / conical) are used in the aquaculture of other crustacean species, and are particularly used for delicate species such as spiny lobsters (*Jasus edwardsii*), fire shrimp (*Lysmata debelius*), cleaner shrimp (*Lysmata amboinensis*) and spot shrimp (*Pandalus playceros*) (Wickins et al., 1996; Calado et al., 2008). The original design optimised the hydrodynamics within the tank and kept delicate organisms from being entangled or touching the aquarium walls, and prevented 'dead spots' in the culture tank where detritus and uneaten feed would encourage bacterial growth, therefore lowering the hygiene levels (Calado et al., 2008). This design has been modified for use with *Nephrops* larvae (Powell et al., 2015). The design enables the phototactic larvae to remain near the water surface in the tank, while the detritus and uneaten feed falls to the bottom where it can be easily siphoned out. The rearing tank design also minimises the damage and abrasion on the fragile spines that protrude from the *Nephrops* larvae. Non-optimal tank designs induce damage to appendages which ultimately may affect swimming behaviour and feeding ability (Calado et al., 2008). The design of the rearing vessels could be improved for the rearing of *Nephrops*, to accommodate development through the larval stages (Z1-Z3), which requires differing designs appropriate for the ontogenetic shifts displayed by the larvae (as discussed in detail earlier).

Other environmental variables that have improved the larviculture of *Nephrops* have focussed upon experimenting with temperature and photoperiod. The intermoult time for *H. gammarus* can be reduced with higher rearing temperatures of up to 19^{o}C. The optimum temperature for rearing *Nephrops* larvae from Z1 to Z3 is around 16^{o}C with a lethal limit around 20^{o}C (Smith, 1987; Dickey-Collas et al., 2000). Lower temperatures prolong the intermoult time and increases mortality (Dickey-Collas et al., 2000). For example, there was a 9-day intermoult period difference with lower temperatures (7-10^{o}C) when compared to higher temperatures (13-17^{o}C) (Figueiredo and Vilela, 1972). At 15^{o}C the Z1 larvae will start moulting into Z2 in around 8 days, from Z2 to Z3 in around 10 days, and Z3 to PL1 in approximately 15 days (Santucci, 1926; Powell and Eriksson, 2013).

Photoperiod has been shown to impact upon the feeding behaviour of larval crustaceans (Pochelon et al., 2009). Light presence induces increased swimming activity for *Nephrops* (Forward et al., 1984). Therefore, the encounter rate with prey would be increased. However

extended photoperiods beyond normal ranges can cause reduced growth, increased intermoult periods, increased lactate and glucose levels, implying higher stress levels (Fitzgibbon and Battaglene, 2012; Farhadi and Jensen, 2015). It seems that for *Nephrops* larvae the change from light to dark periods is more important than the duration of the light and dark periods (Pochelon et al., 2009).

Therefore, techniques must be improved for the mass rearing of *Nephrops* for it to be viable for production. Focussed research activities on *Nephrops* larval and post larval life stages, such as the European funded NEPHROPS project, and open publications describing the techniques used to rear larvae, similar to that of Burton (2003), would further facilitate the development of *Nephrops* larval research and aquaculture. Improving the larviculture techniques could enable further larval research or develop farming techniques for aims described in table 1.1.

1.10. Post Larvae

Within the PL stages (*i.e.* Z4 onwards) the larvae start to become semi-benthic (Figure 1.9), with increasing exploratory behaviour on the substratum and habitat selectivity (Smith, 1987). Full recruitment to the benthos may be after the PL1 stage, when the gills are developed for more benthic habitats (Smith, 1987). The juveniles are thought to reside in similar habitats to the adults (fine grained mud) although catches of juveniles are rare (Cobb and Wahle, 1993). It is clear that the PL are highly selective of the substratum type, and will continue to explore the substratum, and delay their development, until suitable habitat is found (Smith, 1987). The PL are capable of constructing their own burrows (Chapman, 1980; Smith, 1987). PL remain in the burrows and have limited emergence or migration, indicating an early sedentary lifestyle (Cobb and Wahle, 1993). Chapman (1980) indicates that juveniles feed upon the organic material within the burrows, including polychaetes and micro fauna. Emergence from the burrows begins at around 10-15mm CL (Chapman, 1980). As the juveniles grow, the emergence behaviour increases. This is correlated with the development of aggressive and defensive behaviours in relation to predators and conspecifics (Wahle and Steneck, 1992; Cobb and Wahle, 1993). The tendency to emerge from burrows may also be related to starvation levels as found in *Homarus* sp. (Wickins et al., 1996).

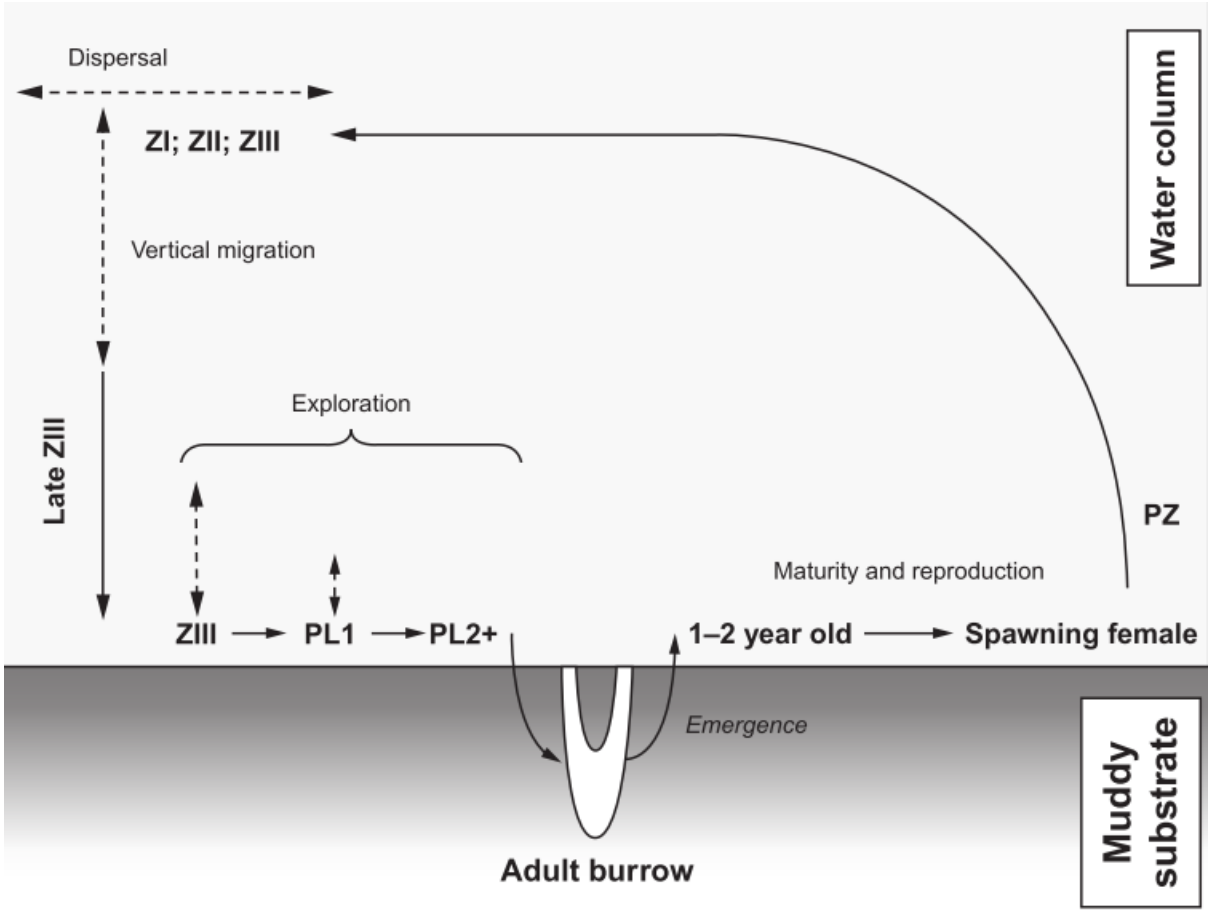

Figure 1.9. The life cycle of *Nephrops* and the theorised recruitment of the larvae to the benthos. Taken from Powell & Eriksson (2013).

A large majority of the PL are susceptible to predation by both fish and larger *Nephrops*. As the habitat is of limited rugosity, the PL are exposed to attacks as seen in tethered *Homarus* sp. and *Jasus edwardsii* (Cobb and Wahle, 1993; Hinojosa et al., 2014). Therefore, it is in the interest of the PL to find shelter in the shortest time. PL3 are able to select suitable habitat and commence burrowing within 40 minutes, but this time is increased in earlier stages, PL1 and PL2 (Smith, 1987). It is suggested that PL excavate a small burrow within an adult burrow, but there is limited evidence of this in the literature (Chapman, 1980; Cobb and Wahle, 1993). It is still unclear how the PL enter the adult burrow, as the risk of predation from the adults residing within the burrow will be high (Smith, 1987). The burrows that the juveniles excavate are usually U or Y shaped, with vertical shafts used for ventilation through the burrow tunnels (Chapman, 1980; Smith, 1987). However, in aquarium trials the PL always inhabited a vacated burrow if one was present (Smith, 1987). Smith (1987) also shows that PL are attracted to dark circles that imitate the adult burrow openings. The mechanisms underlying the selection

of the adult burrows are unclear, although positive thigmotactic, negative phototaxis and rheotactic behaviours are theorised as important factors. Chemical cues may also be a factor as *H. americanus* settle quicker and remain on the benthos in the presence of juvenile conspecifics (Burdett-Coutts et al., 2014). Thus the presence of older conspecifics may attract PL to suitable habitat. In addition, feeding strategies and prey preference may be factors in settlement attraction. Previously starved adult *H. gammarus* have greater frequency of storing food within the burrow, and this may be similar in adult *Nephrops*. Accessing this storage of food may be a reason that *Nephrops* PL enter the adult burrows.

It is unclear whether *Nephrops* initiate burrowing in alternative habitats. For example, *Homarus* sp. burrow under cobbles, tiles and stones when on sand or mud substrate (Berrill, 1974). It is also suggested that the rearing procedure itself may affect the brain function and subsequent decisions related to release procedures and burrowing behaviour (Van Der Meeren, 2005). Therefore investigations into habitat selection by reared larvae may not represent the 'wild' population. In either case it is important for the development of aquaculture of this species that post release behaviour is investigated. Knowledge about habitat selectivity and behaviour is important when developing release protocols.

Growing the larvae post metamorphosis to juvenile stages was adopted in lobster aquaculture to increase the chance of survival post release (Van Der Meeren, 2005). However, as the survival rate of *Nephrops* larvae developing to the PL stages has so far been low, growing *Nephrops* for stock enhancement was largely unsuccessful in the 1960-1970's. Since then, the relatively small number of studies detailing the procedures and techniques for on-growing post larval *Nephrops* still remains limited. However, there have been some technological advancements for use with other crustacean species. These include using individual cells to hold PL, thus reducing cannibalism, and taking up minimal space, at high densities. "Orkney cells" keep the PL separate in a square cell, but at a commercial level would demand high levels of manual labour, time and space (Burton, 2003). The "Aquahive" has relatively recently been incorporated into lobster culture to reduce these high demands (Ellis et al., 2015; Gowland, 2016). The equipment uses individual compartments to house PL, and an upwelling water flow to allow feed to pass through the chambers.

The tank conditions and housing also affect the growth of PL in *Nephrops.* Metamorphosis from Z3 to PL stages can be delayed when they are placed on unsuitable substratum, such as an acrylic based bottom or gravel (Smith, 1987). Other factors such as social interactions and chemical cues may also delay metamorphosis and settlement (Factor, 1995). The optimal solution as suggested by Smith (1987) is to use substrate from the adult grounds. Having the most suitable substrate can improve the survival and growth rate (Jee et al., 2007). However, incorporating sediment into a Recirculating Aquaculture System (RAS, see glossary for a definition) may cause spikes in organic matter and increased turbidity, affecting the water quality within the system. Using aquaria half filled with sediment has been shown to be possible, and has provided positive results in *Nephrops* PL experiments (Eriksson and Baden, 1997).

The low survival to PL stages is the reason for the majority of investigations focusing upon the earlier stages (Z1-Z3), when adequate amounts of larvae are available. In the literature on *Nephrops* culture, only a few sources mention the PL stage (Figueiredo and Vilela, 1972; Hills, 1975; Figueiredo, 1979; Eriksson and Baden, 1997). Smith (1987) indicates high survival at 70% (with low replicate numbers) to PL stage 1, when larvae are held individually in polypropylene bowls with artificial shelters and under low light conditions. Figueiredo and Vilela (1972) grew PL for three months but with low survival, while a later study found survival of 36% to PL2 , 29% to PL3 and 12% to PL4 with high mortality due to increased temperatures above 22^{o}C (Figueiredo, 1979). The PL were kept in individual beakers and fed *Crangon crangon* eggs, small amphipods, isopods and phytoplankton (*Nannochloris* sp.). The duration to reach PL4 was approximately 26.6 days. Hills (1975) however had higher survival from capturing the larvae at later stages such as at Z3. Survival to PL2 was 85%, PL3 was 46% and PL4 was 23% with notes on increased incidence of incomplete moults was noted. PL were fed on a diet of *Mytilus edulis* and *Alloteuthis* sp.

Extensive research has been conducted on the release and nursery behaviours of *Homarus* sp., and the recruitment behaviour contrasts significantly to *Nephrops*. *Nephrops* habitat is much deeper at approximately 20 – 200m, in distinct and discrete habitat patches (Cobb and Wahle, 1993). Routinely diver operated releases are used for *Homarus* sp, but this is not possible for *Nephrops* at depths of below 40m. Other non-diver related techniques use mechanisms that allow the PL to slowly migrate out from a release cassette. This may be

mechanically driven or triggered by some chemical reaction with the water (Van Der Meeren, 2005). When large scale releases are required, surface release may be carried out, however the survival of using this technique may be reduced especially for *Nephrops* when sinking to depths of over 20m (Van Der Meeren, 2005). To date there have been no investigations related to the release of hatchery grown *Nephrops* PL.

Lobster release programmes indicate that lobsters do survive, and are incorporated into the fishery and strengthen a depleted stock (Agnalt et al., 1999). However, the reared larvae have several stressors during packing, transportation and the release to a novel environment which will impact upon their physiology and behaviour (Van Der Meeren, 2005). The use of sedation could reduce the stress levels during transport, and potentially increase the survival upon release. In addition, once released the individuals would most commonly compete in intraspecific and interspecific fighting, as shown in *H. gammarus* (Wickins and Barry, 1996), possibly further adding to physiological stress.

For the purpose of improving the effectiveness of *Nephrops* larval aquaculture it is therefore important to investigate how to develop more successful PL rearing techniques, and to identify effective release protocols that take into account PL behaviours.

1.11. Feeds

Crustaceans produce a wide range of digestive enzymes that enable them to digest a wide variety of food items (Conklin, 1995; Jones et al., 1997; Wickins and Lee, 2002). However, the larval stages are adapted specifically according to their developmental stage, feeding behaviour and the prey available (Wickins and Lee, 2002; Pochelon et al., 2009). In aquaculture the use of live feeds is prevalent for some species to promote both hunting behaviour and enzyme activity that is needed for later stages (Le Vay et al., 2001). Pochelon et al. (2009) indicates that *Nephrops* have different feeding strategies as they develop from Z1 to Z2. Z1 larvae consume large numbers of smaller sized prey whereas Z2 consume large numbers of larger sized prey. The larvae also show feeding plasticity, dependent upon the prey available (Pochelon et al., 2009).

Lobster larvae have low enzyme content when compared to other crustaceans, with a longer digestion time. This allows for higher assimilation of the nutrients, and most are adapted for carnivorous opportunistic feeding strategies i.e. *Nephrops* larvae (Le Vay et al., 2001). The

digestive enzyme content will most likely change and develop along with the development of the larvae, as seen in *Macrobrachium rosenbergii* (Kamarudin et al., 1994). Research on nutrient needs and feeding behaviours of the larvae will add to the development of more adequate diets for improved growth and survival.

The most common practice for crustacean larval culture is to use the brine shrimp, *Artemia* sp (referred to simply as *Artemia* from here on) as the major diet (Wickins and Lee, 2002). Many studies indicate that *Artemia* have low nutritional quality, and that higher survival and growth could be achieved if an alternative with higher nutritional quality was offered (Pochelon et al., 2009). Commonly *Artemia* are enriched with highly unsaturated fatty acids (HUFAs) to increase the nutritional quality of the feed. Developments in non-living, microparticulate diets exists for penaeid and carideans larvae, but studies suggest that non-living feeds, such as frozen mussel and artificial shrimp, incur low survival and growth in *Nephrops* and therefore have limited use in *Nephrops* larviculture (Rotllant et al., 2001). *Nephrops* larvae use an encounter feeding strategy, where they are either carried by the water current, or actively swim and feed upon prey that they encounter opportunistically. There has been no investigation of the specific elements in the hunting behaviour of *Nephrops* larvae, nor of how they capture and consume different prey types. Such knowledge would benefit diet development.

Non-optimal feeds and water conditioning will hinder the development of larvae and PL growth (Wickins and Lee, 2002). It is therefore important to investigate optimising the diet and feeding preferences of *Nephrops* larvae, and trialling alternative feeds to *Artemia*. To date the main feed used for the larviculture of *Nephrops* in all studies is *Artemia*. This may have been provided in conjunction with other feeds such as *Crangon crangon* eggs, mussel, *Mytilus edulis*, *Alloteuthis* sp. various microalgae species, *Fucus spiralis*, commercial microencapsulated feeds and shrimp feed (Figueiredo and Vilela, 1972; Hills, 1975; Smith, 1987; Thompson and Ayers, 1989; Eriksson and Baden, 1997; Dickey-Collas et al., 2000; Rotllant et al., 2001; Powell and Eriksson, 2013).

Probiotics in feed supplements have received extensive attention within the last decade with regards to improving survival and growth in aquaculture produced aquatic organisms, including crustaceans (Daniels et al., 2013). Probiotics enhance the host's immune system

with microbial bacteria and are incorporated into the tissues of the live prey (Dagá et al., 2012). The probiotics also exclude or reduce harmful *Vibrio* spp. and maintain good water quality (Dagá et al., 2012; Daniels et al., 2013). Equally, wild plankton is the 'natural' food source and regularly perceived as the optimal diet for larval growth. Wild zooplankton contains the essential lipids and fatty acids that are essential for *Nephrops* development and contains a range of sizes available for all zoeal stages. One study has found that there were significantly higher concentrations of triacylglycerols, sterols, AMPL and phospholipids, with higher growth and better general condition within *H. americanus* PL1 larvae fed on wild zooplankton when compared to *Artemia* (Gendron et al., 2013). So far, the use of probiotics or wild zooplankton remains uninvestigated within *Nephrops* larviculture.

1.12 Thesis Aims & Objectives

The thesis will provide insight into developing the different aspects of *Nephrops* aquaculture and commercial practices, from the catching phase of the broodstock to the PL release behaviours. The thesis focuses upon the biology, physiology and behaviour from larvae to adult as well as incorporating engineering and husbandry aspects. The lack of knowledge in all aspects of *Nephrops* aquaculture and the relatively low larval survival and growth are the justifications for this thesis. The results will enable investors, managers and fishers to further develop aquaculture techniques for this species. The specific objectives were to:

- Identify appropriate social stress parameters in adult *Nephrops* and how these relate to behavioural and social interactions;
- Assess if a sedative could be developed for use with *Nephrops* broodstock;
- Improve the survival and growth of larvae by examining commercial diets and developing feeding strategies; and
- Gain greater understanding of post larval habitat preferences and behaviours that would benefit release activities.

Each chapter includes a separate introduction, methods, results, and discussion. The appendices give a brief overview of developing hatchery techniques in a commercial setting, focussing mainly on broodstock procurement, transport development and husbandry, as well as pilot scale larval trials. The following describes the title of each chapter.

Chapter 2 – Preliminary broodstock and larval trials.

Chapter 3 – Intraspecific interactions and associated blood chemistry in adult *Nephrops norvegicus*.

Chapter 4 – Evaluation of different concentration doses of eugenol on the behaviour of *Nephrops norvegicus* (published as Cowing et al., 2015).

Chapter 5 – Primary investigations of the effects of feed types and regimes on the growth and survival of *Nephrops norvegicus* larvae.

Chapter 6 – Burrow occupancy influences *Nephrops norvegicus* post larval release behaviour.

Chapter 7 (Conclusion) – The main points from the thesis, wider implications and future perspectives.

Appendix I – Broodstock procurement and transport.

Appendix II – *Nephrops* Fisheries around the UK.

2. Preliminary Broodstock and Larval Trials

2.1. Introduction

Developing hatchery techniques for *Nephrops norvegicus* (*Nephrops*) requires the empirical research. However, the details on relevant methodologies used for this species are insufficient and therefore needs improving in relation to the hatchery set up and routine husbandry methodologies. Using several pilot experiments background information can be quickly gathered, and important results concluded. The ideas and conclusions formulated during these trials is fundamental to the development of the main body of the thesis. The rationale of the pilot studies is to develop the best industry method for gathering and holding broodstock as well as improving husbandry techniques, larval diets, and the hatchery infrastructure (tank design).

It is unclear from the literature what the optimum time of year is to procure *Nephrops* broodstock. Procurement of broodstock during the winter months could be beneficial due to the lower water and air temperatures, resulting in potentially lower stress levels, when compared to summer procurement (Giomi et al., 2008). Procurement of the broodstock during the winter months also allows hatchery personnel adequate time to prepare for the summer larval rearing. In comparison procurement of the broodstock whilst the embryos are in the later stages of development would be more cost effective and less labour intensive. The objectives of the preliminary trials were to identify whether *Nephrops* broodstock can be successfully held over-winter, and to investigate the impacts of this on their general fitness and of the embryos and their development.

Hatchery facilities for *Homarus sp.* stock enhancement activities tend to hold the broodstock individually. Like *Homarus sp.,* previous research has suggested that *Nephrops* have a social structure which is established by social interactions (Katoh et al., 2008). However, this requires more space per individual and is more labour intensive for feeding, cleaning and observation. It would be interesting to identify whether holding *Nephrops* broodstock communally would cause significant stress to individuals, and how this would impact upon embryo development, when compared to holding the broodstock individually.

There is also limited research on the optimal substrate for the broodstock within the holding tanks. It has been shown that the substrate of the holding tank can influence the number of larvae produced by the broodstock in prawns (Nakayama et al., 2008). *Nephrops* are found upon mud substrates, with a clear preference for sediment that allows for the construction of burrows (Johnson et al., 2013). Therefore, holding broodstock on artificial substrates other than mud, that may not permit an individual to perform natural behaviours such as burrowing, may cause increased stress and impact upon embryo development and larval vitality. Therefore, the reproductive performance (number of larvae produced and embryo development) was investigated by holding broodstock on different substrates, including sediment taken from *Nephrops* grounds.

Homarus sp. larvae are collected either by hand or in a collection tank and then further grown through the larval stages in a cylindro-conical tank (conical). Aeration provides a water current that prevents the larvae from settling on the tank bottom, disperses feed evenly through the tank and reduces the rate of cannibalism. However, such velocity of movement would cause excessive damage for *Nephrops* larvae, due to their delicate spines that can be easily damaged. (Powell and Eriksson, 2013). The fundamental knowledge on the appropriate tank design and hydrodynamics for mass culture of *Nephrops* larvae is limited. Powell & Eriksson (2013) provide a good review of the literature on techniques and feeds previously used. The tank design is of clear importance to the success of raising larvae to the post larval (PL) stages which is complex due to the behaviour and morphology of the larvae throughout each larval stage. The *Nephrops* larvae become negatively phototaxic in the later stages of development. Once on the bottom of the rearing tank the larvae can become entangled with other larvae. This could cause undue harm and stress to the larvae but also facilitates the spread of disease, infection, and increase the chance of cannibalism. Cylindro-conical tanks have been used for *Homarus* spp. larval culture and the delicate larvae of other crustacean species (see chapter 1.9. Larvae). Therefore, the survival and development of *Nephrops* larvae should be trialled in several tank configurations, and discussed with relevance to practical elements such as feeding, hygiene and maintenance.

Therefore, several topics have been investigated such as broodstock holding, husbandry, and larval rearing techniques.

2.2. Methods for Pilot Scale Studies

2.2.1. Overwintering Broodstock

Two different overwintering storage types were trialled in CEMS, Scarborough (University of Hull). Broodstock were either placed into a communal tank with several open pipes available as shelter (n=6) or each placed into a closed individual pipe (n=6). Communal tanks held a total of 11 individuals (all adult females) un-banded and artificial shelters were provided to each lobster, made from a section of plastic PVC pipe. The individual pipe housing consisted of a plastic pipe 400mm in length and 112mm width, with mesh ends for continued water exchange. Survival, wet weight and several haemolymph parameters were taken over a period of 91 days. The haemolymph was taken by a 21-gauge needle into a 3ml syringe, inserted into the arthrodial membrane at the base of the fifth walking leg. The haemolymph was then transferred into an Eppendorf tube. The total haemocyte count was determined by a haemocytometer and the haemolymph refractive index was determined by a density-salinity refractometer immediately after withdrawal, similar to the methods used by Lorenzon et al (2011). The haemolymph was diluted 1:1 with deionised water before being placed on the refractometer to adjust to an acceptable range.

Egg development was checked once a week to minimise female disturbance (see introduction, Table 1.3). The individuals were also observed for any damage, legions etc that may indicate deteriorating health, along with measuring the antennae length (mm). A damage index (Table 2.1) was used to assess changes in observed health over time.

Table 2.1. Damage index used to assess changes in observed health over time.

Damage Description	Index Score
Damage to pereopods	0.5
Damage to cheliped	0.75
Missing pereopods	1
Missing cheliped	1
Discolouration on carapace (including lesions)	1

Broodstock were held at a water temperature of 10°C, salinity of 35 psu, pH of 8.15 in a semi recirculating aquaculture system (RAS) unit at CEMS, Scarborough, UK.

2.2.2. Broodstock Substrate

Broodstock were placed on four different substrates at the Orkney Lobster Hatchery (OLH). The four substrates were; plain plastic bottom (n=31); coral maerl (broken up hard branching coral skeleton nodules) (n=28); mud collected from known *Nephrops* fishing grounds, Cava Island, Scapa Flow, Orkney (58°52.6139N 003°08.4821W) using a Van Veen grab (n=5); and suspended in floating boxes (n=27). All substrate types were provided with artificial shelters. The suspended broodstock were held in perforated plastic boxes, a.k.a. velvet boxes (40 x 29 x 15 cm) which were floated in covered 70 l conical tanks. Four boxes could fit within a 70L conical with each box containing a single adult female. Note that the suspended broodstock ran on a different RAS to the one used for the culture of the larvae, but is included for comparison.

The broodstock that were held within plain, maerl and mud substrates were placed within 72×50 l storage boxes, containing two adults each, and two open plastic pipe sections (figure 2.1). The exterior of the boxes was covered with black sheeting and wooden lids were placed on top to reduce light levels coming into the box. The flow rate was 35ml s^{-1}, maintained at a temperature of 16°C with a general rise during June to 19°C. Salinity was 37-40 psu with a pH of 7.94 and dissolved oxygen levels of 94%. A filter was made to be inserted into the outflow to contain the larvae within the storage box. All broodstock (overwintering and substrate trials) were each fed 1 frozen cold-water prawn per week (£1.88 / 250g from Tesco).

Embryo development was observed. Several methods were trialled in the attempt to cause minimal stress. This included using a dental mirror, which caused significantly more disturbance and stress to the female compared to physically removing the female via use of the artificial shelter. The broodstock were inspected by removing them only when they were residing within the artificial burrow, *i.e.* pipe. All embryos dropped during the season were accurately counted by using Sedgewick-Rafter counter chamber method. The data taken daily were the survival of the broodstock, total number of live and dead larvae produced, and embryos dropped (embryos that had not hatched and did not remain attached the adult).

Dropped eggs were placed into a conical referred to as the “the egg tank”, which ran also on the larval RAS.

Figure 2.1. Different substrates for broodstock; from the top left in clockwise motion the substrates were; Plain; Maerl; Velvet box and Mud.

2.2.3. Larval Rearing

The 70 L conical shaped tanks were used for larval stages 1-3. Water input entered via the taps above the tank and the outflow was situated at the top rear of the conical through a banjo filter. Several configurations for water input were trialled to optimise larval survival, as shown in figure 2.2. The method used with *Homarus gammarus* was not trialled, as the aeration would have caused excessive mortality. The straight water input method incorporated flexible tubing attached to the water flow tap, and placed directly below the water surface, propelling water downwards and directly towards the slopping side of the conical (n=15). This method caused the water to flow in a circular motion both in the horizontal and vertical axes. The tee water input configuration added a further circular water projection against the top of the rim of the slopes (n=5). A baseplate was also trialled using

an upturned bowl-like structure, with a series of holes around the parameter. The water entered the top and propelled out through the holes and up against the slopes.

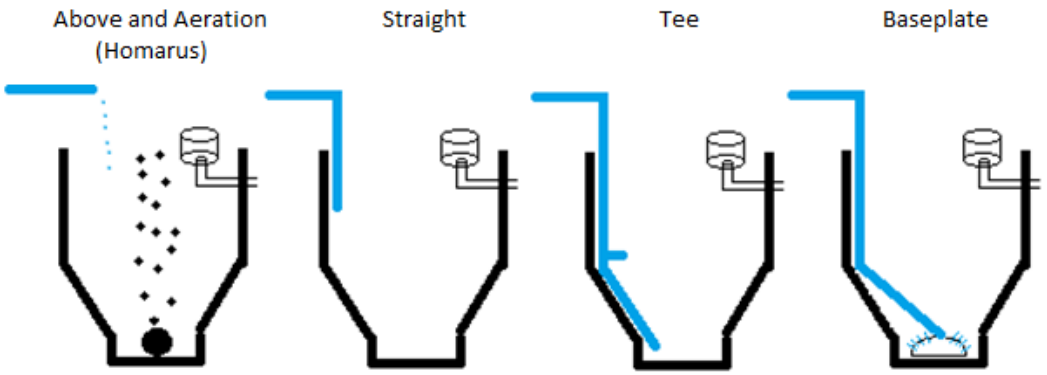

Figure 2.2. Four water input configurations to optimise larval survival within the conical tanks.

The tanks were inspected daily and the hatched larvae were counted. A red light was used to prevent additional stress to the larvae, as ambient surface light intensity can cause damage to the eyes retinal layer (Gaten et al., 2013). Larvae were removed by "water to water" methods using a turkey baster. Larvae were observed within the turkey baster for movement and general health, i.e. observed tail deformities. If a larva did not display any movement within 5 seconds of holding it up to the light, then the larva would be presumed moribund or dead, and discarded. Live larvae were placed into a storage container and then placed into an appropriate conical. Each batch, consisting of 3-4 days of hatchlings, were placed into one conical to reduce cannibalism at subsequent moults (Smith, 1987).

Counts of live and dead larvae were made within the conical every other day. Larvae were removed once a week prior to cleaning and disinfecting the tank with chlorine solution. The filters were removed and cleaned at least twice a day, depending on flow rates and feed build up.

During the initial months, premature larval hatching occurred whilst the system was not running. Therefore larvae were placed into 1000 ml conical flasks and placed on a magnetic stirrer to maintain water movement and prevent larvae clumping on the bottom (n=2). The stirrer speed was adjusted each day depending on observed larval movement.

In addition, different feeds were visually assessed with the different tank hydrodynamics. Both static (copepod mix) and live feeds (rotifers and *Artemia*) were trialled for larval rearing.

The mix of copepodites soft feed (Planktonic AS), stored in chilled aluminium bags, sizes 100-200 µm, 400-700 µm and 1000 µm was provided three times per day. *Artemia* were provided as the methods described in 5.2.3.1. at a density of 3 per ml and the rotifers (*B. plicatilis*) were cultured and fed by the methods described in Millikin (1978) for feeding to blue crab larvae.

The larval RAS unit referred to as "lobster" was maintained at a temperature of approximately 19°C. However, the water temperature of lobster system continually increased to >21°C without controls to cool it down part way through the season. Therefore, a separate system was designed and installed during the later stages of the season which was maintained at a lower temperature (16°C) and is referred to as "Nephrops" and is used for comparison with the "lobster" RAS system. Salinity fluctuated from 37- 40 psu, pH ~8.08 and dissolved oxygen 96%.

2.3. Results

2.3.1. Overwintering Broodstock

Broodstock arrived on the 7th of November 2015, with the highest mortality (27%) within 13 days post transport to CEMS. Mortality averaged at 7% ± 3.4 per month thereafter (figure 2.3). The embryos developed to stage 6 by January and February and stage 7 by the beginning of March. The survival, weight and haemolymph parameters from the different storage configurations were taken over 91 days and the results are shown in Table 2.2.

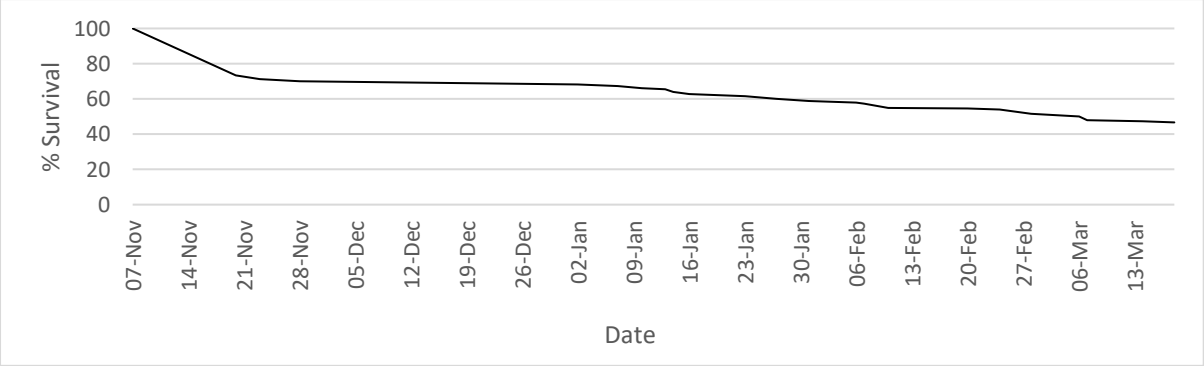

Figure 2.3. % survival of broodstock held over-winter

The space required for communal housing of *Nephrops* that do not have a band placed around the claws (commonly used to stop aggressive behaviour in *Homarus* sp. broodstock) is potentially much greater than for pipe housed broodstock. The pipe housing allows for

approximately double the density of communal tanks, for the same space requirement. Survival was also higher in the pipe housing than the communal tank. However, there was also weight loss, decrease in antennae length and a greater loss in the haemolymph refractive index (Table 2.2.) within the pipe housing. Broodstock within communal housing had greater damage than those kept in the individual pipes. Broodstock in the pipes also showed more healing in previously damaged areas.

Table 2.2. Survival, weight, health and haemolymph parameters for overwintered broodstock kept in two different storage configurations. + = an increase from t0; - = a decrease from t0. ± denotes standard deviation.

Parameter	Communal	Pipe
Potential Density (m^3)	22	48
Survival (%)	67	100
Δ Weight (g)	+ 7.37 ± 8.50	- 0.64 ± 3.02
Δ Damage Index	- 0.33 ± 1.03	0 ± 0.89
THC (t_1) per ml	$5.2 \times 10^6 \pm 2.9 \times 10^6$	$3.1 \times 10^6 \pm 2.3 \times 10^6$
Δ % ($t_1 - t_0$)	+ 102.32 ± 104.29 %	+ 64.30 ± 172.52 %
Δ Antennae Length (mm)	+ 0.34 ± 1.95	- 22.53 ± 31.74
Δ Haemolymph Specific Gravity	- 0.35 ± 0.53	- 0.53 ± 0.57

2.3.2. Broodstock Substrate

In total 44 ovigerous females were placed into the OLH with two shipments arriving in March and May 2013 (figure 2.4). The total number of females in the system was highest during April but rapidly decreased within a month. Broodstock were not available during May, apart from one small shipment of which the majority either died or dropped their eggs prematurely. In total 17 and 27 females were placed into the broodstock and larval systems respectively.

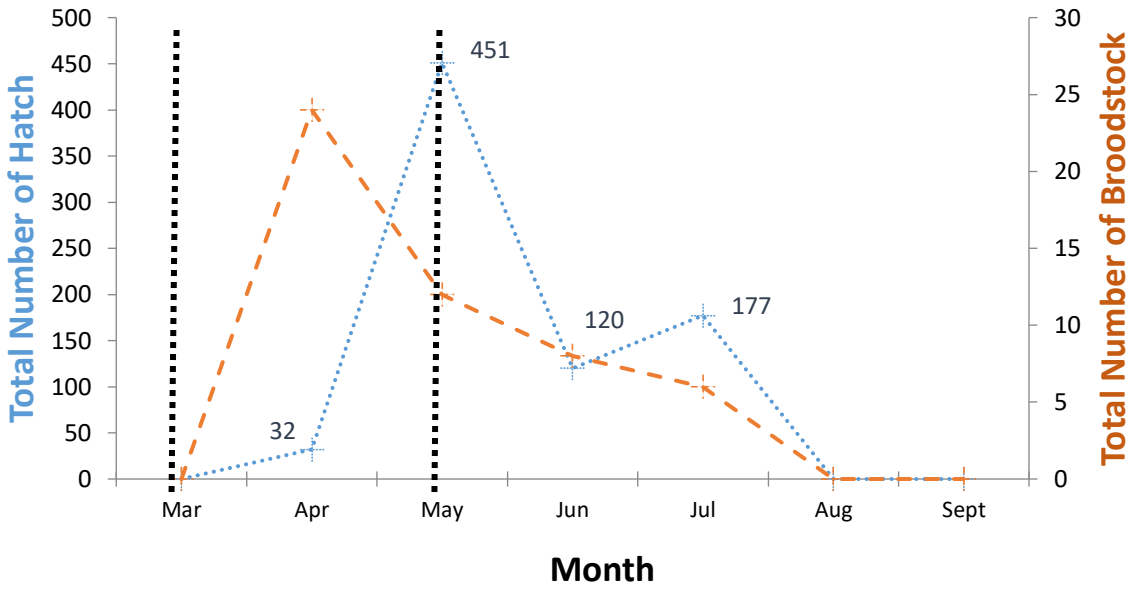

Figure 2.4. Total number of broodstock (dashed orange line) and the total number of hatched larvae (spotted blue line). Note the black dashed line indicates the two broodstock shipments into the OLH in 2013.

Approximately 40% of the broodstock moulted before the release of any eggs or larvae hatching in both systems (Figure 2.5). Fifteen percent of broodstock died within the larval rearing RAS system compared to none in the broodstock RAS system. However, 18% of broodstock in the broodstock RAS system held onto the embryos untill the end of the experiment and did not release any larvae.

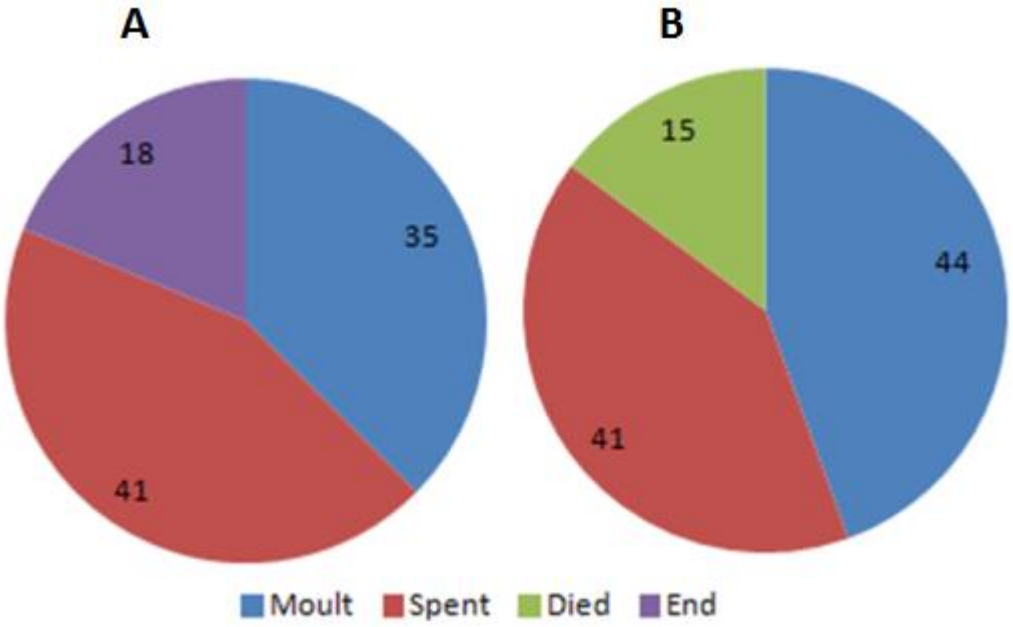

Figure 2.5. Pie chart illustrating the fate of the broodstock in the (A) broodstock and (B) larval RAS systems. Moult = Moulting before embryo release; Spent = removed after embryo release; Died = removed due to mortality; End = Did not release and held onto embryos.

All the substrates produced on average 12 ± 25 larvae per female. Broodstock held in the plain bottom substrate produced 19 ± 34 live larvae per female, the highest number from all substrates, but this was not significantly different when compared to other substrates (Kruskal Wallis, P>0.05). Broodstock held within the maerl substrate had the lowest production of live larvae, producing an average of 1 ± 2 live larva per female. Figure 2.6 illustrates the production of larvae from all the substrates including the egg tank. A total of 61 live larvae (an average of 6 ± 5 per female) and 18 ± 13 dead larvae were found within the egg tank.

The broodstock held in velvet boxes produced the highest number of dead larvae per female (15 ± 45) when compared to plain (1 ± 2), maerl (1 ± 2) and mud (1 ± 1) substrates.

The number of eggs dropped per female was significantly different between the substrates (Kruskal Wallis, H (adjusted for ties) = 9.28, df=3, P=0.026). The highest number of eggs dropped per female were in the velvet box, *i.e.* the larval system with 137 ± 146 eggs dropped per female. The highest number of eggs dropped within the broodstock system was the plain substrate (34 ± 45) when compared to the maerl (3 ± 6) and the mud (0 ± 0).

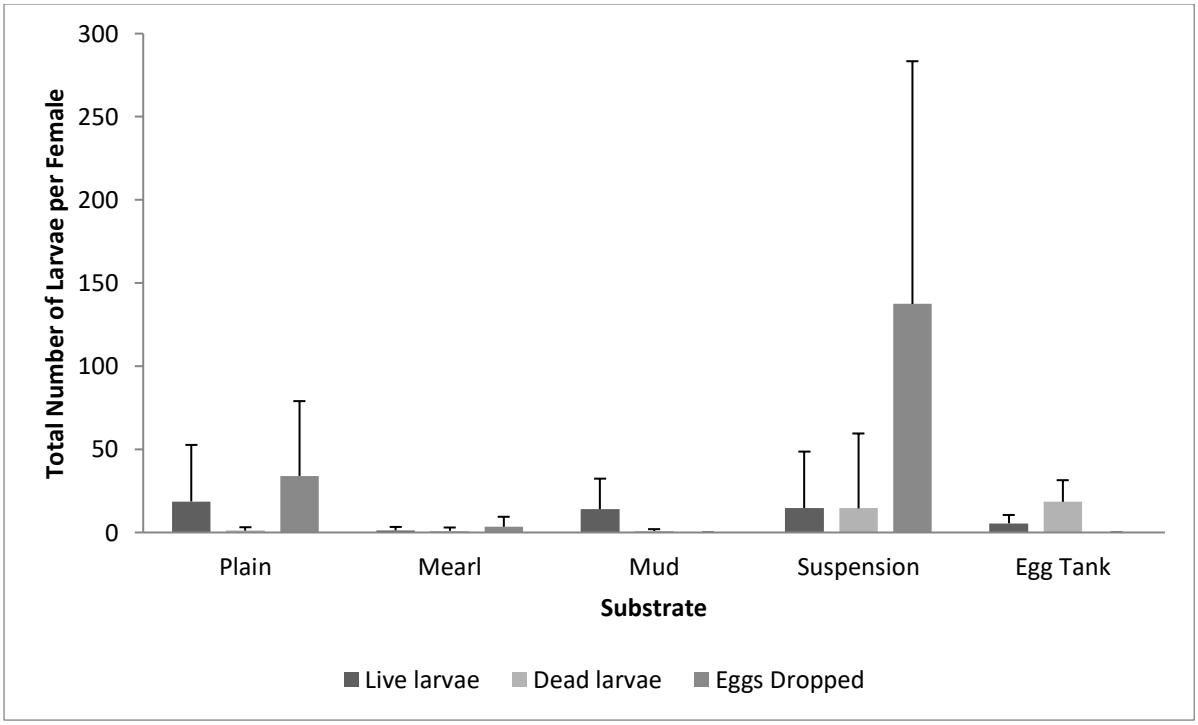

Figure 2.6. Total number of live larvae, dead larvae and eggs dropped per female over different substrates. Error bars denote SEM.

The range of days spawning (Figure 2.7), *i.e.* the number of days from when the first larvae were being released to when the embryos were either all released, or the female was removed from the system, was significantly different between substrates (Kruskal Wallis, H (adjusted for ties) = 7.96, df=3, P=0.047). Overall, broodstock within the velvet boxes were spawning for a longer duration (6d ± 5.3) than broodstock in the mud substrate (2d ± 1.9). With all the substrates combined broodstock performed spawning over an average of 3d ± 1.3.

Broodstock with embryos at development stage 9 held within the mud substrate held on to the embryos for 30d ± 7.8 before releasing, but yet had the lowest number of days before they started spawning (16d ± 7.8) from first entering the hatchery. Broodstock within the velvet boxes held onto stage 9 embryos for the lowest duration of all the substrates (10d ± 11.0). However, the embryo development took longer from first entering the hatchery before they released any larvae (57d ± 16.4).

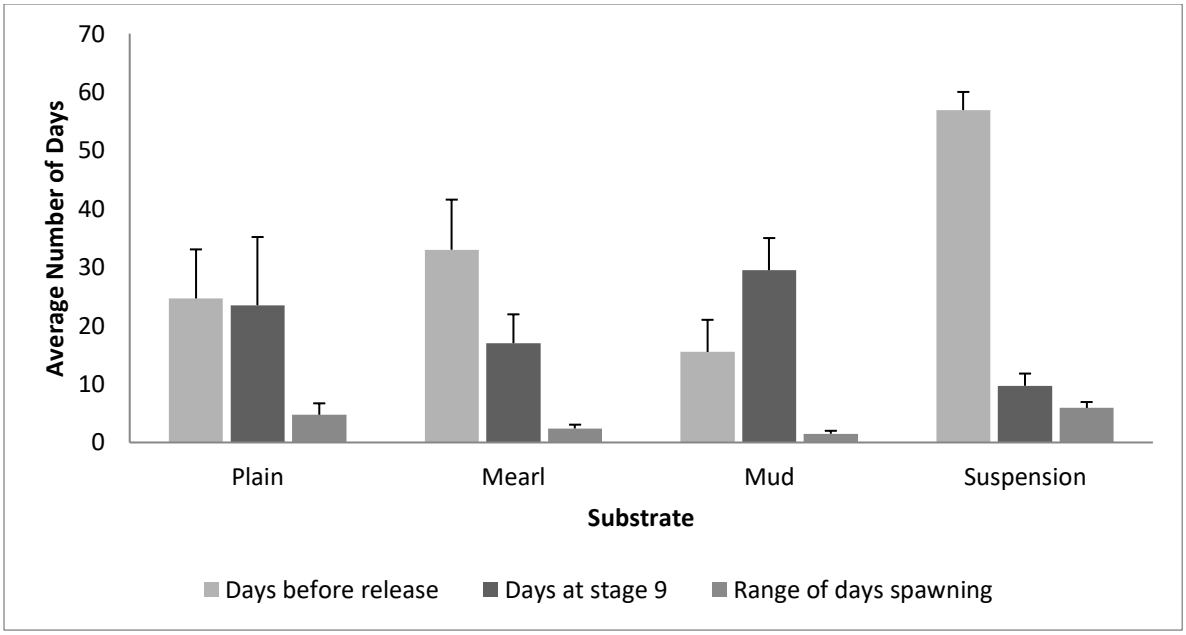

Figure 2.7. The average duration (days) of the broodstock until larvae were first observed; the duration of the embryos at stage 9 and duration of spawning. Error bars denote SEM.

2.3.3. Larval Rearing

Overall, larval survival by day 7 was at 38%, by day 14 it was at 18%, and by day 21 at 3% (Figure 2.8). No larvae survived past day 28. Twenty seven percent of Z1 larvae died within five to seven days of hatching. Within the *Nephrops* culture system the survival of Z1 and Z2 larvae was 100% and 31% respectively. However, within the lobster rearing system Z1 and Z2 survival was 58% and 13% respectively (Figure 2.8). The survival of larvae going from Z2 to Z3 was not different between systems, and usually very low (<3%). The majority of larvae that did survive to Z3 remained at this stage for ~10-12 d. There was no successful development from stage 3 to PL stages.

Larval survival decreased to 0% in conjunction with an increase in the water temperature. There was no significant difference in larval survival between water input configurations and the different system configurations (Kruskal Wallis, $P>0.05$). The survival of larvae within the straight water treatment was only minimally higher than within the tee water treatment (Figure 2.8).

Using a magnetic stirrer to gently agitate the water gave good results with regards to Z1 survival (86%), but no larvae developed further to Z2.

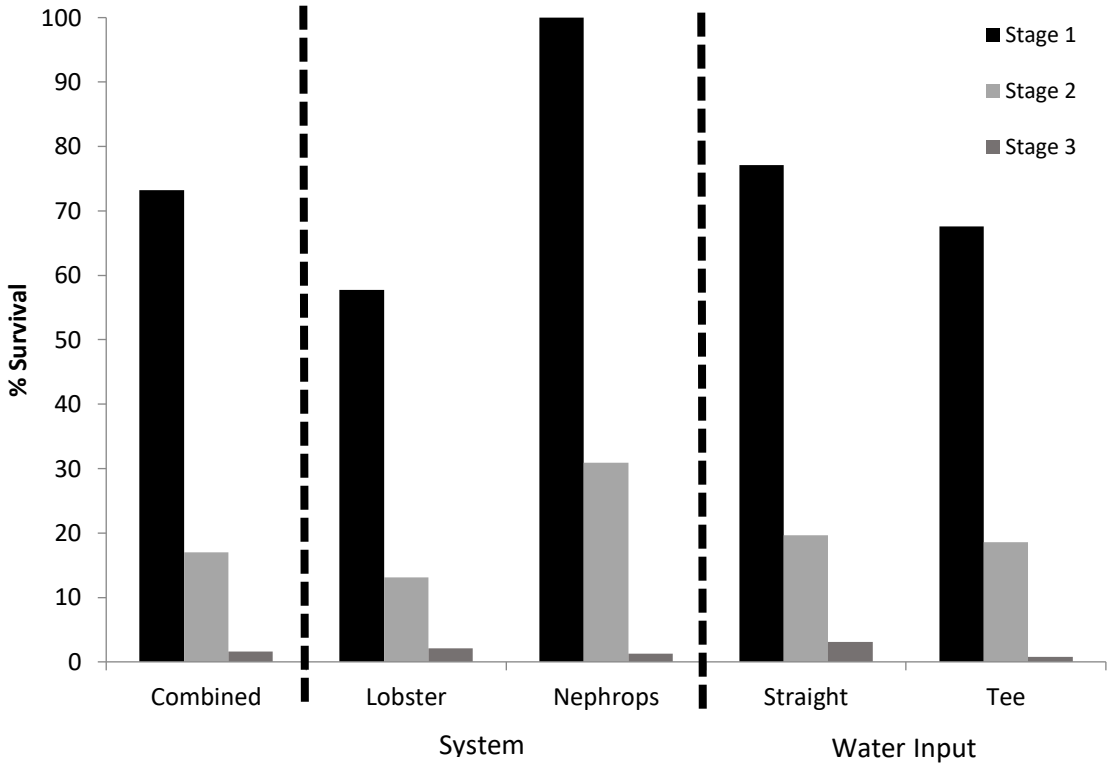

Figure 2.8. Larval survival for each stage compared between different rearing systems and water configurations.

The baseplate water configuration performed unsatisfactory whilst trialling with *Homarus* larvae. The objective of keeping larvae and feed from the bottom succeeded but the flow through was so high that it caused larvae to clump onto the filter. Therefore, the trials were not continued, and no data are shown.

The practical assessment of the diets for use with *Nephrops* larviculture revealed that the static copepod mix diet had a high sinking velocity. In conjunction with a low water velocity, this caused the uneaten feed to accumulate in a large mass within the tanks. This caused considerable difficulties in maintaining hygiene levels within the larval tanks. Within 1 hr the majority of the feed had collected at the bottom or on the sides of the conical. Within 24 hr post feeding, none of the feed was dispersed within the water column. Feed would coalesce into large aggregations (>1 cm) and cause the larvae to become entangled (Figure 2.9). In addition, an occurrence of a mass mortality of all larvae (both *Nephrops* and *Homarus gammarus*) occurred when using one batch of the copepod mix feed.

However, the *Artemia* and rotifers remained within the conical, dependent upon the filter size, with minimal coagulation on the tank bottom and sides. The use of different filter sizes also allowed the user control over the duration the larvae were feeding: small mesh retained the feed before a larger mesh size allowed the live feed to escape through the outflow.

Figure 2.9. Copepod feed build up within the conical over 24 hours. Note the large nodules of feed after 24 hours indicated by the red circles.

2.4. Discussion

2.4.1. Broodstock

The highest mortality of broodstock held over-winter was within the first 13 days post transport. Quantifying the stress parameters within *Nephrops* during capture has been well documented (Harris and Andrews, 2005; Ridgway et al., 2006a, 2006b; Lund et al., 2009; Albalat et al., 2010). Using the same techniques, the stress parameters identified within *Nephrops* could be quantified during both capture and the period in time when the broodstock are placed within communal holding facilities. It would be interesting for the advancement of aquaculture of this species, as well as the ecology and behavioural sciences, to investigate how stress metabolites interact with capture and social stress. It would also be beneficial to develop a method or protocol that could reduce this mortality post arrival at the aquaculture facilities. It is also a recommendation that broodstock are procured in close proximity to the aquaculture facilities to reduce transport time, and that the location of the facility is in an area where *Nephrops* fishing takes place, and therefore broodstock are readily available.

Holding broodstock individually overwinter allowed for a higher density and control over each individual. However, there was concern over the antennae damage and holding an individual

in a small compartment for extended periods of time. The pipe itself was difficult to maintain with the need for retrieving, opening and closing the mesh ends for checking of the individual and providing or removing feed. There was a large increase in THC in both storage configurations which may be a general indication of acute stress. The higher THC increase in communal storage may be due to the continuous physical activity involved with conspecific interactions. The un-natural density at which the individuals are held may cause cumulative physiological stress, even when the hierarchical structure limits agonistic behaviours (Katoh et al., 2008). Jussila et al. (1997) indicates that THC below 4 x 10^6 cells/ml is an indication of poor health. Therefore, the t_1 THC indicates that individuals held in the pipe storage for 79 days had low circulating haemocytes and suggests reduced cellular immune competence (shown in Table 2.2). The haemolymph refractive index can be directly linked to total protein content (Ozbay and Riley, 2002). The decrease in total protein in broodstock held in the pipes could indicate an overall lowering of general health and an increase of susceptibility to disease (Fotedar and Evans, 2011).

It is proposed that individual cell compartments should be used, but with a space that is large enough to allow the individual to roam. The cells should have a mesh bottom for water exchange and detritus removal and an open top for easy maintenance, access and observation.

The high percentage of broodstock that moulted during the trials at OLH (>40%) was likely due to the sudden increase in water temperature to >19^{o}C, with the majority of females remaining ovigerous and therefore losing the entire brood. The optimal temperature for the development and survival of *Nephrops* larvae is 15-17^{o}C (Dickey-Collas et al., 2000; Powell and Eriksson, 2013; Thompson and Ayers, 1989). The temperature of 19-20^{o}C found in the system during the summer months is at the upper thermal tolerance limit for this species (Powell and Eriksson, 2013). There is limited scientific evidence of a safe temperature regime for the successful development of the embryos and broodstock. Therefore, it is suggested that *Nephrops* broodstock are held in ambient water temperatures according to the time of year and depth from which the broodstock are sourced.

The results indicate that broodstock held within the plain substrate produced the highest number of larvae. In contrast, broodstock within the velvet boxes produced the least number

of viable larvae. The cause of the low larval viability within the velvet box is also probably due to the higher water temperature, as they were within the larval (Lobster) system (>19^{o}C) rather than the broodstock system (16^{o}C). Although the temperature may increase the rate of egg development, the rate itself may be too high for successful embryonic development and subsequent healthy larvae.

The muddy substrate replicates to some extent the adults natural environment (although still under aquarium conditions) where they can display some natural behaviours such as constructing burrows (Campbell et al., 2009). However, problems may arise when using mud in a RAS facility due to the difficulties in acquiring and transporting the quantity needed, and maintenance issues that arise in holding sediments with a high organic load (Chapman and Rice, 1971; Campbell et al., 2009; Sardà and Aguzzi, 2011). Therefore, future research could investigate the possible use of artificial substrate to replicate the characteristics of silt/clay mud typically found within *Nephrops* grounds. It is possible that beneficial bacteria found within the mud could assist in maintaining a healthy embryo, as it has been shown in other marine invertebrates (Peyton et al., 2004) but it remains unclear whether this is found within *Nephrops.*

Embryos that had fallen from the adult and placed within the egg tank did contribute to the live larvae production. However, the majority of the embryos and the larvae that did hatch were not viable. It is unclear whether the embryos were dropped because they were un-viable or whether leaving the protection of the adult caused the larvae to become un-viable. Embryo loss may have several causes including mechanical abrasion, direct removal from the female, increased pleopod fanning, parasitism and adult stress (Balasundaram and Pandian, 1982; Shields, 1994; Brillon et al., 2005).

Broodstock tended to spawn over a range of three consecutive days, similar to the findings of Smith (1987). Spawning was over several months from April to August.

2.4.2. Larval Rearing

Using the mass production methods to rear *Nephrops* resulted in low survival to Z3, which is similar to the findings from Smith (1987). The highest number of larvae produced was during May and again in July. There was no successful metamorphosis to the PL stages. There were

no significant improvements in survival due to the water system (Lobster and Nephrops) or water input method (Straight and Tee).

Due to the temperature rise and water quality issues, there was high mortality. However, even within the Nephrops system, which was maintained at a lower temperature, the survival was still low.

Other factors such as feed could have influenced the low survival. Overall the copepod feed diet caused mortality to the larvae due to "clumping" of the feed both in the bottom of the tank and on the filters. Although copepods are considered to have nutrient profile that is better suited than other live feeds such as *Artemia*, the issue of inert feed causing major mortality is a clear problem for *Nephrops* larviculture. Live feeds, such as *Artemia*, rotifers, copepods would be recommended. Mass mortality of *H. gammarus* larvae was observed due to poor quality inert feed, which highlights the potential for intruding harmful bacteria and parasites within the feed. In total 78% (6 batches) were lost from filter loss which occurred on one single evening. The conical had to be siphoned daily to remove large coagulates of uneaten feed, within which the larvae would become entangled. In comparison, the *Artemia* and rotifer diets flowed freely out of the tank. The major problem of the *Artemia* and rotifer diet was that the filter mesh diameter was too large, and the majority of the feed was able to flow from the rearing tank within ~2 hours. To improve on this different filter sizes should be selected. Small diameter mesh could be used to trap the live feed within the rearing tank and replaced with larger mesh to allow the feed to be flushed from the tank. Clearly *Nephrops* larval rearing is difficult, with major bottlenecks due to system design and diets. Further experiments should primarily assess the appropriateness of different live feeds for use with *Nephrops* larval rearing before further investigation.

It must also be noted, as previously discussed, that the high temperature during embryo development may have produced un-viable larvae and may explain the insignificant differences between systems and water configurations. Temperature directly affects yolk absorption and conversion efficiency and therefore impacts upon larvae tissue development and subsequent larval characteristics (Brillon et al., 2005). Abnormalities and deformities caused by high temperatures have been shown to impact upon embryo and larval

development in other crustaceans (Rhodes, 1981; García-Guerrero et al., 2003; Sibert et al., 2004; Brillon et al., 2005).

Observations of the hatched larvae indicate that a considerable proportion displayed abnormal behaviours and were morphologically deformed. Deformity manifested itself in a number of ways including cramped/curled telson, reduced swimming behaviour and low numbers of larvae remaining near the surface. Viable larvae should actively swim and 'capture' feed (Pochelon et al., 2009; Powell and Eriksson, 2013). Instead the majority of larvae in these trials did not display swimming behaviour and remained passive in the water flow within the conical. A proportion also remained curled after hatching which was most notable for the larvae hatched from within the egg tank. These deformities may have been caused by a number of factors including temperature, but also emaciation, nutrient deficiency, genetic expression, embryonic development associated with broodstock health, infectious microbes and water quality issues (Johnson, 1995).

In conclusion, the experiments have given insight into developing *Nephrops* aquaculture facilities. Broodstock are better maintained individually, but they also need to be easily accessible and manageable. Use of substrate does have an impact to the overall health and subsequent larval supply. The larvae cannot withstand high temperatures, nor inadequate feed. Development in the rearing vessels (specifically hydrodynamics and filtration to assist larval and feed circulation and retention) and the feed type will benefit the survival and growth. The pilot data and discussion provided gives a background and baseline of understanding that will benefit the experimental procedures developed in the chapters of this thesis.

3. Intraspecific Interactions and Associated Blood Chemistry in Adult *Nephrops norvegicus*.

Abstract

Conspecific interactions within species that establish hierarchal dominance, often involves agonistic interactions that can be costly to the individual. The principles that govern social interactions can affect the behaviour and physiology within the individuals involved. This study examined the intraspecific interactions of a commercially important species, *Nephrops norvegicus*, staging two individuals of different sex and size combinations. The behaviours were examined in conjunction with physiological parameters. The baseline t_0 and t_1 haemolymph levels of protein, glucose, lactate, ammonia, total haemocyte counts (THC) and the refractive index (RI), were examined. Behavioural interactions did cause significant changes in THC, lactate and ammonia levels but not in protein, RI and glucose levels. Frequent behavioural patterns, both individual and in relation to the opponent's display, were identified with a significant majority of behaviours being submissive rather than aggressive. Aggressive behaviours correlated with THC and protein levels, whereas submissive behaviours correlated with lactate and ammonia levels. There were significant differences between sex and size combinations. Female baseline levels were significantly lower than those of males in all haemolymph parameters. Males with male opponents and treatments where the opponent was of symmetrical size exhibited a higher frequency of aggressive behaviours, whereas females with female opponents and asymmetrical size exhibited a higher frequency of submissive and exploratory behaviours. Carapace length also correlated with THC, glucose, protein and RI levels. RI levels correlated with haemolymph protein levels. Thus, the results indicate a relationship between physiology and conspecific interaction. The key metabolites involved and the implications of these findings for future studies are discussed.

Abbreviations:

RHP= resource holding potential; THC= Total Haemocyte Counts; refractometer analysis (RI); MMSS= male vs male same size; MMDS= male vs male different size; FFSS= female vs female same size; FFDS= female vs female difference size; MIXSS= mixed sex same size.

3.1. Introduction

From a practical perspective knowledge of social behaviour in crustaceans is important as it may inform practices to improve survival after disturbance from fishing, live transport and holding operations (Katoh et al., 2008; Stoner, 2012). There is growing interest in the principles that govern social interactions and how these behavioural patterns and rules flow between individuals (Sumpter, 2006). Crustaceans often exhibit agonistic behaviour when competing for limited resources such as shelter, food and mating opportunities (Chapman and Rice, 1971; Katoh et al., 2008, 2013). From these interactions, behavioural responses can be either submissive, where an individual will retreat from the opponent, aggressive, where the individual exhibits agonistic behaviours towards the opponent and neutral, neither aggressive or submissive (Stoner, 2012). Additionally, *Nephrops norvegicus* (referred from here onwards as *Nephrops)* establish dominance hierarchical relationships resulting from these interactions (Katoh et al., 2008). The behavioural responses are influenced by intrinsic and extrinsic factors (Briffa and Sneddon, 2007; Moore, 2007). Extrinsic factors include the use of chemical signalling and pheromones received from the sender (Katoh et al., 2008). Intrinsic factors are, for example, the sex, size and experience of the individual (Moore, 2007; Stoner, 2012).

It is known that *Nephrops* exhibit sex-based differences in behaviour. Males typically show higher levels of aggression, and are found with a higher ratio of damaged claws compared to females (Chapman and Rice, 1971; Farmer, 1975; Briones-Fourza et al., 2015). Females appear to be less active and spend less time exploring out of the burrow than males, especially during the incubation period (Farmer, 1974a). In addition, size is a key factor in determining hierarchical structure and access to resources (Parker, 1974; Thiel and Baeza, 2001; Moore, 2007). The largest individuals are usually the most dominant in most Crustacea, but other factors such as the individual's resource holding potential (RHP), experience, general fitness and the resource value can influence the outcome of social interactions (Parker, 1974; Huntingford et al., 1995; Briffa and Sneddon, 2007).

However, there are also physiological adaptations and responses that can play an important role in how an individual behaves (Briffa and Sneddon, 2007). For example, the concentration of serum lactate and the energy stored (such as glycogen) is integral to the decision-making process of attacking or defending in Hermit crabs (Briffa and Elwood, 2001, 2002). However

there has been limited research on how interactions with conspecifics correspond to the internal physiology of *Nephrops*. It would therefore prove useful to use multiple physiological parameters to comprehensively quantify the consequences of social interaction.

Haemolymph parameters have been widely used to evaluate the effects of fishing related procedures, live transport and holding operations in commercially valuable crustaceans (Stoner, 2012). There are several commonly measured haemolymph parameters that indicate changes in homeostasis in response to a wide range of stressors. These parameters are ammonia, glucose, lactate, total protein and haemocyte counts and density (Ridgway et al., 2006a, 2006b; Fotedar and Evans, 2011; Stoner, 2012; Bernardi et al., 2015). However, the subject is complex and baseline values can show pronounced variation (Taylor et al., 1997). Stoner (2012) provides a comprehensive review of how different stressors have caused different physiological responses. For example, haemolymph glucose levels either decrease or increase from stressors such as exposure and temperature, baseline levels can be variable in parameters such as Total Haemocyte Counts (THC), (Ridgway et al., 2006a) and protein levels can vary over the moulting cycle (Chang, 1995), making interpretation difficult.

This chapter aims to investigate the appropriateness of the suggested parameters for assessing physiological changes in association with behavioural interaction within *Nephrops*. It also aims to investigate the behavioural patterns, responses and inter-sequence behaviours between different conspecifics in relation to sex and size. Specifically, this investigation will assess the haemolymph parameters in response to intraspecific interactions similar to those experienced in commercial situations such as in creels and holding facilities.

3.2. Methods

3.2.1. Animals

Adults of mixed sex and sizes (min CL 32.8 mm, max CL 51.8 mm; Appendix 3A) were creel caught (July and August 2014) in the Gullmarsfjord, Sweden, and brought to the Sven Lovén Centre for Marine Sciences (Kristineberg; period emersed <30 mins), where they were housed in individual cells and under dimmed lights (by covering the lights with layers of black plastic). The carapace length (CL), sex and moult stage, using pleopod staging criteria (Aiken, 1973; Waddy et al., 1995), were taken upon arrival. The *Nephrops* were held in a flow through

system, with water taken from a depth of approximately 60 m with a temperature of (mean ± S.D) 17^{o}C ± 0.21 and a salinity of 33 PSU ± 0.11.

3.2.2. Experimental Procedure

Preliminary observations were performed to develop the ethogram (Appendix 3A; Table 3.8), and the behaviours were also grouped into three categories; exploring, aggressive and submissive. The sequences of behaviours were further categorised as "start", "intermediate" and "end", indicating respectively the behaviours exhibited at the beginning of an interaction, behaviours after the start, and the concluding behaviours before separation. The behavioural trials consisted of different sex and size combinations of two individuals (Table 3.1). Overall there were five treatments with five replicates in each and five controls for each sex. Behavioural observations and haemolymph parameters (glucose, lactate, protein, ammonia, total haemocyte count and the refractive index) were taken from each individual per trial.

Table 3.1. Sex and size combinations for behavioural interactions. Different sized symbols represent different sized individuals. ♂ = Male; ♀ = Female.

	Same Size	Different Size
Different Sex	♂ ♀ **MIXSS**	
Same Sex	♀ ♀ **MMSS**	♀ ♀ **MMDS**
Same Sex	♂ ♂ **FFSS**	♂ ♂ **FFDS**

Individuals were selected depending upon sex and size characteristics and the haemolymph was taken (t_0), the individual was then marked with permanent marker for identification purposes. There was an average CL difference of 0.5 mm (S.D = 0.54) within the Same Size (SS) treatment and 8.6 mm (S.D = 4.19) within the Different Size (DS) treatment. The individuals were then placed in a 60L tank (the arena) (L60 x H34 x W30) and left to acclimatise for 20 minutes behind a separating barrier. After the acclimatisation period, the barrier was removed and the sequence and duration of behaviours of each individual were continuously noted for a total of 30 minutes. Individuals were then removed from the arena and the haemolymph was again taken (t_1), and the animals returned to known cells in the holding

tank. The dominant and subdominant individuals were noted (referred to as winner/loser respectively). Individuals were only used once throughout the experiment. The t0 and t1 haemolymph was taken from a control group (n=5) subjected to the same methods as above, however absent from conspecific interaction.

A total of 400 µl of haemolymph was drawn by a 23-gauge needle into 1ml syringes, inserted into the arthrodial membrane at the base of the fifth walking leg, and then placed directly onto ice. One syringe withdrew 100 µl haemolymph into an equal volume of ice cold anticoagulant (see Appendix 3A) and used for total haemocyte counts (THC) and refractometer analysis (RI). A further 300µl of undiluted haemolymph was used for biochemical assays (Powell and Rowley, 2008).

Haemolymph was allowed to clot on ice and was centrifuged (10,000 x *g*, 5min), to remove haemocytes and large blood clotting proteins to produce serum. The supernatant was taken and stored as 100µl aliquots at -20 °C and used within 7 days after being defrosted once only. For glucose, ammonia and lactate assays, 100 µL of serum was mixed with 150µl 5% Trichloroacetic acid (TCA) to deproteinise the serum. The serum was then centrifuged once more to remove protein as a precipitate. After appropriate dilution, metabolite concentrations were determined in triplicate in 96 well plates (Nunclon™ MicroWell) via assay kits and processed in a spectrophotometer (Labsystems iEMS 96 well plate reader MF). Arbitrary absorbance readings were measured and correlated to the known standard curves (Ridgway, 2005; Ridgway et al., 2006b).

3.2.2.1. Glucose

Serum glucose levels were determined using the Amplex® Red Glucose/Glucose Oxidase Assay Kit. Reagent aliquots (200µl) was added to 50 µl of serum and incubated at room temperature for 30 minutes. Glucose levels were determined using calibration curves produced prior to the experiments. The absorbance was read at ~560 nm.

3.2.2.2. Lactate

Serum lactate values were determined using a Sigma-Aldrich Lactate Assay Kit. The haemolymph was centrifuged, deproteinised and stored as above. The reagent was added to the 50 µl sample and incubated at room temperature for 30 minutes. The samples were

calibrated against a standard curve of lactate using an absorbance of 570 nm (Bergmann et al., 2001).

3.2.2.3. Ammonia

Ammonium concentrations were determined via the Indophenol method (Boltz and Howell, 1978). The haemolymph was centrifuged (10000 g, 5 min), and the supernatant taken and diluted with 1:5 3% sodium chloride solution. Ammonia reagent 1 (50 µl; see Appendix 3A) was added to 100 µl of serum and mixed, then 50 µl of reagent 2 (see Appendix 3A) was added and incubated for 30 minutes at 37^{o}C. The samples were calibrated against a standard curve of ammonium chloride using an absorbance of 635 nm.

3.2.2.4. Protein

Serum protein concentrations were determined by using the BCA^{TM} Protein Assay Kit in accordance to the manufacturer's guidelines. The serum protein levels were determined by adding samples of non-deproteinised serum with BCA working reagent, diluted 100 fold in 3% sodium chloride and MilliQ water solution, into each well and incubated at 37 ^{o}C for 30 minutes and allowed to cool to room temperature. Samples were calibrated against a bovine serum albumin (BSA) standard curve using an absorbance of ~562 nm. Protein concentration data were analysed for correlation with RI (nD).

3.2.3. Statistical Analysis

All data were tested for normal distribution (Kolmogorov-Smirnov Test) and equal variances (Levene's test). If the data were not normal they were either transformed or tested with a non-parametric test (Dytham, 2003). Data were analysed using R software and packages (R Core Team 3.1.2, 2015).

A Kruskal Wallis and post hoc test were used to test differences in behavioural pattern frequencies and inter-individual sequence behavioural probabilities between treatments. They were also used for moult stage analysis and all haemolymph level change between treatments. Percentage data were arcsine transformed.

A *t*-test was used to test differences in protein, THC and RI haemolymph levels between control and treatment, between t_0 and t_1 levels and between sexes and size classifications (larger individual, smaller individual) and sex treatment (same sex, opposite sex). If normality

could not be met through transformation then a Wilcox test was used (glucose, lactate and ammonia parameters). A Wilcox test was also used to test differences in following behavioural patterns and behavioural frequencies between sexes and size classifications (Lehner, 1996).

A chi square *r* X *k* test of independence was used to assess if there was significant association between two following behaviours from different individuals (inter-individual sequences) (Lehner, 1996). A Fishers Exact test was used to test differences in the frequency of behavioural sequences and the number of winners between two variables. An ANOVA and Tukey HSD post hoc test was used to test the total durations of behavioural categories (aggressive, submissive and exploratory) between treatments.

Behavioural scores were derived by using the sum of categorical behaviours and multiplying by the total duration.

To test correlations between behavioural scores, sex, size and haemolymph level change (Δt), the Pearson's Correlation Coefficient (r_p) and Spearman Rank Correlation Coefficient (r_s) were used when data met the assumption of each test.

Several general linear models were formulated as a predictive model that best approximated the different factors such as sex, size, behaviours and haemolymph levels on the fight outcome (winning) and the amount of aggression or submission displayed within the encounter. The Alkaike information criterion was used to rank and compare candidate models.

Several models were tested to predict the outcome of the individual from the interaction period (win / lose) based on behavioural data alone (shown below).

Model 1 (Aggression)

$$X = (fA + fMS + fT) \times (tA + tMS + tT)$$

Model 2 (Aggression and Submission)

$$X = ((fA + fMS + fT) \times (tA + tMS + tT)) - ((fAV + fQ + fTF) \times (tAV + tQ + tTF))$$

Model 3 (Aggression and submission)

$$X = (fA + fMS) - fTF$$

Model 4 (Aggression and submission)

$$X = (fA + fMS) – (fAV + fTF)$$

Model 5 (Aggression and submission)

$$X = (fA + fEX + fMS) – fTF$$

Model 6 (Submission)

$$X = (fAV + fQ + fTF) \times (tAV + tQ + tTF)$$

Model 7 (Exploring)

$$X = fEX \times tEX$$

Model 8 (Aggression, exploring and submission)

$$X = (fA + fEX + fMS + fT) – (fAV + fQ + fTF)$$

Where X = dominance score; f = total frequency of behaviour; t = total duration of behaviour; A= approach; AV = avoid; EX = explore; MS = meral spread; Q = quite; T = touch; TF = tail flipping.

3.3. Results

3.3.1. Behavioural Patterns

Some major behavioural patterns were seen (Figure 3.1) with significant differences in the frequency of behavioural sequences between treatments (Kruskal Wallis, $P<0.05$). Individuals would approach and touch the opponent following exploration of the arena. Individuals then either avoided or began to display meral spread. There was a significantly higher frequency of aggressive behavioural patterns such as “meral spread” followed by “touch” in the treatments with similar sized individuals (1.47 ± 0.26) when compared to different sized opponents (0.15 ± 0.15; Kruskal Wallis, $\chi^2 = 23.53$, df=4, $P<0.05$). Females of Similar Sizes (FFSS) exhibited a higher frequency of “meral spread” followed by “touch” behaviours (1.83 ± 0.50) than Females of Different Sizes (FFDS; 0 ± 0), and similarly Males of Similar Size (MMSS) was higher (2.1 ± 0.46) than Males of Differing Sizes (MMDS) (0.3 ± 0.3). MMSS was also significantly higher than FFDS.

Following interaction, the individuals would either further “explore” the arena or exhibit “quiet” behaviours. Individuals in the FFDS (10.6 ± 1.68) and Mixed Sex of the Same Size (MIXSS) (9.8 ± 1.57) showed a significantly higher frequency of “exploratory” behaviours following “quiet” behaviours when compared to MMSS (3.1 ± 0.55; Kruskal Wallis, $\chi^2 =17.92$,

df=4, P<0.05). The same was attributed for "quiet" periods following "exploratory" periods (Kruskal Wallis, χ^2 = 12.17, df=4, P<0.05). Overall, males exhibited a significantly higher frequency (1.92 ± 0.48) of "approaching" followed by "exploring" when compared to females (0.44 ± 0.18; Wilcox test, W = 213.5, P<0.05).

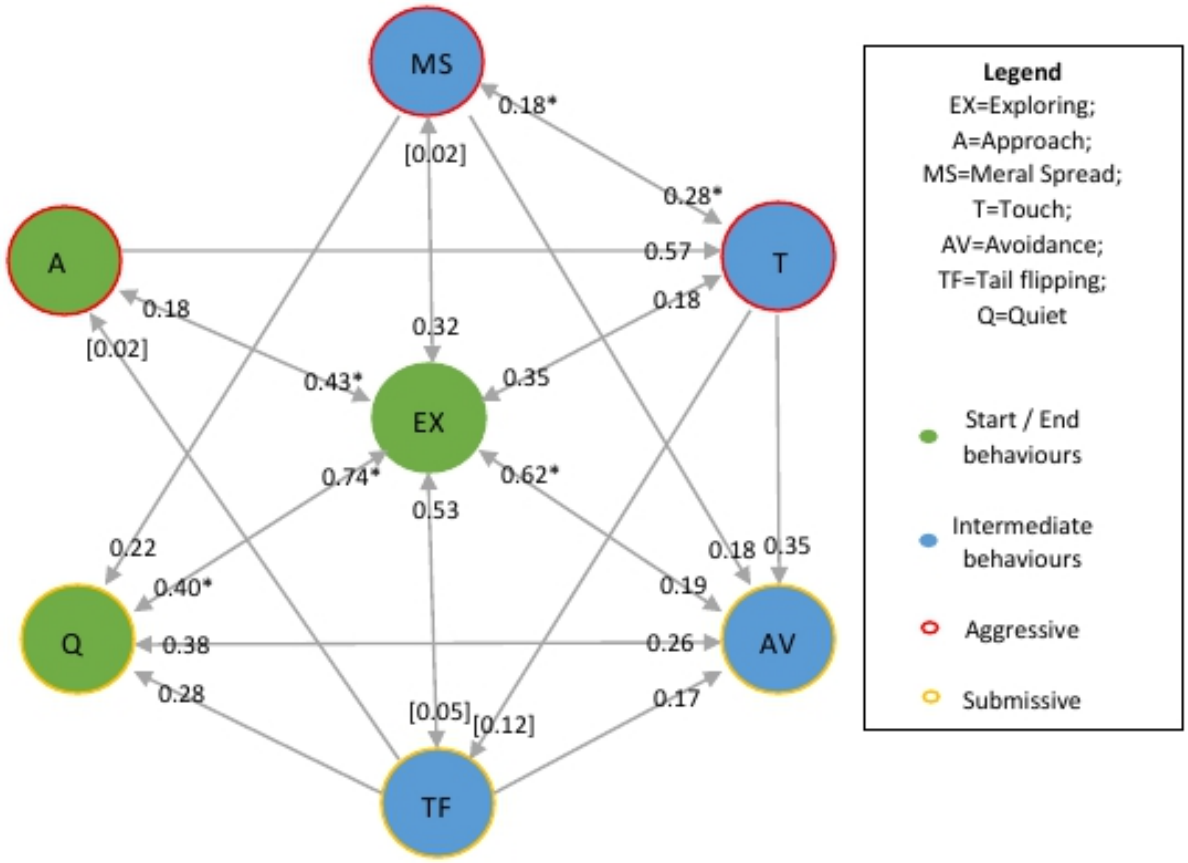

Figure 3.1. Major behavioural sequence probabilities of individuals (n=52). Only sequence probabilities above 18% are indicated to highlight overall key behavioural patterns. Sequence probabilities below 18% are shown in brackets are included for figure completeness. Asterisks indicate where significant differences lie between treatments (P<0.05).

Inter-individual sequences indicate how the preceding behaviour (t_0) of individual A influences the behaviour of the opponent B (t_1; Figure 3.2 - 3.5). The frequencies of the observed inter-individual sequence behaviours are significantly different from random (Chi-squared χ^2= 1249.24, df=21, P<0.001). Overall the behaviour of individual A at t_0 had a significant influence on the way the opponents responded (Kruskal Wallis, χ^2= 26.15, P<0.001; Figure 3.2). Aggressive behaviours from individual A (t_0) had a significantly higher probability

that the opponent would respond (t_1) with an aggressive behaviour (4.40 ± 0.65), when compared to individual A displaying submissive behaviours (t_0) (1.67 ± 0.32). The submissive response was significantly higher in frequency when males exhibited aggressive behaviours (5.68 ± 1.10) compared to females (2.11 ± 0.63; Wilcox test, W=204, P<0.05). If individual A exhibited submissive behaviours at t_0, the frequency of the opponent responding with submissive behaviours was significantly higher (3.98 ± 0.33) than responding with aggressive behaviours (1.67 ± 0.32).

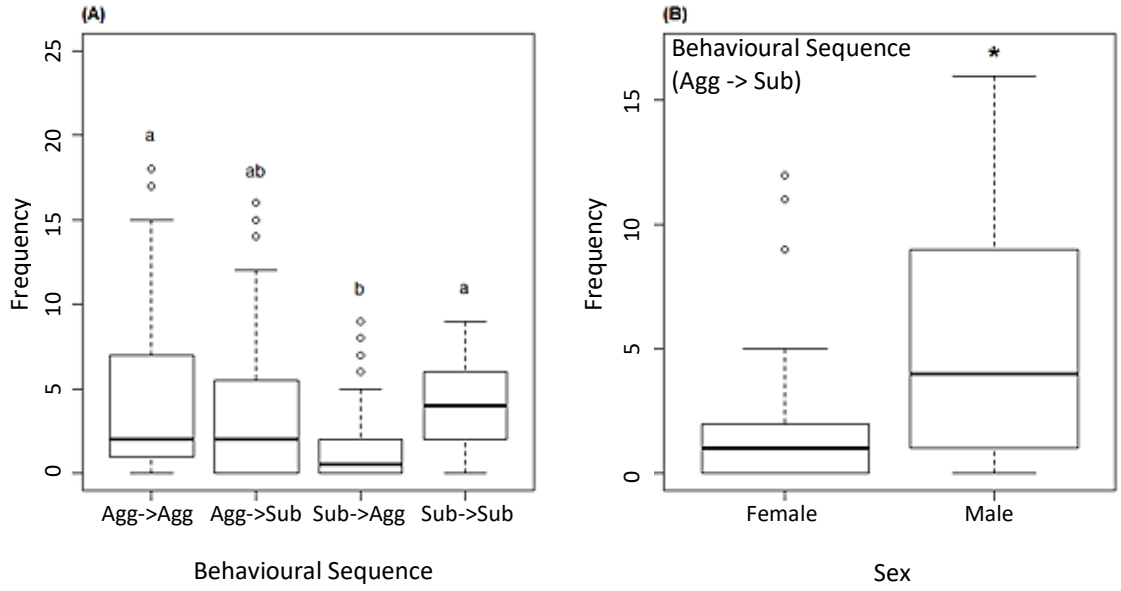

Figure 3.2. Summary statistics per 30 min interaction period. (A) Frequency of inter-individual behavioural sequences (the behaviour of individual A at t_0 followed by the behaviour of individual B at t_1) n=52. (B) Frequency of t_1 submissive behaviour from t_0 inter-individual behaviour sequence of aggression compared between sexes; Female n=27; Male n=25. Agg = Aggressive behaviours; Sub = Submissive behaviours. Different letters indicate where significance lies between behavioural sequences. Asterisk indicates the higher significant difference between sexes.

Figure 3.4 shows inter-individual behaviour between opposite sexes. When males would "approach" (t0), the female "avoided" significantly more in frequency than males when females "approached" (Fishers Exact Test [FET], P< 0.01). However, when a female did "approach" the males, the males responded significantly more by "touching" (FET, P< 0.001) or "tail flipping" (FET, P< 0.05). Once "touching" the males responded significantly more by

“avoiding” females (FET, P< 0.001), in contrast to females which continued to “touch” males (FET, P< 0.001).

Interestingly females approached males significantly more when males were “avoiding” (FET, P< 0.05). When males were displaying “quiet” behaviour, females “approached” (FET, P< 0.05) or displayed “quiet” (FET, P< 0.05) behaviours significantly more times than males in response to females.

Figure 3.5 illustrates the inter-individual interactions between different sized opponents. Smaller individuals responded to larger opponents with significantly more submissive behaviours such as “Quiet” in response to “Exploring” (FET, P< 0.001) and “Tail Flipping” (FET, P< 0.05) in addition with “Tail Flipping” in response to “Approaching” (FET, P< 0.001).

However, large individuals responded significantly more to the smaller opponents “exploring”, by also “exploring” (FET, P< 0.05). Larger opponents also displayed significantly more aggressive behaviours in response to the behaviours of smaller opponents. For example “Approaching” in response to being “approached” (FET, P< 0.05), “touching” in response to being “touched” (FET, P< 0.001); “Approaching” smaller opponents in response to their “avoidance” behaviours (FET, P< 0.05) and “touching” in response to the smaller opponents “quite” behaviours (FET, P< 0.05).

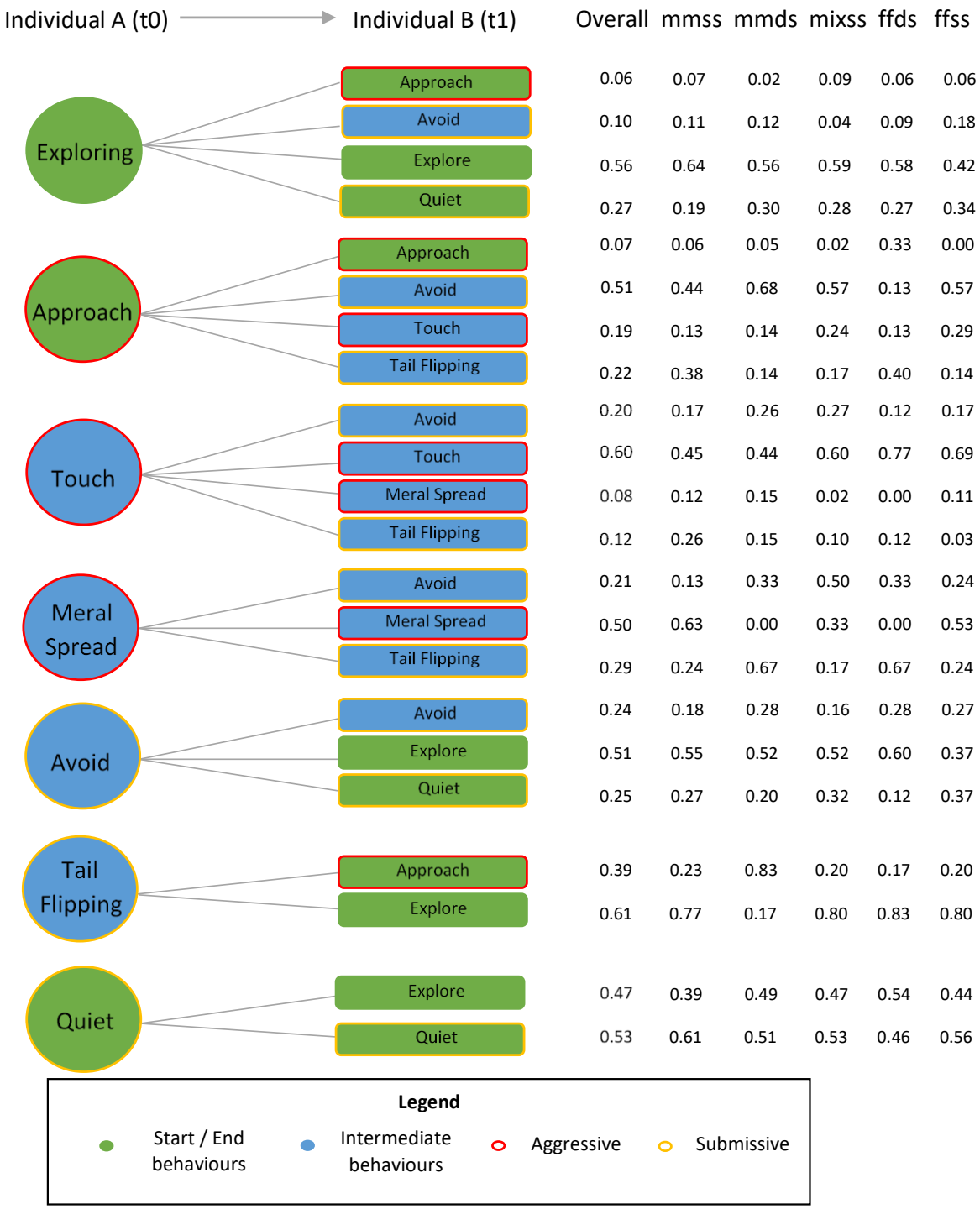

Figure 3.3. The responses of individuals (t1) to the behaviour of an opponent (t0) within treatments (referred to as inter-individual sequences; n=52). Numbers indicate sequence probabilities, for example that when animal A is exploring, animal B reacts by approaching them 6%, avoiding them 10%, exploring 56% or remaining quiet 27% of the time. Responses are also broken down into the interaction categories (mmss = male-male, same sizes; mmds = male-male different size; mixss = male-female; ffds = female-female, different size; ffss = female-female, same sizes).

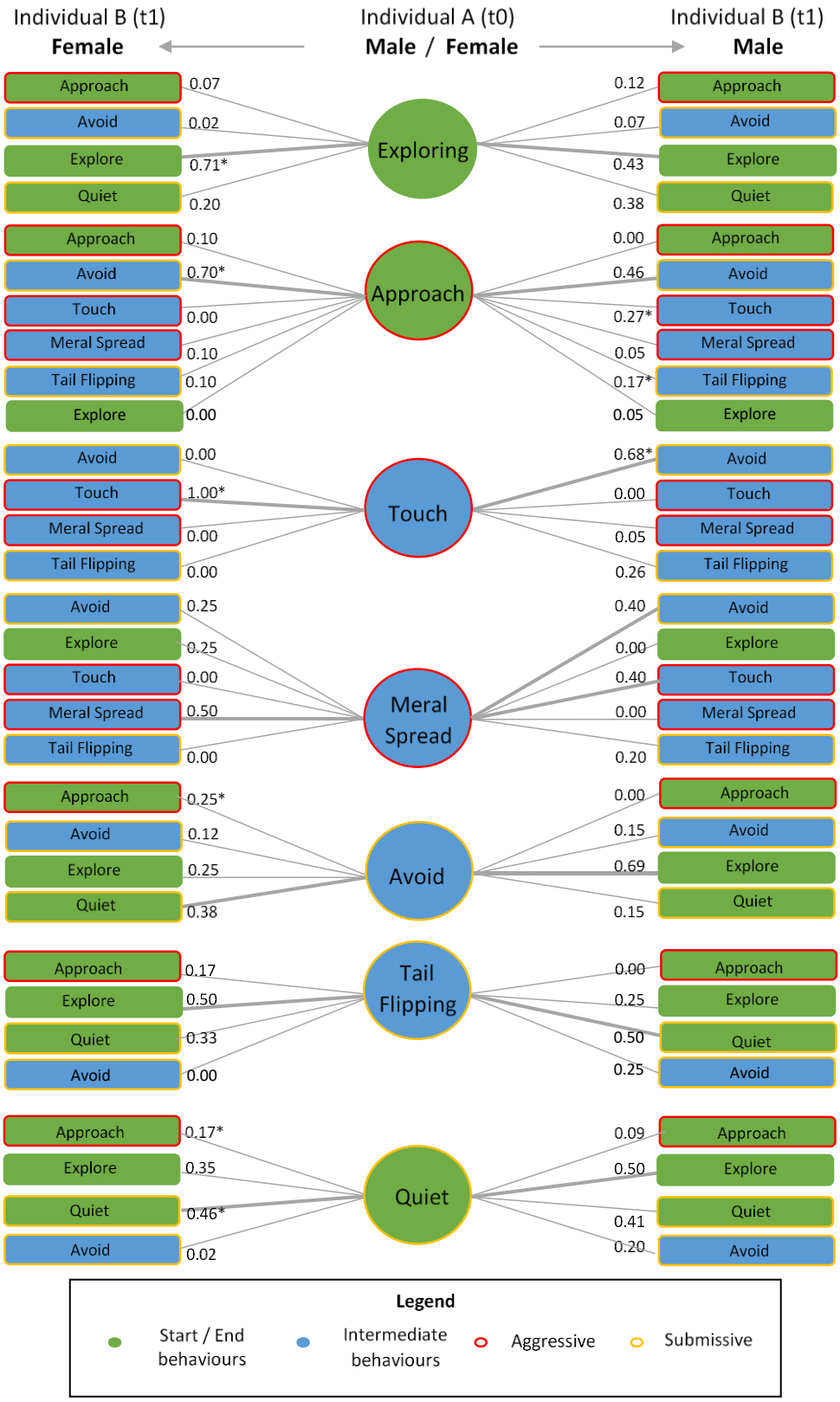

Figure 3.4. Inter-individual sequences between opponents of different sex (n=5). Numbers indicate the sequence probabilities of individual B following the behaviours displayed by individual A. Asterisks denote a significantly higher frequency of that specific behavioural response when compared to the opposite sex (Fishers-Exact test p<0.05).

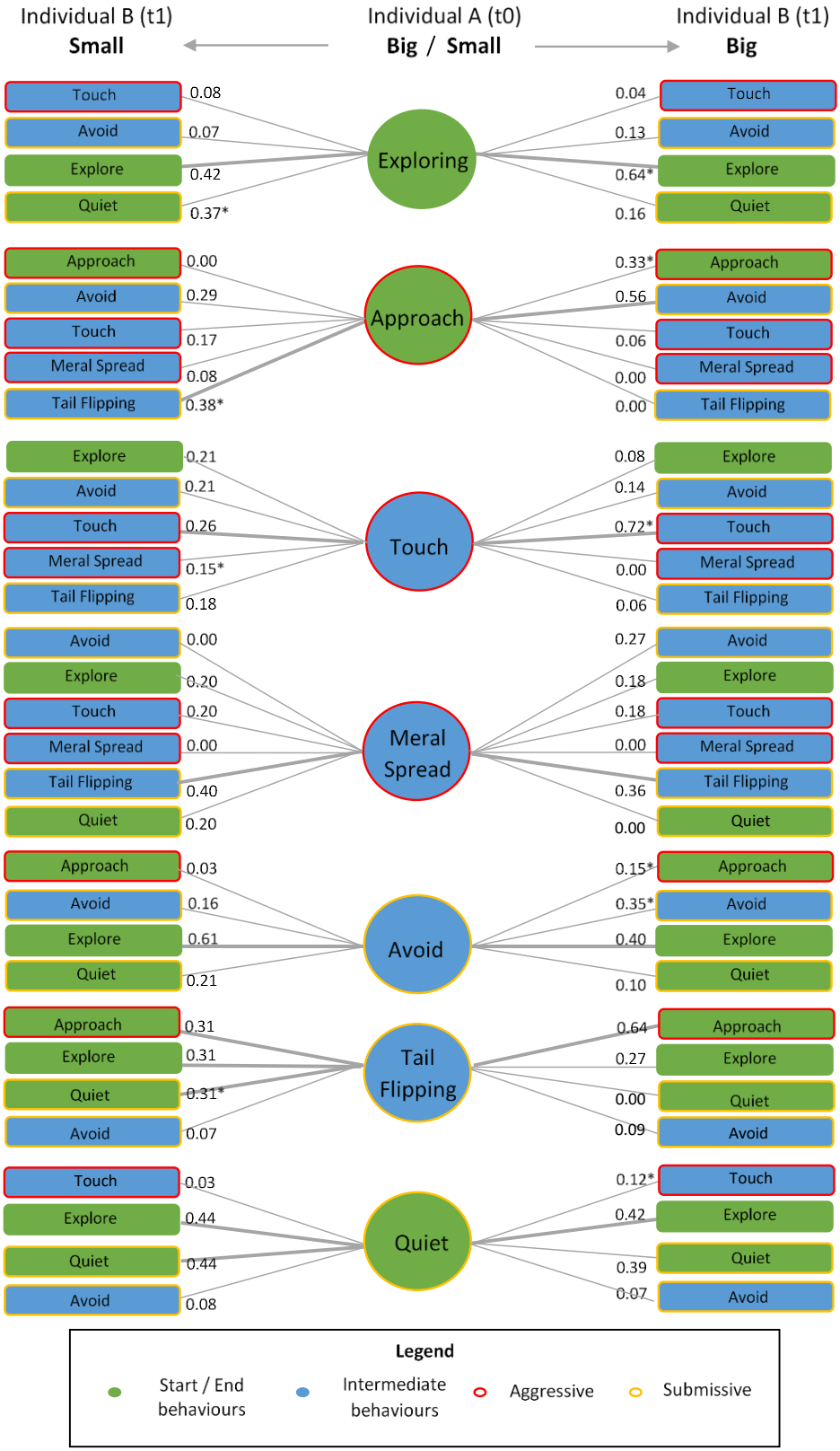

Figure 3.5. Inter-individual sequences between opponents of different size (n=10). Numbers indicate the sequence probabilities of individual B following the behaviours displayed by individual A. Asterisks denote a significantly higher frequency of that specific behavioural response when compared to the opposite size (Fishers-Exact test $p<0.05$).

3.3.2. Behavioural Frequencies & Durations

There was a significant difference between treatments in the frequency of “meral spread” and “quiet” behaviours (Figure 3.6; Kruskal Wallis, χ^2= 14.82 & 12.34; df=4, P<0.05). Within the MMSS treatment individuals displayed on average a significantly higher frequency of “meral spread” behaviour (7.0 ± 2.29) when compared to FFDS (0.5 ± 0.22), and significantly fewer “quiet” behaviours (7.4 ± 1.33) when compared to MIXSS (16.3 ± 2.10).

There were specific differences between different sexed and sized opponents (Figure 3.6). Males approached their opponent significantly more often (5.88 ± 1.07) than females did (2.78 ± 0.68; Wilcox test, W=204, P<0.05). “Meral spread” behaviours were significantly more frequent when the opponent was of a similar size (3.88 ± 0.88) when compared to different sizes (1.15 ± 0.50; Wilcox test, W=169, P<0.05). Additionally, opponents of the same sex but different sizes also displayed a significantly higher frequency of “quiet” behaviours (13.50 ± 1.15) compared to opponents of a similar size (9.14 ± 1.05; Wilcox test, W=317, P<0.05). The treatments with different sexed opponents displayed a significantly higher frequency of “quiet” behaviours (16.30 ± 2.10) than same sex opponents (11.0 ± 0.82; Wilcox test, W=310, P<0.05). Specifically, it was the females that displayed “quite” and “avoidance” behaviours significantly more than the males in the mixed sexed treatment (Figure 3.7; Fishers Exact Test, P<0.01).

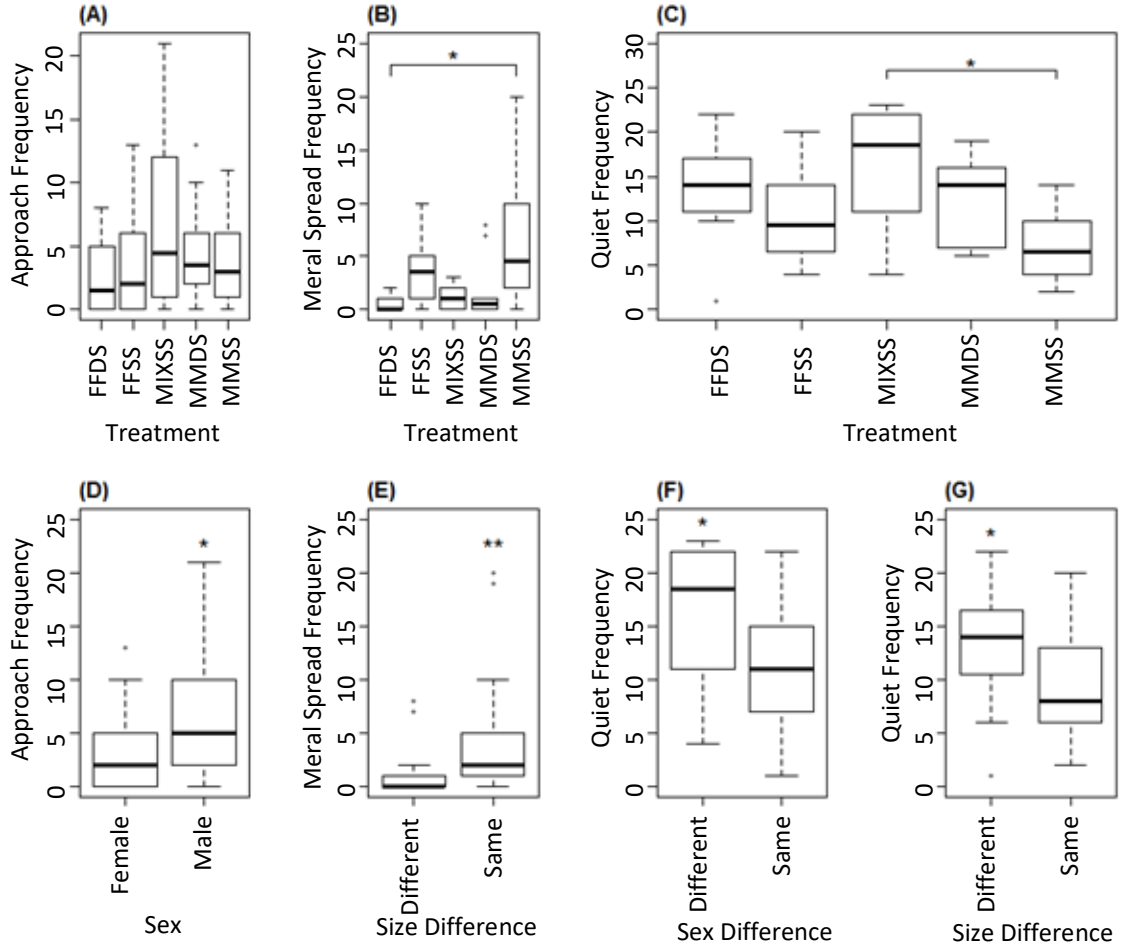

Figure 3.6. Median frequency of displayed behaviours during the 30 minutes interaction period between treatments (A-C; n=10), sexes (D&F; n=26) and sizes (E&G; n=26 & 22). A-C show the median from both individuals grouped together within the same treatment. Lines indicate where a significant difference lies between treatments (A-C; Kruskal Wallis, <0.05). Asterisks above the data indicate a significantly higher frequency (D-G; Wilcox test, * = <0.05; ** = <0.01). FFDS = female-female, different sizes; FFSS = female-female, same sizes; MIXSS = male-female, same sizes; MMDS = male-male, different sizes, MMSS = male-male, same sizes.

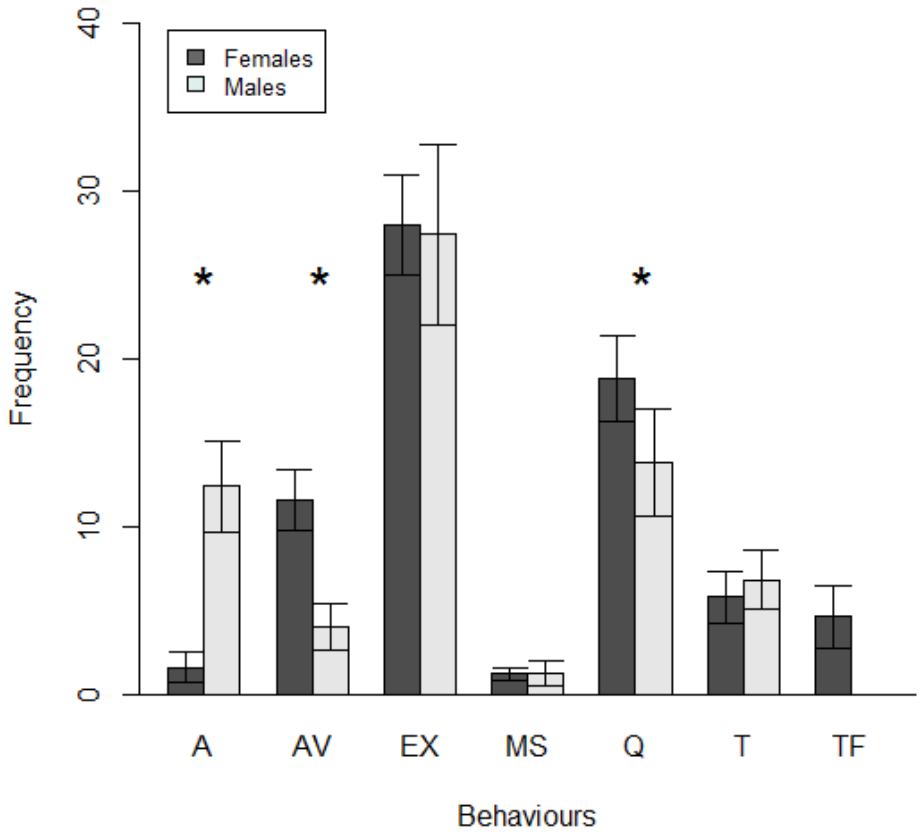

Figure 3.7. Average frequency of behaviours per 30-minute interaction period in the mixed sex treatment (MIXSS) between males and females. A= Approach; AV = Avoid; EX = Explore; MS = Meral Spread; Q = Quite; T = Touch; TF = Tail Flipping. Asterisks denotes a significant difference in total frequency between sexes (Fishers Exact Test, P<0.05).

The majority of the time was spent exhibiting submissive behaviours (48.59% ± 3.05) which was significantly higher than exploratory (38.24% ± 2.70) and aggressive behaviours (13.17% ± 1.69; ANOVA, P<0.001), with the latter being exhibited significantly less than the other two behavioural categories (ANOVA, P<0.001; Figure 3.8). The only significant differences in the duration of behavioural categories (Appendix 3A; Figure 3.14) were for aggressive behaviours (Kruskal Wallis, χ^2= 11.5, P<0.05). Aggressive behaviours were exhibited for a longer duration in the MMSS treatment (385.6 s ± 84.92) compared to the FFDS (78.4 s ± 26.58), and when opponents were of similar size (298.1 s ± 42.91) compared to opponents of different sizes (141.2 s ± 29.27).

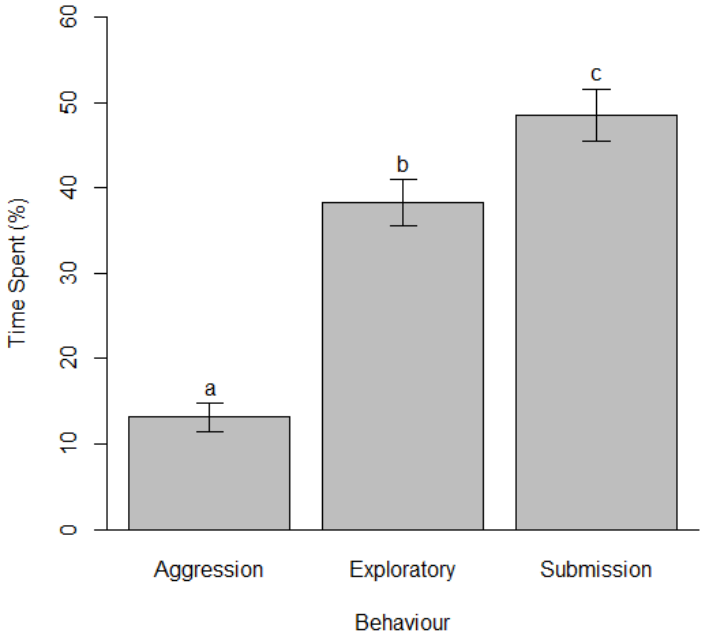

Figure 3.8. Time spent (%) during the 30-minute interaction period of exhibited behaviours from all treatments (mean ± SEM) (n=52). Different letters indicate where significant differences lie between behavioural categories (ANOVA, P<0.05). N.B. Data were arcsine transformed for statistical analyses, but the original data are shown.

3.3.3. Behavioural and Haemolymph Change Correlations

There were some significant correlations between behavioural scores (aggression, submission and exploration) and the haemolymph physiological changes (Figure 3.9). Aggression scores were significantly correlated to **THC** (Pearson's correlation, r_p=-0.47, df=50, P<0.001) and **Lactate** (Spearman Correlation, r_s= -0.31, df=52, P<0.05) haemolymph level changes (Δt).

This was especially prevalent in males (r_p=-0.65; -0.40, df=23, P<0.05) but not significantly correlated within females for Δt **THC** and **RI levels** respectively (r_p, df=25, P>0.05). Aggression scores within the MMDS correlated to Δt **THC** levels (r_p=-0.75 & 64, df=8 & 18, P<0.05) but not in any other treatment (P>0.05). For male-male encounters, high aggression scores also correlated negatively with protein levels (r_p=--0.58; -0.70, df=18; 8, P<0.05).

With regards to size, both small and large individuals had significant correlations of Δt **THC** levels with aggressive scores (r_p=0.46 & 0.48, df=24, P<0.05).

For **lactate**, females and smaller sized individuals showed a significant correlation with aggression (r_s= -0.40 & -0.46, df=27 & 26, P<0.05), but males and larger sized individuals did not (r_s=-0.28 & 0.18, df=25, P>0.05). Additionally, Δt **ammonia** level significantly correlated with aggressive behaviours within the FFDS treatment (r_s=0.68, df=10, P<0.05).

Submission scores were significantly correlated to Δt **THC** (r_p=-0.29, df=50, P<0.05), **RI** (r_p=-0.28, df=50, P<0.05), **lactate** (r_s=0.27 df=52, P<0.05) and **ammonia** (r_s=0.31, df=52, P<0.05) levels. The total duration of submissive behaviours was found to be significantly correlated to **ammonia** levels at t1 (r_s=0.31, df=52, P<0.05), especially for small sized individuals (r_s= 0.45, df=26, P<0.05).

Submissive behaviours displayed by smaller sized opponents were correlated to Δt **RI** (r_p=-0.40, df=24, P<0.05), **lactate** (r_s=0.51, df=25, P>0.05) and **ammonia** (r_s=0.42 & 0.45, df=26, P<0.05) levels. However, the opposite was found for **THC** (r_p=-0.56, df=24, P<0.01) and **protein** (r_p=--0.41, df=24, P<0.05) for which it was significantly correlated with the larger opponents.

Exploratory score behaviours negatively correlated to Δt **glucose** levels r_p=-0.28, df=49, P<0.05) and t1 **protein** levels (r_p=--0.29, df=50, P<0.05).

In addition, CL was significantly correlated to Δt **protein** (r_p=0.30, df=50, P<0.05), **THC** (r_p=0.31, df=58, P<0.05), **glucose** levels (r_p=-0.30, df=49, P<0.05) and **RI** levels at t1 (r_p=0.28, df=50, P<0.05). (See Appendix 3B-3G).

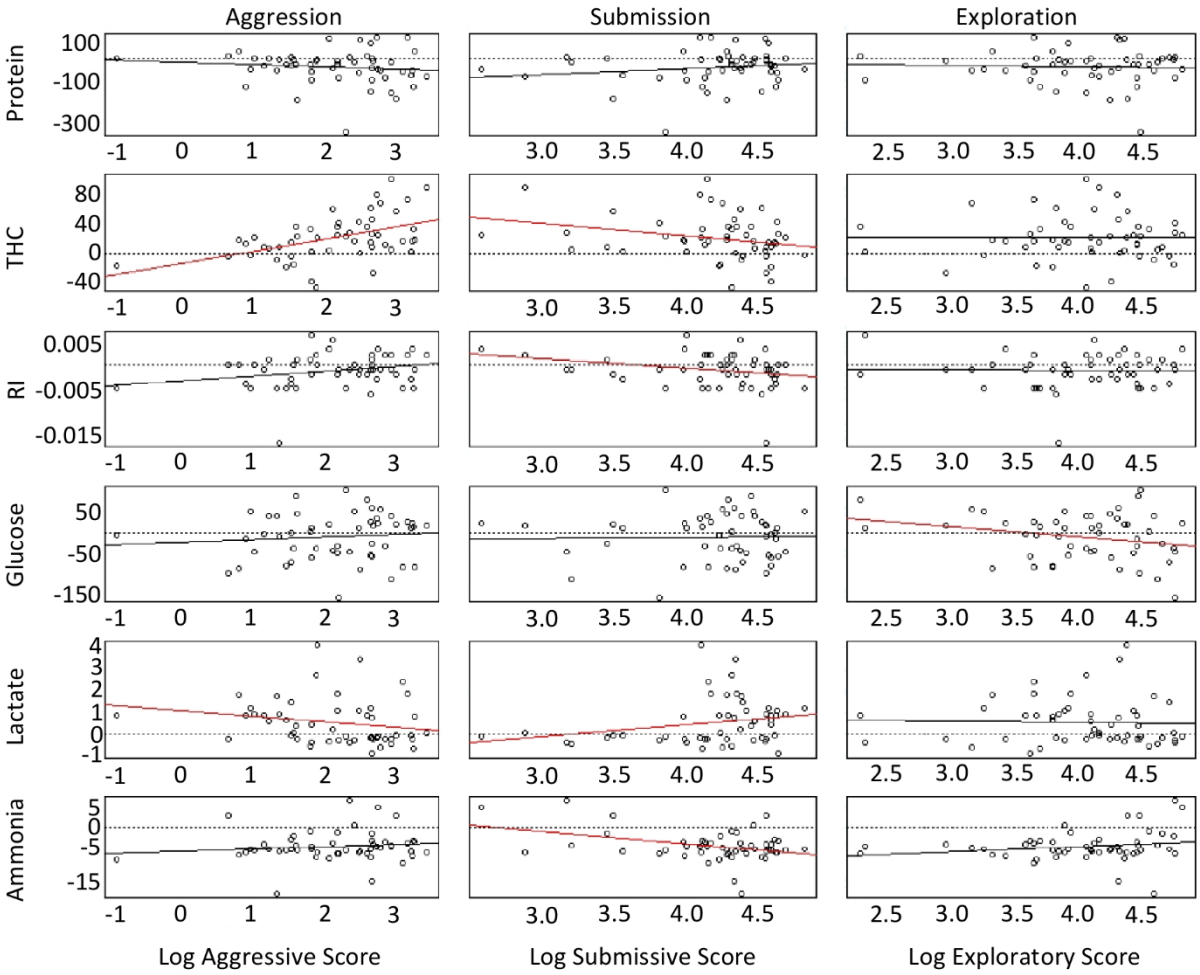

Figure 3.9. Behavioural correlations with haemolymph level change (Δt). N.B. Dotted line indicates zero. Coloured lines indicate a significant correlation ($r_{s,p}$, P<0.05) N=52.

3.3.4. Fight Outcomes, Related Haemolymph Levels and Behaviours

The only significant difference found in haemolymph parameters between winners and losers was indicated in THC levels (Figure 3.10). There were no significant differences in THC levels at t0 between winners and losers (Wilcox Test, W=317.5 P>0.05). However, t1 THC levels and the Δt THC levels, (shown in Appendix 3C, Figure 3.18), were significantly different between winners and losers (Wilcox Test, W=203 & 169.5, P< 0.01 & 0.05 respectively).

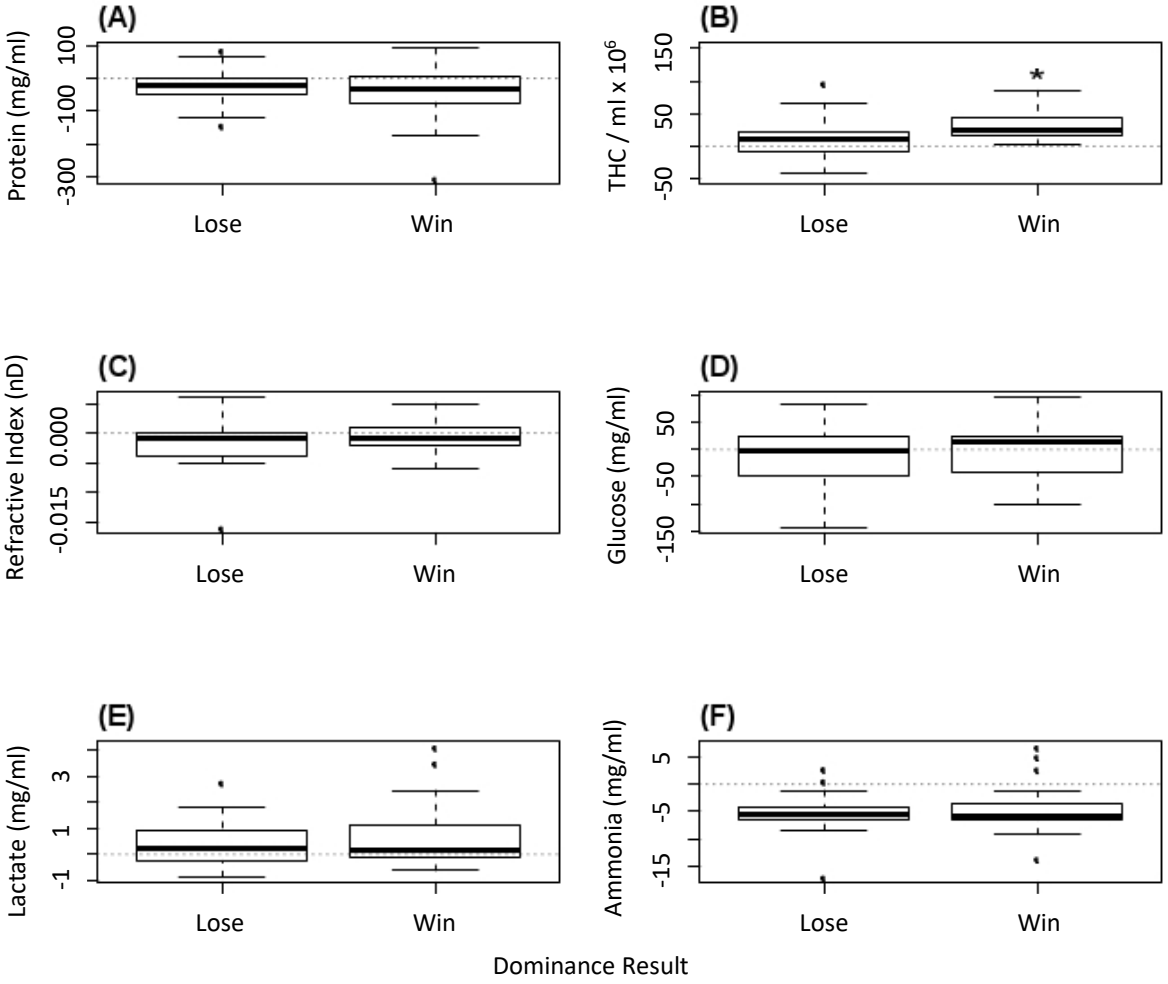

Figure 3.10. Δt haemolymph level between individuals that won and lost during the interaction period. (A) Protein; (B) THC; (C) RI; (D) Glucose; (E) Lactate; (F) Ammonia. The dashed line indicates zero. Asterisk denotes a significantly higher Δt haemolymph parameter.

The general linear models are shown in tables 3.2, 3.3 and 3.4. The top ranked general linear models based upon the AICc values to predict the interaction outcome (i.e. the dominant individual) included selected haemolymph parameters (THC t1 and t2) and behaviours (approaching and tail flipping). Models that included the CL and sex data performed relatively well compared the first ranked model (ΔAIC_c <2) indicating that these parameters may be important in the interaction outcome. The result of using all the haemolymph parameters in the model resulted in an ΔAIC_c of above 2. This may indicate that not all haemolymph parameters are useful in predicting the interaction outcome.

The best ranked models for predicting the amount of aggression and submission displayed during an interaction were based upon haemolymph levels only. There was a significant relationship determined by regression analysis between the amount of aggression displayed and THC t1 and t2, protein t1 and t2, ammonia t1 and glucose t2 haemolymph parameters (Z test; $P<0.05$). Similar results are shown in Table 3.4 for predicting the amount of submission displayed. The following haemolymph parameters were considered to have a significance regression in the top ranked model (Z-test; $P<0.05$); THC t2, protein t1, SG t1 and ammonia t2.

Both the sex and size of the individual may have had some influence upon the amount of aggression displayed, although the model AIC score was above >2 from the first ranked model.

Table 3.2. Ranked General Linear models based upon the AICc values to predict the interaction outcome (i.e. the dominant individual).

Model	AIC_c	Null Deviance (d.f)	Residual Deviance (d.f)	Number of Fisher Scoring Iterations	Significant parameters within the model (P<0.05) and the effect it has on the dependant (+ / -)
Selected haemolymph + Selected behaviours #1	56.27	72.09 (51)	46.27 (47)	5	THC t2 +
Selected haemolymph + Selected behaviours #1 + sex	56.50	72.09 (51)	44.50 (46)	5	THC t2 +
Selected haemolymph + Selected behaviours #1 + CL	58.24	72.09 (51)	46.24 (46)	6	THC t2 +
Selected haemolymph + Selected behaviours #1 + CL + Sex	58.49	72.09 (51)	44.49 (45)	6	THC t2 +
Selected Behaviours #1	59.94	72.09 (51)	53.94 (49)	5	Approach +
Selected Behaviours #2	60.36	72.09 (51)	52.36 (48)	5	Approach +
All Behaviours	65.89	72.09 (51)	49.89 (44)	5	None
Selected haemolymph	67.08	72.09 (51)	61.08 (49)	4	THC t2 +
Full	68.80	70.68 (50)	28.80 (31)	9	None
All Haemolymph + Behaviours	69.18	70.68 (50)	33.18 (33)	7	THC t1 - THC t2 + Ammonia t2 - Tail Flipping –
CL	74.90	72.09 (51)	70.90 (50)	4	None
Sex	75.39	72.09 (51)	71.39 (50)	3	None
Cl + Sex	76.60	72.09 (51)	70.60 (49)	4	None
All haemolymph	77.09	70.68 (50)	55.09 (40)	5	THC t2 +
Size + all haemolymph	78.99	70.68 (50)	54.99 (39)	5	THC t2 +
Sex + all haemolymph	79.03	70.68 (50)	55.03 (39)	5	THC t2 +
All haemolymph + CL + Sex	80.97	70.68 (50)	54.97 (38)	5	THC t2 +

Full = All haemolymph parameters + All behaviour counts + sex + CL; Selected haemolymph = THC t1 + THC t2; Selected behaviours #1= Approach + Tail Flipping; Selected behaviours #2 = Approach + Avoid + Tail Flipping

Table 3.3. Ranked General Linear models based upon the AIC_c values to predict the amount of aggression displayed by an individual

Model	AICc	Null Deviance (d.f)	Residual Deviance (d.f)	Number of Fisher Scoring Iterations	Significant parameters within the model (P<0.05) and the effect it has on the dependant (+ / -)
All Haemolymph (except RI t1 and t2)	458.21	349.87 (50)	229.47 (42)	5	Intercept + THC t1 – Protein t1 + Ammonia t1 + THC t2 + Protein t2 – Glucose t2 –
All Haemolymph	460.62	349.87 (50)	227.89 (40)	5	Intercept + THC t1 – Ammonia t1 + THC t2 + Protein t2 – Glucose t2 –
Full	461.43	349.87 (50)	224.69 (38)	5	Intercept + THC t1 – Ammonia t1 + THC t2 + Protein t2 – Glucose t2 –
Selected Haemolymph #1	521.87	349.87 (50)	305.13 (48)	5	Intercept + THC t2 + Glucose t1 –
Sex	549.50	350.11 (51)	330.43 (50)	5	Intercept + Sex
Sex + CL	550.26	350.11 (51)	329.19 (49)	5	Intercept + Sex
CL	563.63	350.11 (51)	344.56 (50)	5	Intercept + CL +

Full = All haemolymph parameters + sex + CL; Selected Haemolymph #1 = THC t2 + Glucose t1.

Table 3.4. Ranked General Linear models based upon the AIC_c values to predict the amount of submission displayed by an individual

Model	AICc	Null Deviance (d.f)	Residual Deviance (d.f)	Number of Fisher Scoring Iterations	Significant parameters within the model (P<0.05) and the effect it has on the dependant (+ / -)
All Haemolymph	472.12	249.58 (50)	201.13 (40)	4	Intercept + THC t2 – RI t1 + Protein t1 – Ammonia t2 –
Full	475.99	249.58 (50)	201.00 (38)	4	Sex (female) THC t2 – RI t1 + Protein t1 – Ammonia t2 –
Selected Haemolymph #1	483.48	249.58 (50)	226.49 (47)	4	Intercept + THC t2 – Ammonia t2 –
CL + Sex	508.60	252.24 (51)	248.99 (49)	4	Sex (female)
CL	506.70	252.24 (51)	249.09 (50)	4	Intercept +
Sex	509.19	252.24 (51)	251.58 (50)	4	Intercept (female) +

Full = All haemolymph parameters + sex + CL; Selected Haemolymph #1 = THC t2 + glucose t1 + ammonia t2.

Overall males were winners in 60% of the interactions (Table 3.5). Within the mixed sex treatment (MIXSS), males were determined as winners in all replicates (100%; Fishers Exact test, P<0.01). The fight outcome between different sized opponents had contrasting results depending upon the sexes. Within the male treatments (MMDS), smaller individuals won in 60% of the encounters, whereas this was the opposite for females (FFDS; 40%).

Table 3.5. Fight outcomes. The winning % between different sexes and sized opponents. Asterisks denotes where a significant difference lies between the frequency of individuals who won between the two variables (Fisher Exact Test, P<0.05).

Treatment	Sex	Size	% Win
Overall (all)	Male		60
	Female		40
		Big	54
		Small	46
Mixed Sex (MIXSS)	Male		100*
	Female		0
Same Sized (MMSS, FFSS, MIXSS)	Male		56
	Female		44
Different Sized (MMDS, FFDS)		Big	50
		Small	50
Male Different Sized (MMDS)		Big	40
		Small	60
Female Different Sized (FFDS)		Big	60
		Small	40

Several models (as described in 3.2.3), were used to predict the fight outcome of the interaction (win or lose) which was noted by the observer. Table 3.6 indicates that model 1 predicted the outcome of the interaction with 100% accuracy. The equations are solely based upon the behaviours observed and not on the size or sex of the individuals. Equation 1 is based upon using the frequency and duration of aggressive behaviours, excluding submissive and exploratory behaviours.

Table 3.6. Model error and % of correct predictions of the dominant individual based upon behavioural data.

Model	% Error	% Correct
Model 1	0	100
Model 2	15.38	84.62
Model 3	15.38	84.62
Model 4	7.69	92.31
Model 5	23.08	76.92
Model 6	15.38	84.62
Model 7	46.15	53.85
Model 8	7.69	92.31

3.3.5. Intrinsic Factors and Physiological Change

There were also some significant physiological changes in the haemolymph levels resulting from behavioural interactions (Figure 3.11). Table 3.7 illustrates a summary of the major

results from each haemolymph parameter and differences between intrinsic features such as sex and size. Haemolymph parameter results are discussed in further detail.

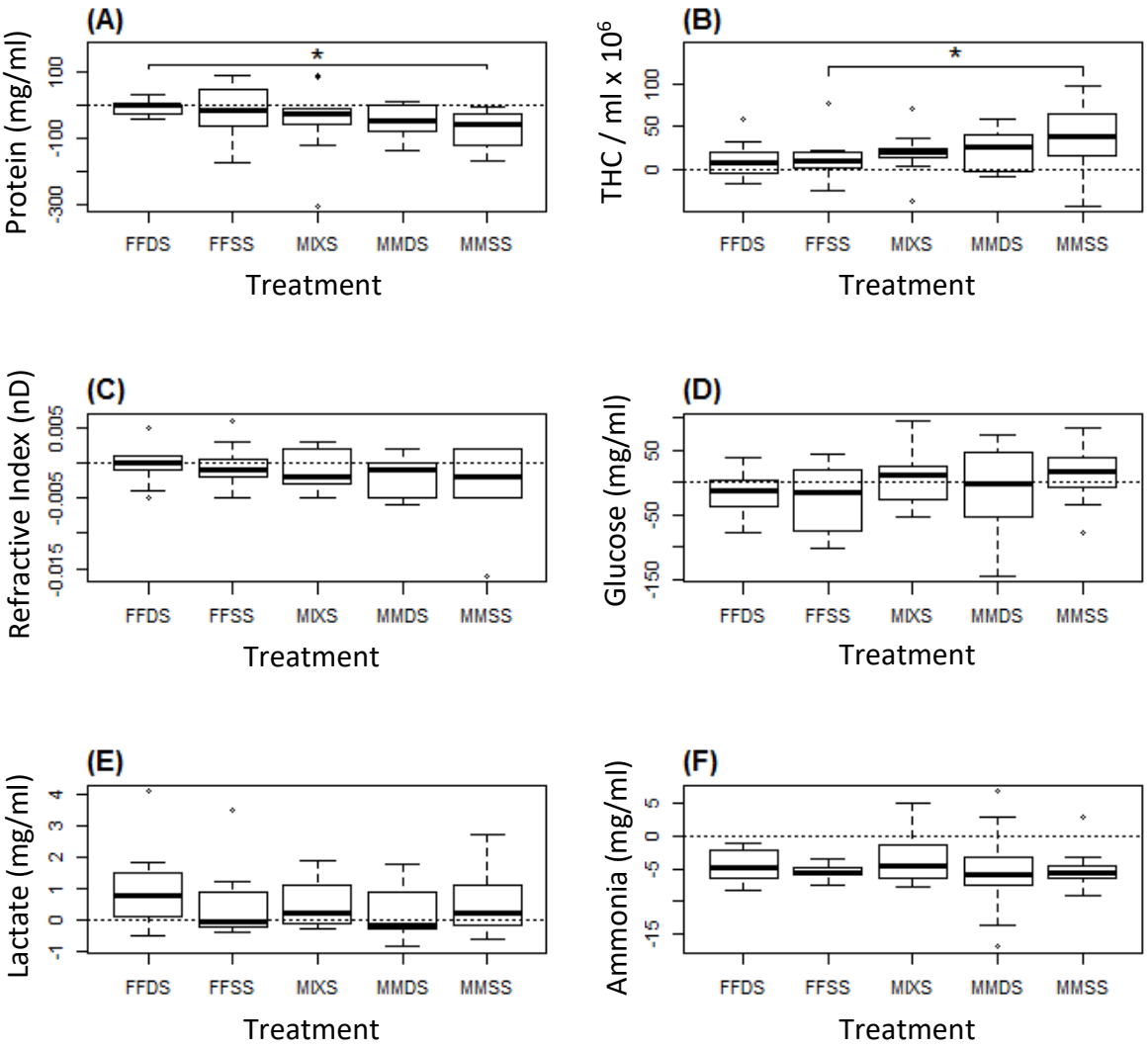

Figure 3.11. Δt haemolymph levels between treatments. Median values are from grouped individuals from the same treatment. (A) Protein; (B) THC; (C) RI; (D) Glucose; (E) Lactate; (F) Ammonia. The dashed line indicates zero.

Table 3.7. Summary of the physiological changes. Letter / symbols in the brackets indicate the greatest level of change*.

There was a significant difference between;						
	Protein	THC	RI	Glucose	Lactate	Ammonia
Control & treatments	N	Y	N	N	Y	Y
Overall t_0 & t_1 levels	N	Y	N	N	Y	Y
Level change (Δt) between sexes (♂ & ♀)	Y (♂)	Y (♂)	N	N	Y (♂)	N
Level change (Δt) between sizes (Same & Different)	Y (D)	Y (S)	N	Y (S & D)	Y (S)	N
Level change (Δt) between different sized opponents (Big vs Small)	N	Y (Big)	N	N	N	N
Level change (Δt) between sex of opposition (♂ v ♂ / ♀ vs ♀ / Mix)	Y (♂ v ♂)	Y (♂ v ♂)	N	N	N	N
Level change (Δt) between treatments	Y (MMSS-FFDS)	N	N	N	N	N
Increase / Decrease from baseline level following behavioural interaction	Decrease	Increased	Decrease	Variable	Increased	Decrease
Appendix Reference	3B	3C	3D	3E	3F	3G

*** Y= Yes; N= No; ♂= Male; ♀= Female; D = Different sized opponent; S= Same sized opponent; Big = Larger sized opponent; MMSS = Male with male opponent of same size; FFDS = Female with female opponent of different size.**

3.3.5.1. Protein

There was no significant difference in **protein** levels from t_0 to t_1, and no significant difference to the controls (t-test, $P>0.05$). Average haemolymph protein levels did decrease from a mean of 514.06 ± 148.43 mg/ml at t_0 to 482.79 ± 127.38 mg/ml at t_1 (Appendix 3B). There was a significant difference in protein levels between sexes. Females had significantly lower protein levels at t_0 (461.84 ± 121.42 mg/ml) and t_1 (451.03 ± 104.57 mg/ml) than males (569.91 ±

156.13 & 516.73 ± 142.00). Males however had a significantly greater decrease in protein levels from t_0 to t_1 (53.17 ± 72.45 mg/ml) than females (10.79 ± 57.37 mg/ml). The decrease in protein levels was most apparent between males and females when the opponents were of a different size (48.53 ± 48.59 & 5.84 ± 19.73 mg/ml respectively; t-test = 20.57, df=11.89, P<0.05), but not significantly different when opponents were of a similar size (66.83 ± 91.04 & 17.04 ± 73.63 mg/ml; t-test =1.67, df=26.97, P>0.05). There was a significant difference in the protein level change (Δt) between treatments (Kruskal-Wallis, χ^2=9.66, df=4, P<0.05), with both individuals within the MMSS having significantly reduced protein levels (-80.85 ± 57.32 mg/ml) compared to the individuals within the FFDS treatment (-5.84 ± 19.73 mg/ml). Males who had male opponents had a significantly greater reduction in protein levels (MM; -59.84 ± 52.07 mg/ml) than females with female opponents (FF; -9.84 ± 56.80 mg/ml; Kruskal-Wallis, χ^2=7.86, df=2, p<0.05).

3.3.5.2. THC

Overall, **THC** significantly increased from t_0 (78.84 ± 26.16 ml x 10^6) to t_1 (97.58 ± 37.40 ml x 10^6) (t-test, t=3.18, df=105.58, P<0.01; Appendix 3C). There was no significant difference between the control and trial THC for t_0 and t_1 (t-test, P>0.05), although there was a significant difference for THC change (t_0-t_1) with the control increasing by 4.00 ± 10.63 ml x 10^6 and the treatment groups 21.01 ± 28.75 ml x 10^6 (t-test, t=3.10, df=26.92, P<0.01). Whilst there was no significant difference in t_0 THC between males and females (t-test, P>0.05), the t_1 THC and Δt THC was significantly different between sexes (t-test, t=2.96 & 2.89 ml x 10^6, df=41.90 & 50.70, P<0.01). Males had significantly higher THC (111.71 ± 44.27 ml x 10^6) than females (84.37 ± 23.42 ml x 10^6) at t_1, and a significantly larger Δt THC (28.88 ± 29.97 ml x 10^6) than females (9.26 ± 21.62 ml x 10^6). There was no significant difference in Δt THC between treatments (Kruskal-Wallis, χ^2= 7.51, df=4, P>0.05), however male vs male treatments had a significantly higher Δt THC (31.50 ± 32.91 ml x 10^6) than female vs female treatments (12.14 ± 23.31 ml x 10^6; Kruskal-Wallis, χ^2= 6.38, df=2, P<0.05). Additionally, males had a significantly higher Δt THC when the opponent was of similar size (MMSS; 38.40 ± 34.09 ml x 10^6) when compared to females (FFSS; 9.5 ± 23.96 ml x 10^6; t-test, t=2.74, df=24.75, P<0.05). However, this was not significantly different between the sexes when the opponents were differently sized (t-test, P>0.05).

3.3.5.3. Refractometer Analysis

Haemolymph **RI** levels (nD) did not significantly change from t0 different to t1 (t-test, t=1.13, df=118, P>0.05; Appendix 3D), or from the control levels (t-test, P>0.05). Males did have significantly higher RI (1.035 ± 0.004 nD) than females (1.032 ± 0.004 nD) at t_0 (t-test, t= 2.46, df= 46, P<0.05). However, this was not significantly different for t_1 (t-test, P>0.05) or Δt RI (Wilcox test, P>0.05).

3.3.5.4. Glucose

There was no significant difference between t_0 (56.01 ± 41.41 mg/ml) and t_1 (53.01 ± 32.22 mg/ml) **glucose** levels (Wilcox test, W=1825.5, P>0.05; Appendix 3E). There was a significant difference in t_0 glucose levels between treatment (61.08 ± 42.45 mg/ml) and controls (30.15 ± 23.02 mg/ml) (Wilcox test, W=122, P<0.01), but not for t_1 and Δt (Wilcox test, P>0.05). Males had significantly lower glucose levels at t0 (52.44 ± 43.91 mg/ml) compared to females (69.39 ± 40.08 mg/ml; Wilcox test, W=431.5, P<0.05), but not at t_1 or Δt (Wilcox Test, P>0.05). There were no significant differences between treatments in glucose level change (Δt) (Kruskal-Wallis, χ^2=7.55, df=6, P=0.27).

3.3.5.5. Lactate

Lactate levels significantly increased from t_0 (0.48 ± 0.59 mg/ml) to t_1 (1.23 ± 1.44 mg/ml; Wilcox test, W=1225, P<0.001; Appendix 3F). There was a significant difference between trials and control levels for t_0, t_1 and Δt (Wilcox test, W=153.5, 399, 420, P<0.05). Trial lactate levels had a significantly lower change (Δt; 0.57 ± 1.06 mg/ml) when compared to controls (1.67 ± 1.19 mg/ml). Males had significantly higher lactate levels at t_0 (0.58 ± 0.48 mg/ml) when compared to females (0.35 ± 0.22 mg/ml; Wilcox test, W=228.5, P<0.05), but not at any other times (t_1 and Δt; P<0.05). However, when males had similar sized opponents the lactate levels at t_1 were significantly higher (1.38 ± 1.16 mg/ml) than females which had similar sized opponents (0.77 ± 1.13 mg/ml; Wilcox test, W=72, P<0.05).

3.3.5.6. Ammonia

There was a significant decrease in **Ammonia** levels from t_0 (5.98 ± 3.26 mg/ml) to t_1 (1.96 ± 4.02 mg/ml) (Wilcox test, W=2983.5, P<0.05; Appendix 3G). There were significant differences found between trial (6.99 ± 2.28 mg/ml) and control (0.15 ± 1.19 mg/ml) ammonia levels at t_0 (Wilcox test, W= 0, P<0.001), but not at t_1 (Wilcox test, W= 206, P>0.05). Δt Ammonia

significantly increased in the control trials (0.58 ± 1.70 mg/ml), but individuals within the treatments had reduced ammonia Δt by 4.82 ± 3.88 mg/ml (Wilcox test, W=425, P<0.001).

3.3.5.7. Haemolymph Correlations

There was a significant correlation found in the Δt values between different blood parameters (Figure 3.12). For example, as (Δt) lactate levels increased, (Δt) protein levels decreased (r_s=0.35, df=52, P<0.05) and (Δt) ammonia levels increased (r_s=-0.43, df=52, P<0.01). In addition, as THC level differences increased as did the glucose level differences (r_s=0.36, df=52, P<0.01).

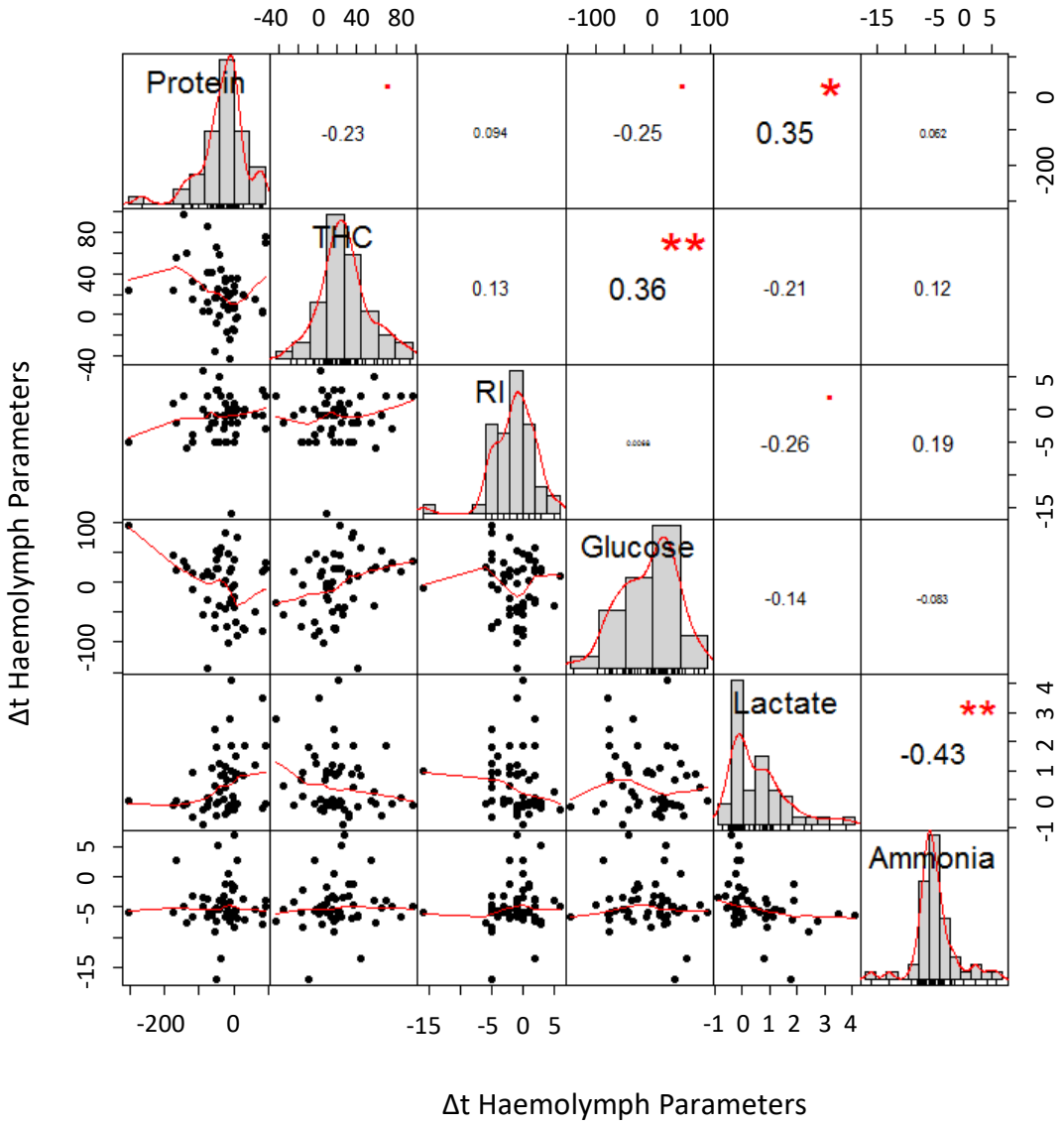

Figure 3.12. Distribution of the Δt values for each haemolymph parameter are shown on the diagonal. The bottom left are bivariate scatter plots with a fitted line. The top right indicates the value of the correlation. Asterisks indicate significance levels (r_s; ** = 0.01; * = 0.05; . = 0.1.

There was a significant correlation of protein concentration (mg/ml) and the refractive index (r_s=0.79, df=102, P<0.001, shown in Appendix 3D. RI).

3.3.6. Moult Stage and Carapace Length

Overall there was no significant difference in moult stages between the treatments (Kruskal Wallis, χ^2= 5.16, df=5, P>0.05), with 51% of individuals at stage C_4, 43% at stage D_0 and the rest at later stages (D_{1-3}). The moult stage had no significant influence on all physiological parameters (Kruskal Wallis, P<0.05), except for lactate level change within the mix sex treatment (MIXSS; Kruskal Wallis, χ^2= 5, df=1, P<0.05). Individuals at stage D_0 (n=2) had increased t_1 lactate concentration, whereas individuals at moult stage C_4 (n=5) had decreased t_1 lactate concentration following behavioural interactions. There was a significantly higher number of observed males at the D_0 moult stage (68%) than females (17%; χ^2=8.05, df=1, P<0.05), but no significant difference was found between any other moult stages (P>0.05).

There was a significant difference in CL between the opponents in the different sized treatment and between males and females except for in the MIXSS treatment (Mann Whitney U-Test, P<0.05; Figure 3.13). There was no significant difference in CL between individuals within the same sized treatments (MMSS, FFSS MIXSS), or the range of sizes used within all treatments (Mann Whitney U-Test, P>0.05).

3.4. Discussion

3.4.1. Sex and Size

There were some significant differences in serum metabolite parameters between sexes. Overall, t_0 female metabolite concentrations were significantly lower in comparison to males across all parameters (except for glucose and ammonia). Males also had significantly greater Δt protein, THC and lactate blood parameters when compared to females. There is limited evidence of measured physiological differences between sexes. Uglow et al. (1986) indicates that differences in survival between sexes could be attributed to the female breeding cycle, with starvation causing lower protein levels. Although there was no significant difference in moult stage between the treatments, the variance between sexes was reflected in the baseline levels in this study and how they adapt to given behavioural situations. Therefore, potential differences in sex should be acknowledged in future physiological, stress related

studies, as well as the implications it could have for shellfish holding operations and live transport.

Carapace length (CL) was significantly correlated with Δt THC and glucose levels, and t_1 protein and RI levels. Similar findings have been seen in other crustaceans (Briffa and Elwood, 2001).

3.4.2. Behaviours

This study has indicated major behavioural differences in conspecific interactions with different sexed and sized individuals. *Nephrops* exhibited behavioural patterns such as to approach and then avoid opponents. A large majority of the time was spent exhibiting submissive behaviours, and submissive behaviours were usually reciprocated by the opponent, rather than aggressive behaviours. The matched filter hypothesis states that receivers benefit from tuning into the honest signals from the sender. Exhibiting subdominant behaviours (de-escalation signals) is a beneficial strategy for both individuals as less energy is used in fighting, and the potential of injury and death is reduced (Parker, 1974; Briffa and Sneddon, 2007). However, an aggressive behaviour has 50% chance of it being reciprocated with either an aggressive or submissive behaviour.

It seems that body size is a key component in assessing RHP during contests, as also found in hermit crabs (Yasuda et al., 2012). The fighting ability, otherwise known as resource holding potential (RHP), is related to experience and the size of weapons which often determines the outcome of fights (Parker, 1974; Katoh, 2011). Similar sized opponents escalate levels of aggression to determine which individual is dominant until the subdominant individual retreats (Parker, 1974). In this study females that had female opponents of different sizes exhibited the least amount of aggression, whereas when opponents were of similar size and/or male, then more aggression was evident. Where opponents differ in body size, it is usually accepted that the larger individuals are dominant. However, this is determined not only by RHP but several other factors including, the advantage gained from the fight (positive reinforcement such as hierarchical gain) and the costs of fighting (consequences). This has been shown in dyadic male - male hermit crab contests, where damaged individuals still decided to escalate fights with intact conspecifics (Lehner, 1996; Katoh et al., 2008, 2013; Yasuda and Koga, 2016). In addition, personality traits could also influence on how an individual behaves in a given situation, and therefore the outcome of an interaction. For

example, measuring the amount of shyness-boldness (*i.e.* risk-taking) behaviours have been used to investigate personality within other decapods (Gherardi et al 2012) such as fiddler crabs. Reaney and Backwell (2007) showed that "bold" males, defined by the duration of time spent in the shelter (burrow) and the duration exploring following the introduction from an aerial predator, was correlated to mating success and aggressiveness. Such consistent individual traits (*i.e.* personality) have been shown in *Carcinus maenas* (Fürtbauer, 2015) with relation to physiological condition, where haemolymph density is correlated with boldness (*i.e.* lower haemolymph density correlated with more exploratory behaviours). Understanding the role of behavioural patterns and inter-individual personality traits linked with physiological processes has important consequences in the design and analysis of future investigations for the development of *Nephrops* fisheries and aquaculture. For example, shy individuals may be less represented in creel capture experiments than bolder (more exploratory) individuals (Gheradi et al., 2012).

Larger opponents in this study displayed more aggressive behaviours in response to smaller opponents that were displaying aggressive behaviours such as touching. Aggressive displays involve meral spread and pushing behaviours where both individuals press the chelae against the opponents to assess the opponent's size and strength (Katoh et al., 2008). However, neutral behaviours tended invoke neutral behaviours (neither aggressive or submissive). Interestingly, opponents of different sizes showed contrasting dominance outcomes. For example, within the male same sex and different size treatment (MMDS), smaller individuals were more dominant than the larger opponent, which was the opposite within the female treatment (FFDS). Adey (2007) showed similar results, where smaller individuals would win agonistic encounters over larger sized conspecifics within the creel. This was attributed to the winning individual being already established within the creel prior to the contestant's arrival (also termed the residence effect).

As with the crayfish *Cherax destructor* (Walter et al., 2011) it seems that female *Nephrops* primarily rely on signalling, without the need for escalation, whereas males tended to escalate fights. As the individuals in this study did not have any known prior knowledge of each other, the individuals send signals to reflect their dominance, sex and fighting ability, i.e. body size, which increases in aggression until one decides to break off (Bradbury and Vehrencamp, 2011). Within the present study it is suggested that males used more sequential steps than

females, as the former had a higher frequency of approaching the opponents (Arnott and Elwood, 2009). The results indicate that males were dominant in the majority of the interactions, and females were more submissive with interesting inter-sequence behaviours between the two sexes (MIX treatment). For example, males were dominant in all of the interactions but both sexes responded to submissive behaviours with both aggressive and submissive behaviours.

The models also suggest that the key behaviours that determine the outcome of dominance are “approaching”, “meral spread” and “touching”. These sequential assessments by means of external stimuli, both aggressive and submissive, could be contributing factors that modify the internal physiology of the individuals (Bradbury and Vehrencamp, 2011; Lehner, 1996).

The present study has demonstrated that *Nephrops* use a range of strategies in relation to conflict resolution (Arnott and Elwood, 2009; Bradbury and Vehrencamp, 2011). It seems that sex and size variables influence the way the individual gathers information within social interactions. Different models such as the self-assessment and cumulative assessment models could be used with *Nephrops* to determine when an individual decides to escalate and retreat (Arnott and Elwood, 2009). It is however unclear whether it is the stress caused by the opponent or self-assessment that influences the decision of whether to continue in engaging with the opponent. *Nephrops* would therefore be an ideal candidate species to further test game theory models within crustaceans.

3.4.3. Haemolymph Parameters

Behavioural interactions significantly influenced the internal physiology in *Nephrops*. The THC, lactate and ammonia levels significantly changed following behavioural interactions.

In this study **THC** increased from t_0 and became significant different between winners and losers. Increased aggression caused increased Δt THC levels, compared with increased submissive behaviours which caused lower Δt THC levels, and in some instances, losers even displayed a reduction in THC. Males with male opponents, and those that were of a similar size had increased THC more than females with female and different sized opponents. An increase in THC has been shown in other Crustacea to be a consequence of handling, injury and a functional response to high temperatures (Le Moullac and Haffner, 2000; Jussila et al., 2001). There were also some relationships between other haemolymph parameters. The

haemolymph glucose concentration and THC seem to be correlated. Possible reasons for this are that released haemocytes are partially responsible for CHH (Crustacean Hyperglycemic Hormone) exocytosis, which in turn controls glucose levels (Lorenzon, 2005). Also, an increase in glucose concentration from previous stressors can also cause fluctuations in THC (Lorenzon, 2005).

However, there is limited evidence of how haemocytes are altered in relation to behavioural interactions, as most literature has focussed upon environmental contaminants and physio-chemical parameters (Le Moullac and Haffner, 2000). This study then highlights the potential of using THC to assess physiological changes in relation to behavioural activity in *Nephrops*.

Lactate levels significantly increased following behavioural interactions. Again males with similar sized opponents had higher levels of lactate increase than females and those with opponents of different size. Submissive behaviours had a stronger relationship with lactate levels than aggressive behaviours. Smaller individuals had a higher correlation in lactate increase than larger individuals. However, is it unknown whether submissive behaviour is the cause or effect of increased lactate levels. Typically, smaller sized opponents tended to exhibit more retreat behaviours when interacting with a larger opponent. *Nephrops* display an intrinsic escape response to perceived threats which uses rapid movements of the tail and abdominal musculature to propel the individual away, as found in most decapod crustaceans (Katoh et al., 2008, 2013). CHH (crustacean hyperglycemic hormone) is a member of the neuropeptide family synthesised in the eyestalk and has a main function (among many) of regulating energy levels within the haemolymph. CHH is released and targets tissues to mobilse glucose from glycogen (glycogenolysis), leading to, in some instances, hyperglycemia and hyperlipidemia (Fanjul-Moles, 2006). The requirement of energy is as a primary response to variable stressors is found within many decapod crustaceans (Stoner, 2012). Studies suggest that both glucose and lactate levels independently influence and regulate CHH mobilisation (Morris and Airriess, 1998; Fanjul-Moles, 2006). An increase in lactate resulting from glycolytic flux is correlated to an increase in CHH levels (possible positive feedback), whereas the opposite is found for increased glucose levels (*i.e.* lowering CHH levels). Thus, it is suggested that lactate is an independent interactive control for accelerated glycolysis under anaerobic conditions (Morris and Airriess, 1998). Therefore, lactate build-up is not only the metabolic end product due to anaerobic metabolism, but also potentially a separate process

for glycogenolysis. The build-up of substances such as lactate have been influential in the decision-making process of retreating during fights in hermit crabs (Briffa and Elwood, 2001), and may be a possible explanation of the increased lactate in submissive individuals within this study (Ridgway et al., 2006a).

Behavioural interactions caused **Ammonia** levels to significantly decrease. Again, submissive behaviours were linked to increased Δt ammonia levels when compared to aggressive behaviours. Within the literature ammonia levels typically increase with stressors such as physical disturbance, emersion and temperature increase (Giomi et al., 2008; Hunter and Uglow, 1993; Jacklin, 2005). Increased activity whilst submerged has been shown to increase the excretion rate of ammonia in *Cancer pagurus* (Regnault, 1987; Danford et al., 2001). This may explain the decreasing levels shown in this study, as the individuals were active when near other individuals, for both aggressive and submissive behaviours. This could have implications towards the way *Nephrops* alter their physiology during fishing practices, especially whilst within a creel (pot) with conspecifics. The creel environment is unique in the fact that it allows new individuals to enter and disappear, each time potentially changing the established hierarchical dominance causing more interaction and therefore physiological debt. The ammonia levels could then theoretically fluctuate throughout the capture process, declining whilst in the creel, increasing whilst emersed and then again decreasing when re-immersed as seen in previous investigations by Bernasconi and Uglow (2008). Adey (2007) concurs with other findings that whilst individuals are attracted to and remain in the vicinity of the creel, a low percentage of *Nephrops* locate the creel entrance, causing a relatively low catch rate. In addition, size selectivity occurs in and around the creel, with larger sized individuals displacing smaller sized individuals following an agonistic interaction (*i.e.* creel self-selection). Improving the creel design based upon behavioural data could minimise social stress and improve post capture and transport survival in *Nephrops*. For example, the red king crab creel fishery improved the creel design to be more selective of target individuals whilst increasing the catch rate (Zhou and Shirley, 1997).

There was no significant physiological change observed within glucose, protein and RI levels following behavioural interactions.

Glucose is used as an indicator of stress from a number of stressors in many crustaceans (Stoner, 2012). For example, increased glucose haemolymph levels imply high levels of exercise, emersion and disease (Lund et al., 2009; Stoner, 2012). In this study increasing exploratory behaviours were linked with reduced Δt glucose levels (less reduction). Glucose levels can be very variable, as previously described in other crustacean studies (Lorenzon, 2005; Ridgway et al., 2006b; Bernardi et al., 2015). Some question whether Crustaceans are hyperglycemic given glucose levels can return to baseline levels under prolonged stress (Lorenzon, 2005; CrustaSea, 2006). In terms of behaviour, low glucose levels are thought to be a key factor in determining whether the individual should fight or flee. In this study females and individuals in the different sized treatments, were found to have lower glucose concentrations as found in submissive hermit crabs (Briffa and Elwood, 2001), whereas within the MMSS treatment, the glucose levels increased, along with aggressive and exploratory behaviours. The higher levels of activity and aggression could be the reason for higher levels of glucose in this treatment, whereas lower levels could be a key factor in determining the exhibition of submissive behaviours. A similar explanation could be applied to Δt **protein** levels. Previous research indicates that changes in protein levels are associated with transporting oxygen and providing energy to the tissues in response to stressors (Cheng et al., 2003; Lorenzon et al., 2008). Therefore, the mode in which glucose and protein are metabolised could be complex in relation to the different stressors and the opponent, and further investigations should be conducted.

RI showed little change from behavioural interactions. However, haemolymph density (*i.e.* the RI of whole haemolymph by the use of a refractometer) is used to determine protein concentrations in other crustaceans as it is a method that is quick, cheap, portable, easy and non-destructive in comparison to colour-metric analysis. Haemolymph protein levels have shown to respond to the moulting and reproduction cycle, hypoxia and nutritional condition within other crustacean species (Leavitt and Bayer, 1977; Lorenzon et al., 2008; Lorenzon et al., 2011; Ozbay and Riley, 2002; Stoner, 2012). The present study is the first to indicate that using the RI taken from *Nephrops* could be used as a proxy for haemolymph protein concentration and therefore used to assess general health in future applications.

3.4.4. Summary

This study provides evidence that behavioural interactions are costly to both the dominant and submissive individuals, as it causes physiological changes following both aggressive and submissive behaviours. In summary there were behavioural differences between treatments, with males and similar sized opponents showing more aggression. There were also physiological differences between treatments, with certain key parameters being influential in how the individual behaves. Aggressive behaviours seem to be correlated with THC and protein Δt, whereas submissive behaviours seem to correlate with lactate and ammonia Δt. Glucose seems to correlate to both with different responses depending upon the situation. Size also seemed to affect both the behaviour and the physiology dependent upon the opponent. However, size was not a clear factor for predicting the dominant or subdominant individual. Relationships between the physiological parameters remain a complex issue, with different reactions to that induced by fishing stressors.

The results have implications for the fishing industry, whether it be within the creel or in a holding facility; holding unrestricted individuals in a relatively small space will cause physiological changes. The behaviours and physiological changes may be adaptive for the given situation of being trapped in an enclosed space with other individuals. This work has future implications for telescoped populations where fishing activity has selected larger individuals, leaving a population of smaller and similar sized individuals.

3.5. Acknowledgements

I would like to the following people; Susanne Eriksson for the support and guidance throughout, and Tony Roysson (skipper of the fishing vessel Tärnö) for the supply of live *Nephrops*. Thank you to the funding received by ASSEMBLE grant agreement no. 227799 at the Sven Loven Centrum för Marina Vetenskaper, Kristineberg.

Appendix 3A. Supplementary Data

Table 3.8. Haemolymph reagents and marine anticoagulant

Agent Name	Components
Indophenol reagent 1	Phenol 1g Sodium nitroprusside 5mg ddH_20 50ml
Indophenol reagent 2	Sodium hydroxide 0.5g Sodium hypochlorite 1 ml ddH_20 50ml
Marine anticoagulant	Sodium chloride 2.63g Glucose 1.80g Trisodium citrate 0.88g Citric acid 0.55g EDTA (FW 372.24) 0.37g MilliQ ddH_20 100ml

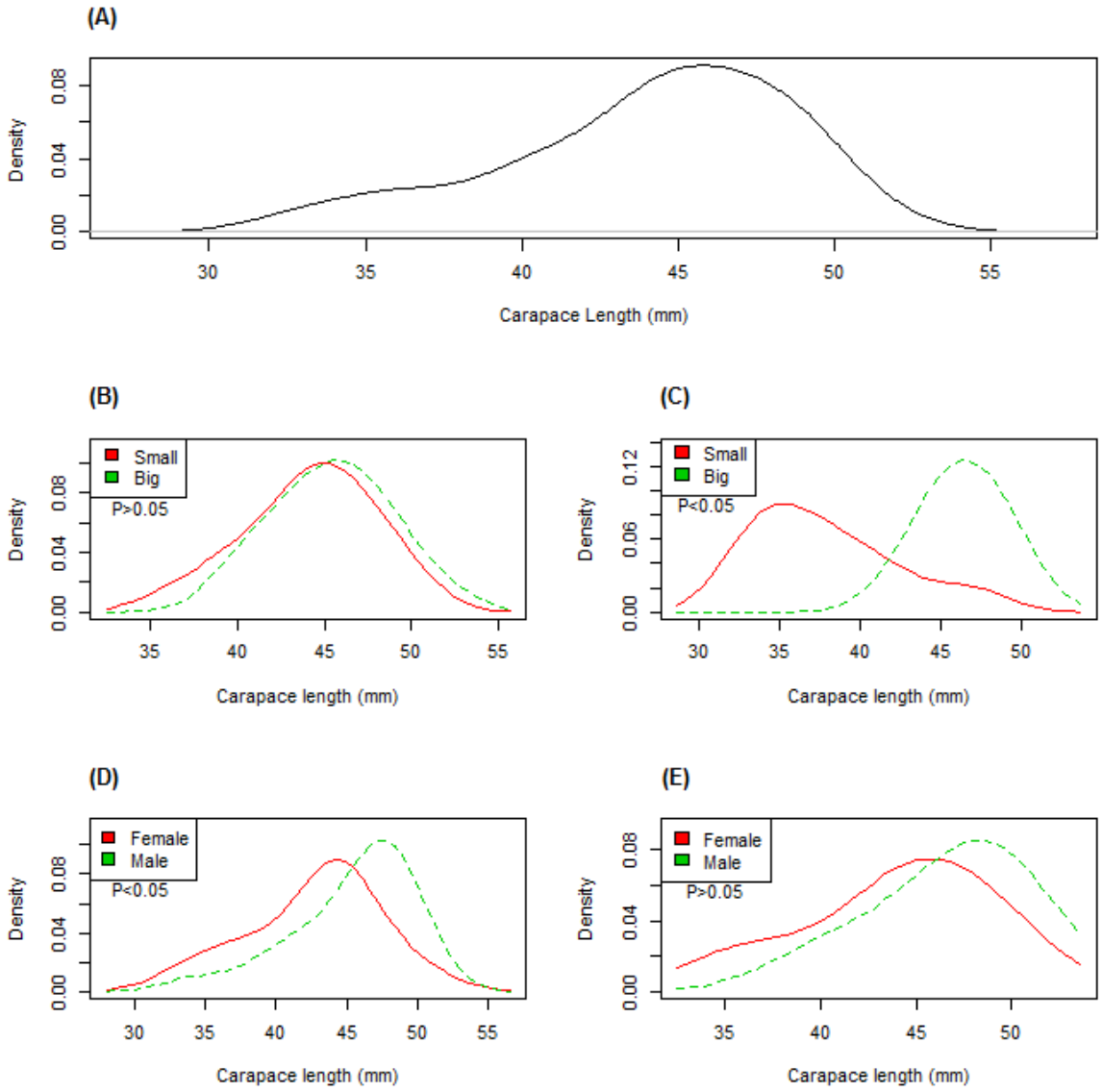

Figure 3.13. Carapace length (mm) for (A) overall; (B) between similar sized opponents; (C) different sized opponents; (D) different sexed opponents and (E) the controls. P values are displayed within the figure (Mann Whitney U-Test).

Table 3.9. Ethogram of behaviours and grouping categories.

Grouping Behaviour	Key of behaviour	Description
Exploring	EX - Exploring	Walking in the arena without contact of the opponent or walking in the direction towards the other individual. Out of reach from the opponent. This also included the individual walking into the corner of the arena and using the pereopods to walk against the side (corner climbing).
Aggressive	A – Approach	Movement towards the opponent, within reach of the cheliped. Opponents can approach face on, sideward, or from behind. Usually with clear intent to make contact with the opponent.
	MS – Meral Spread	High on legs, meral spread (horizontally spread chelipeds). Combatants push each other face to face in meral spread position. Smacking, pushing, cheliped grabbing, punching are also included.
	T - Touch	Any part of the body comes into contact with the opponent.
Submissive	AV – Avoid	Keeping a distance away from the opponent, body pressed to the ground. Usually walking away from the opponent. Includes walking backwards from the approach of the opponent.
	Q - Quiet	No locomotion or limited activity such as cleaning. The individual is low to the floor.
	TF – Tail Flipping	Rapidly moving away from the opponent such as fleeing backwards and tail flipping.

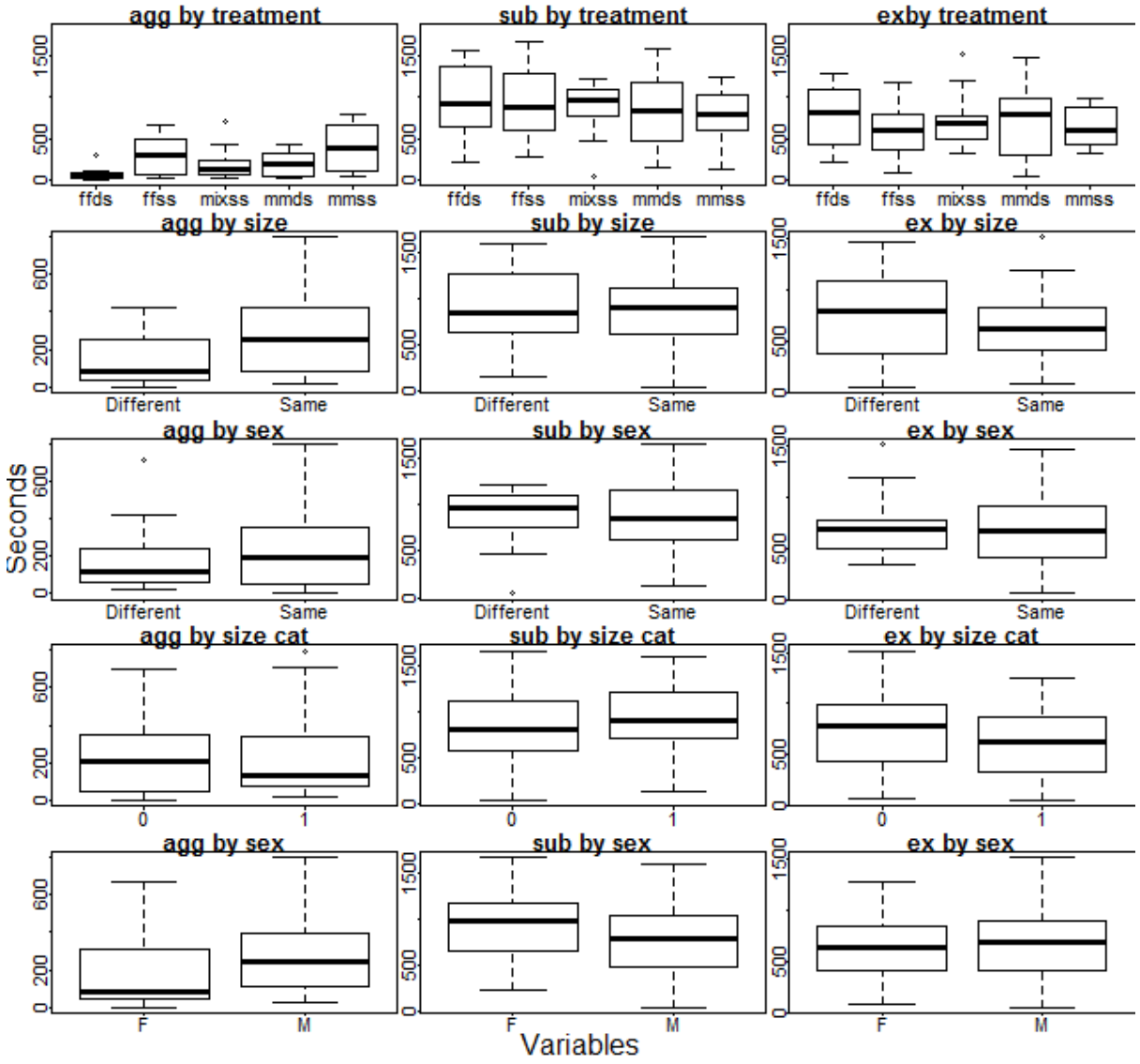

Figure 3.14. Duration (seconds) of aggressive (agg), submissive (sub) and exploratory (ex) behaviours between treatments, sex and size differences. ffds = female vs female of different sizes; ffss = female vs female of same sizes; mixss = mixed sex and same sized; mmds = male vs male of different size; mmss = male vs male of same size; Different = Individuals that were in the treatments of either opposing size or sex; Same = Individuals that were in the treatments of either the symmetrical size or sex; 0 = smaller opponent; 1 = larger opponent; F = Female; M = Male.

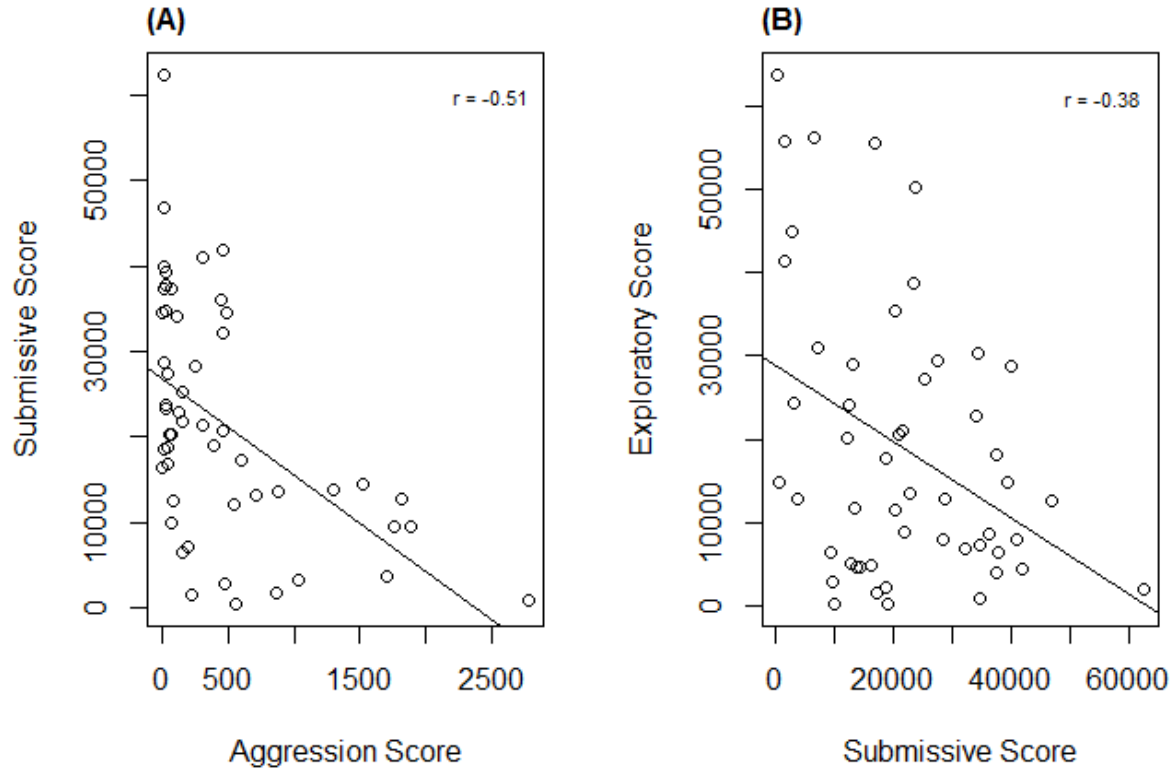

Figure 3.15. Correlations between (A) aggressive and submissive behavioural scores and (B) submissive and exploratory behavioural scores.

Appendix 3B. Protein

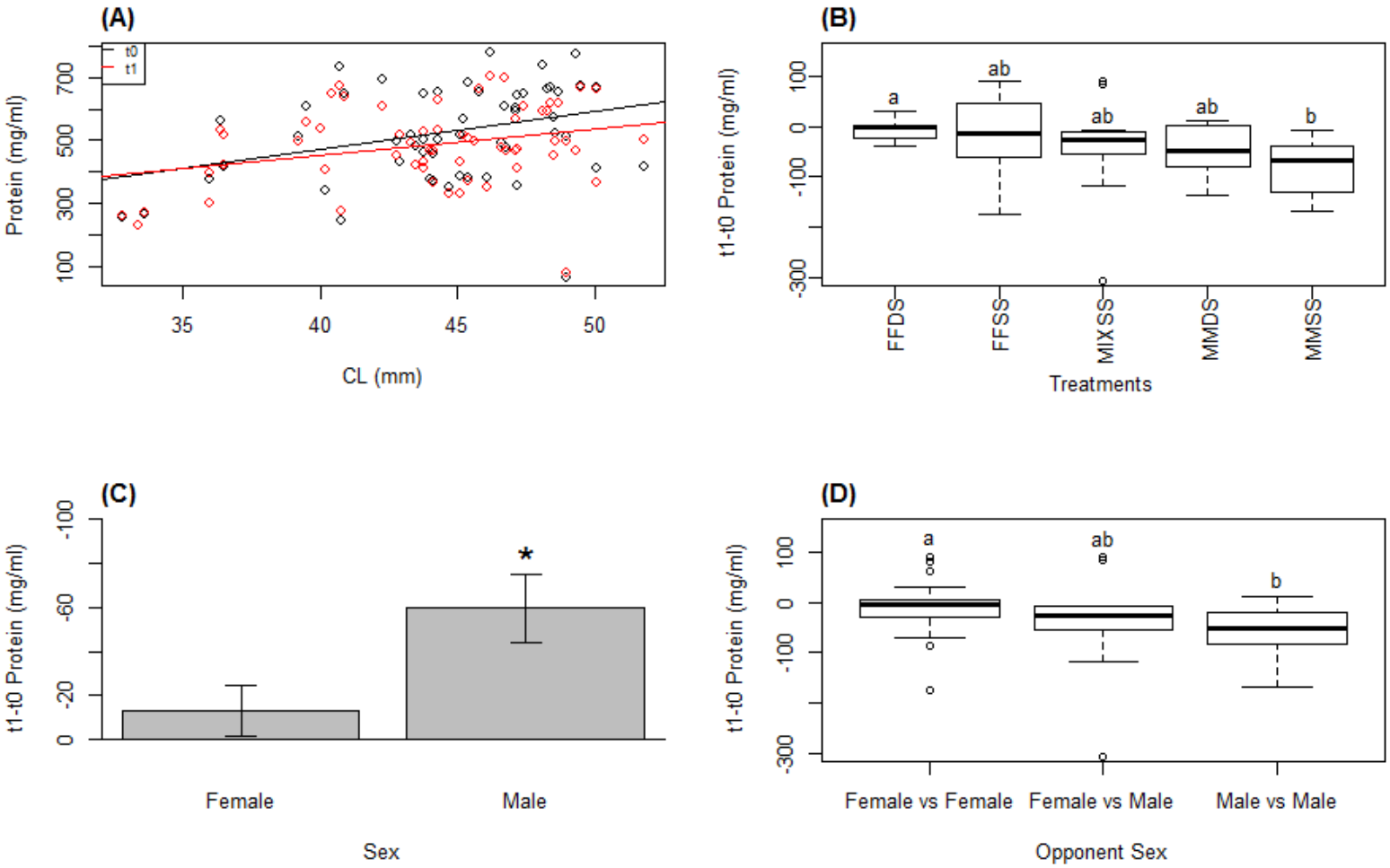

Figure 3.16. Haemolymph protein levels in relation to physiological parameters (A) Carapace length (mm); (B) Behavioural treatments; (C) Sex; (D) Opponent sex. Letters indicate where significance difference lies between parameters (Kruskal-Wallis, P<0.05). Asterisks indicates a higher significant difference (t-test, P<0.05).

Appendix 3C. THC

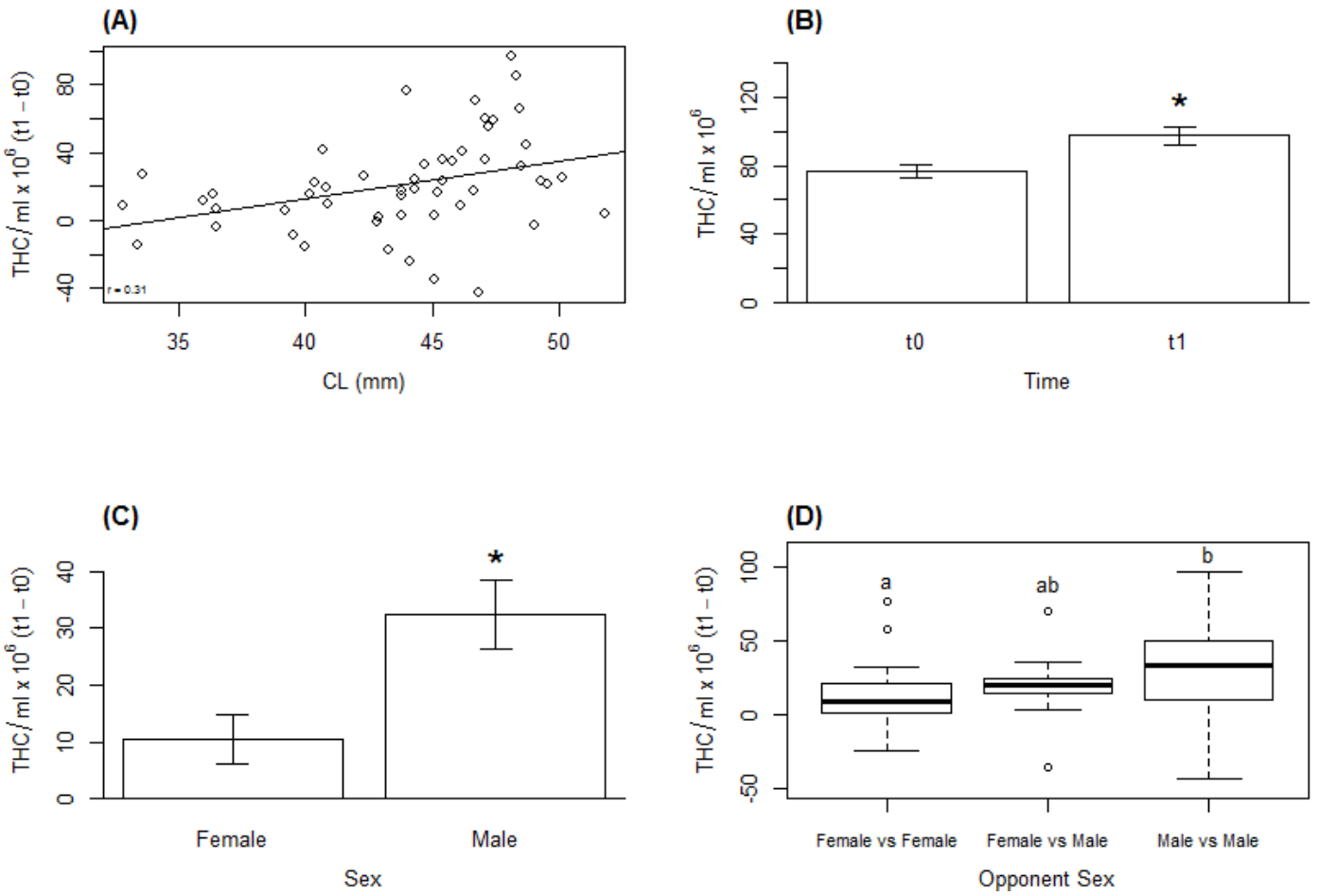

Figure 3.17. Physiological factors that affect Total Haemocyte Counts (THC). (A) Carapace length in correlation with Δt THC; (B) THC levels at t0 and t1; (C) Δt THC between sexes and (D) Δt THC within different treatments. Asterisks indicate where significant difference occurs (T-test, P<0.05). Letters denote significant difference (Kruskal-Wallis, P<0.05).

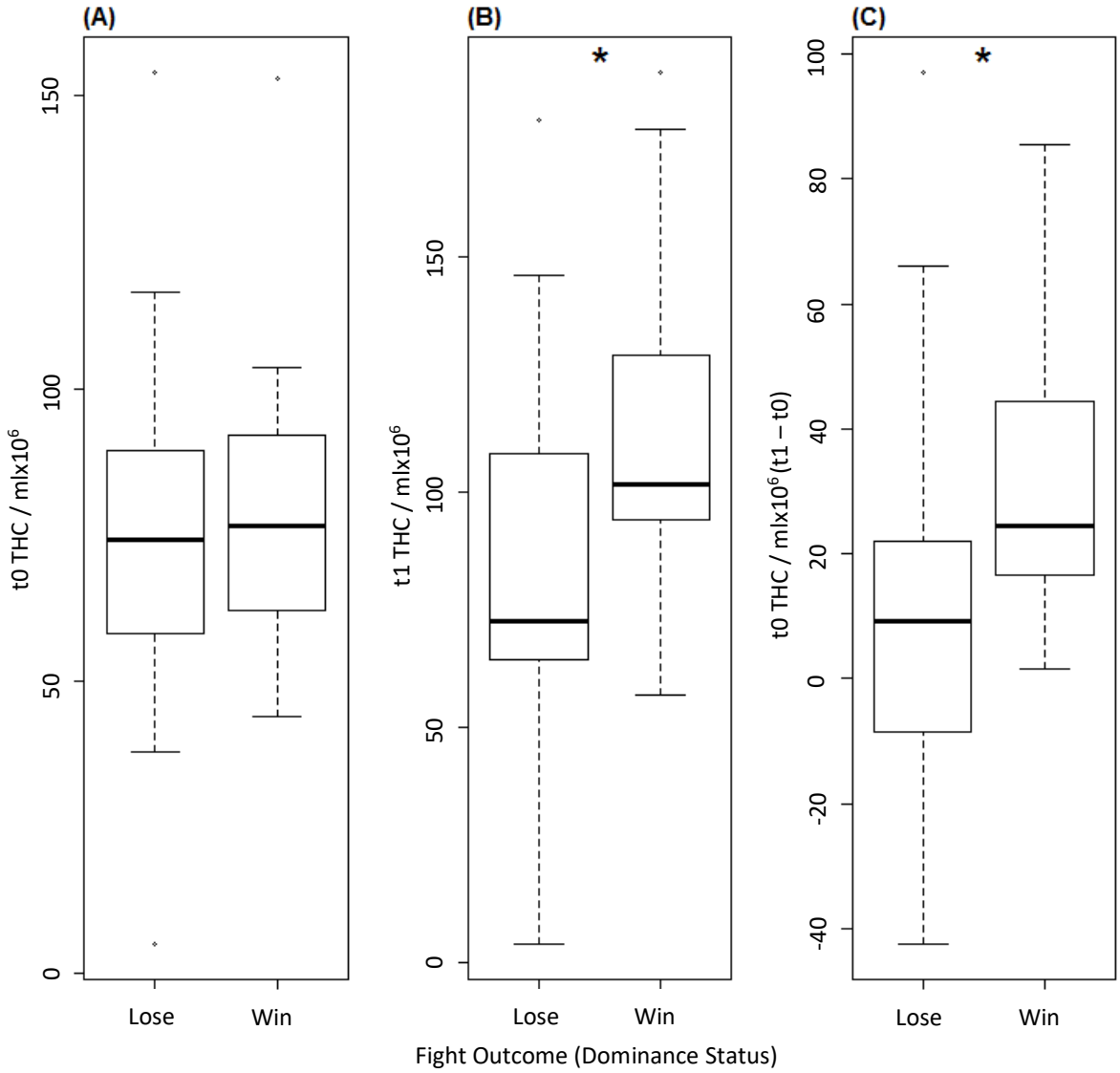

Figure 3.18. THC levels at (A) t0; (B) t1; and (C) Δt between winners and losers of the 30-minute interaction period. Asterisks denote significant differences in THC levels (Wilcox Test, P<0.05).

Appendix 3D. RI

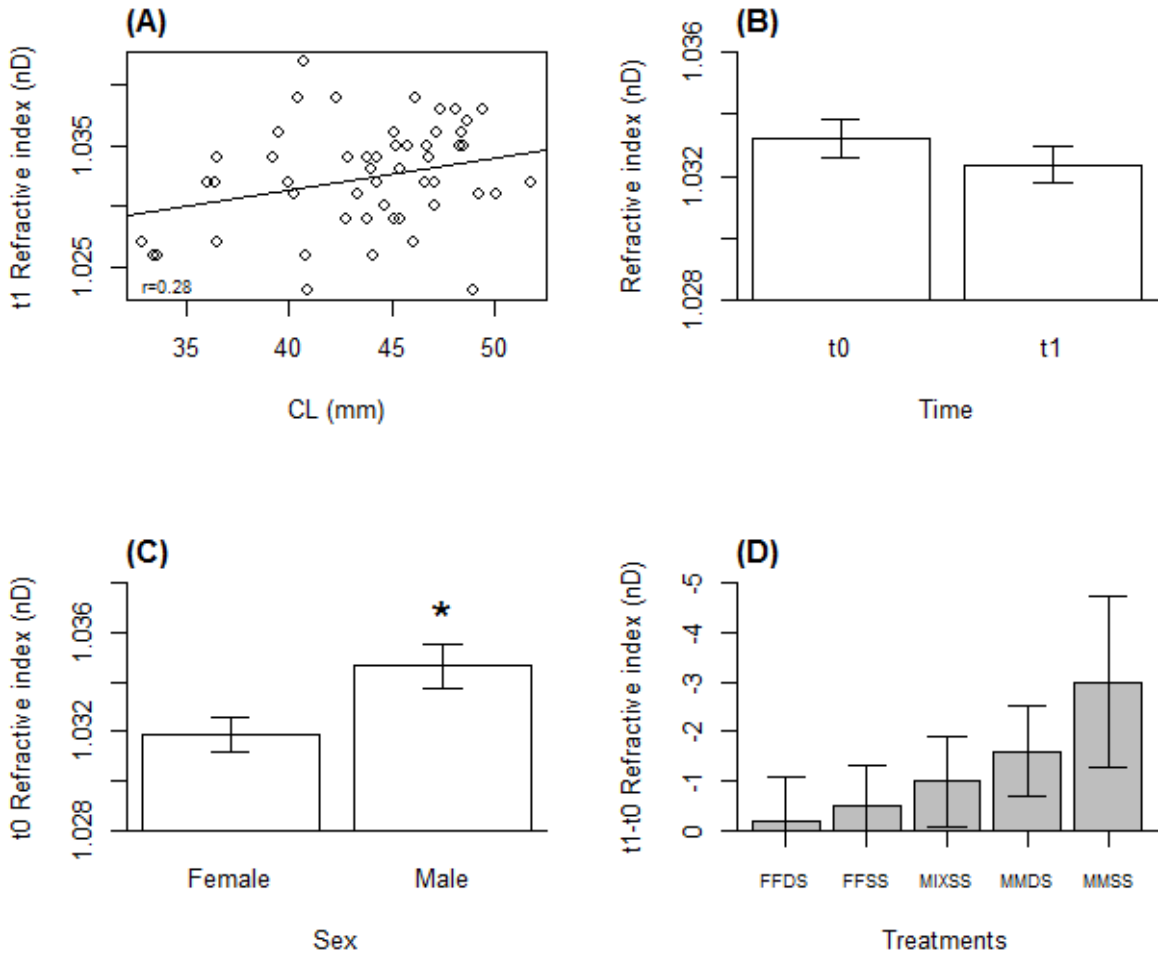

Figure 3.19. Haemolymph refractive index (A) correlated to the Carapace length at t1; (B) between times t0 and t1; (C) between sexes at t0 and (D) Δt between treatments. Asterisk indicates where significant differnces lie (T-test, P<0.05).

Figure 3.20 illustrates the significant correlation of protein concentration (mg/ml) and the refractive index (r_s=0.79, df=102, P<0.001).

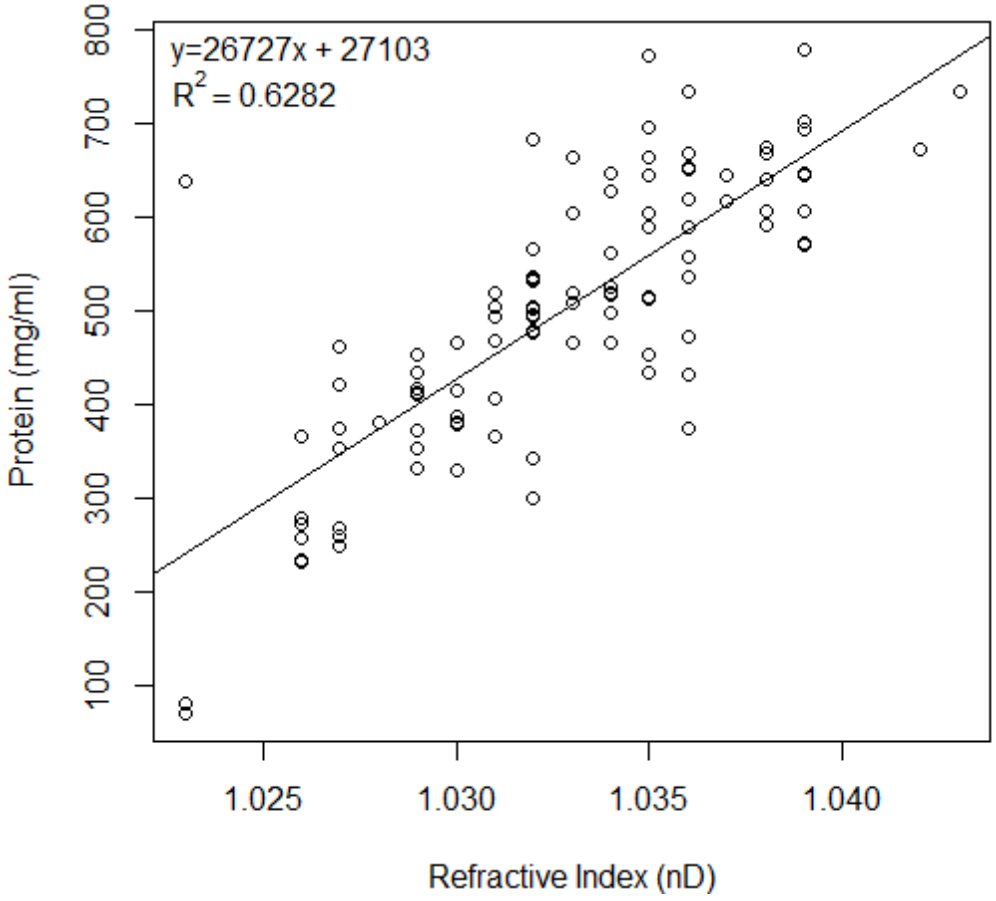

Figure 3.20. Correlation of protein concentration (mg/ml) and refractive index (nD) (n=102).

Appendix 3E. Glucose

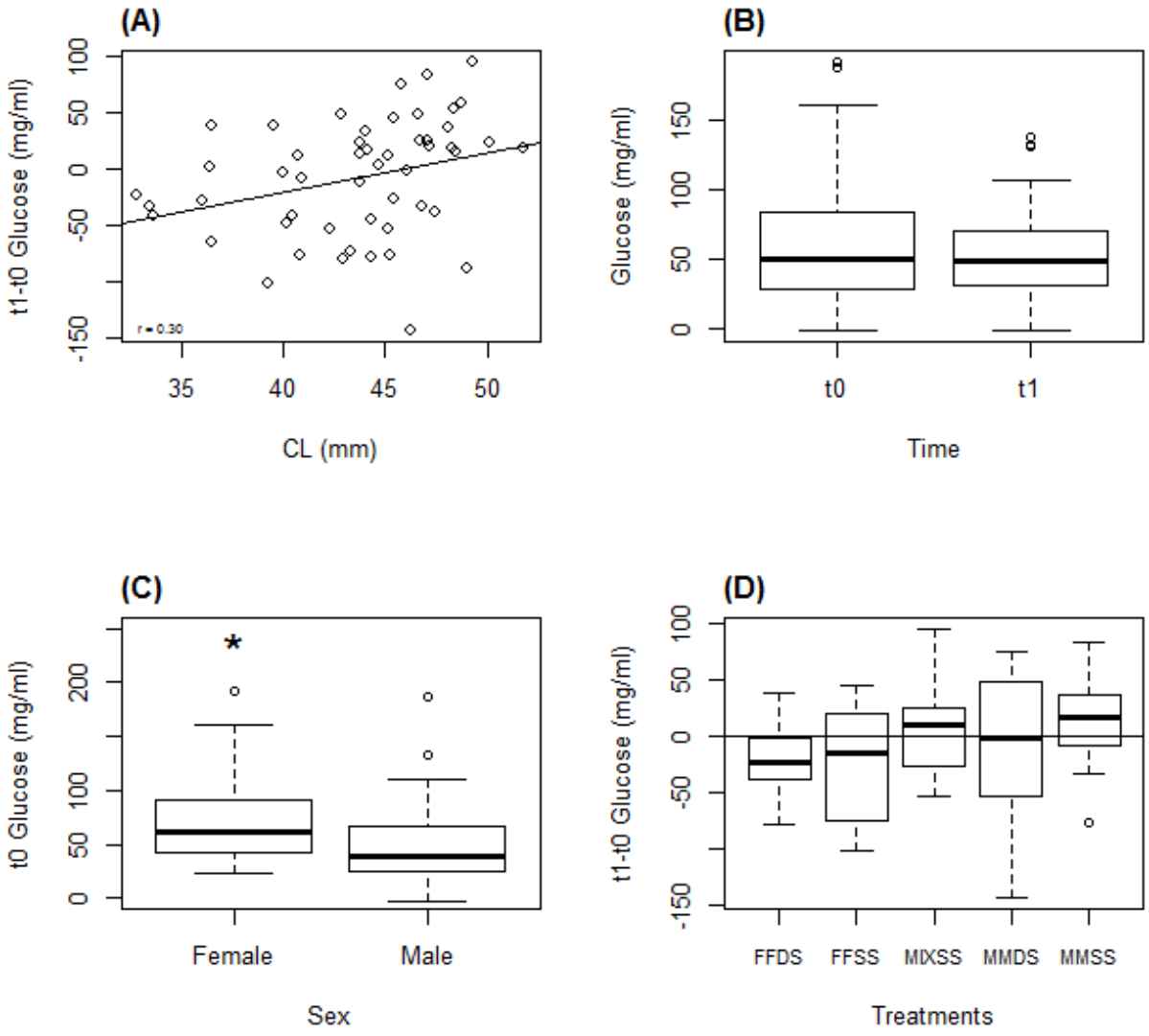

Figure 3.21. Glucose (A) correlated with CL; (B) levels at time t0 and t1; (C) between sexes at t0 and (D) Δt between treatments. Asterisk inidactes signficant difference between sexes (Wilcox test, P<0.05).

Appendix 3F. Lactate

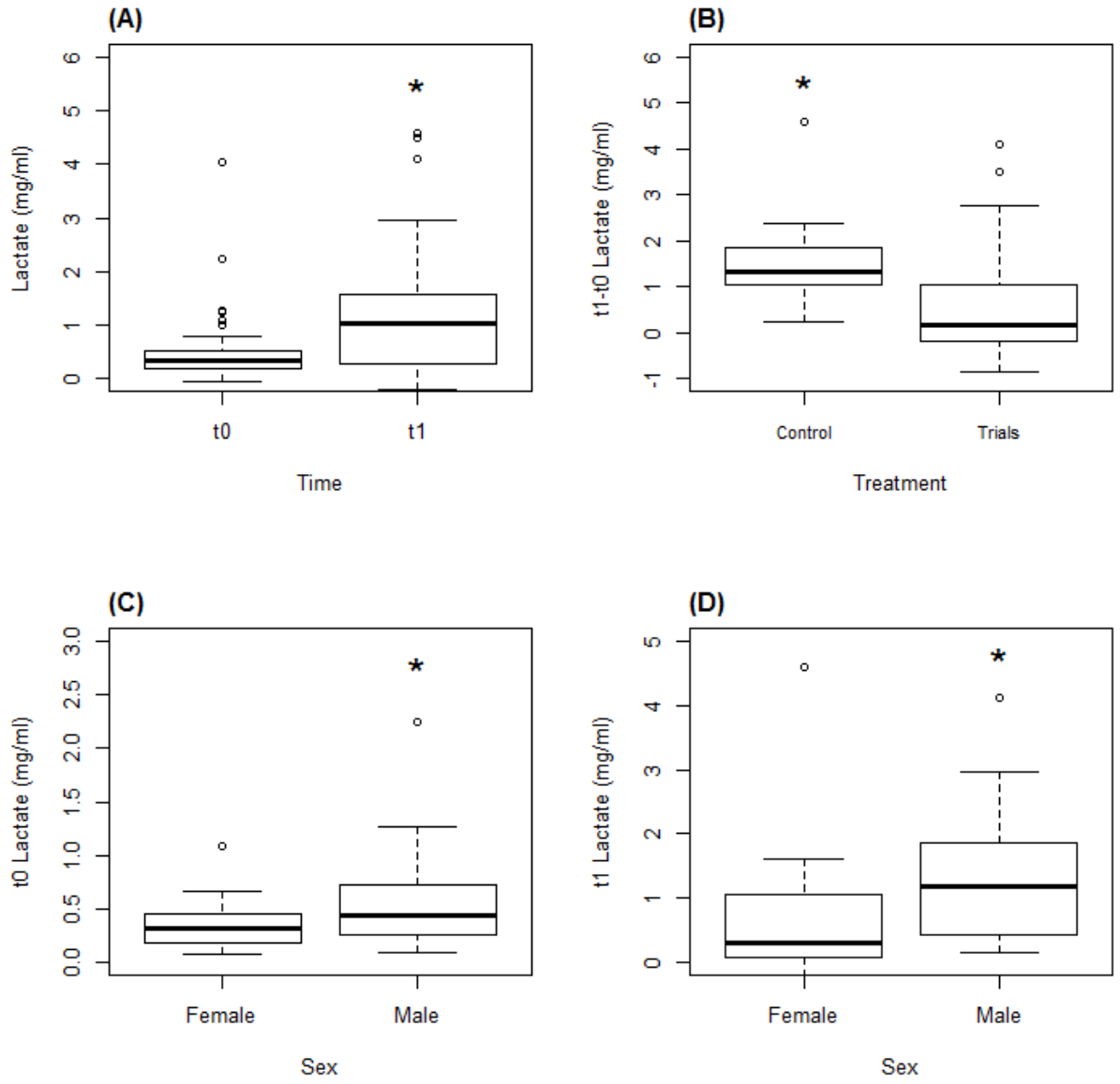

Figure 3.22. Lactate levels (A) at t_0 and t_1; (B) Δt between control and trials; (C) t_0 levels between sexes and (D) t_1 levels between sexes.

Appendix 3G. Ammonia

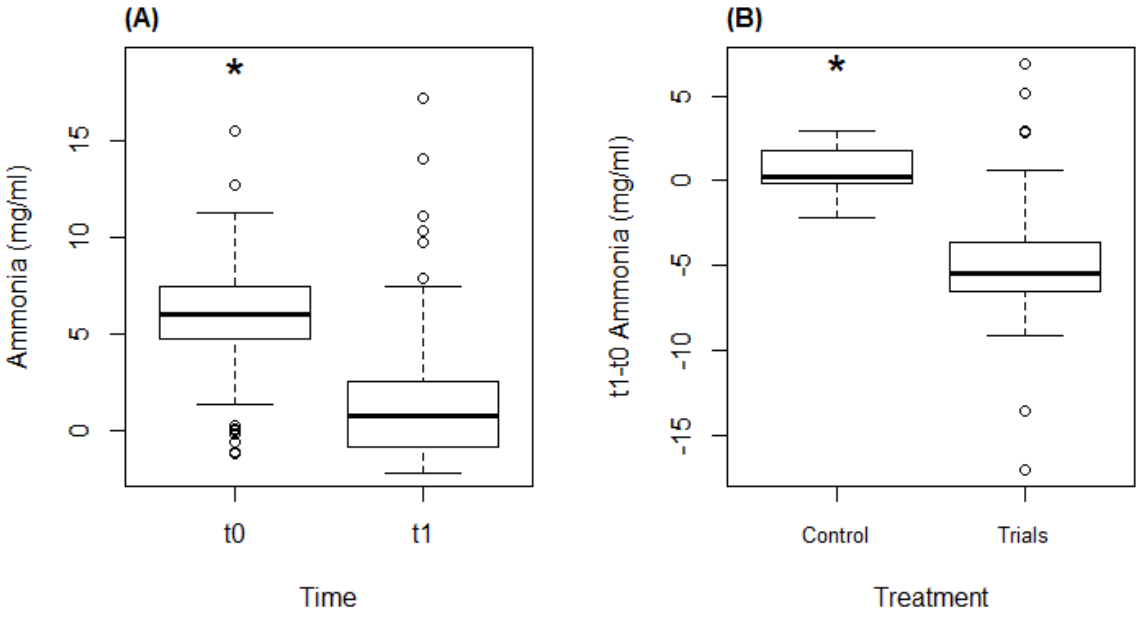

Figure 3.23. Ammonia levels (A) between t0 and t1; (B) Δt ammonia levels between control and treatments trials.

4. Evaluation of Different Concentration Doses of Eugenol on the Behaviour of *Nephrops norvegicus*.

Abstract

Eugenol was tested for use as an anaesthetic on *Nephrops norvegicus* at three different concentrations (300, 600, 900 µL/L). Monitoring included the time taken to reach each sedation stage. Sedation stage was assessed by passive behaviours and reaction to direct stimulation. A concentration of 300 µL/L induced full anaesthesia in 56% of individuals taking on average (median time seconds ± SD) 849 ± 200 s. Concentrations of 600 and 900 µL/L induced full anaesthesia in 100% of individuals taking 439 ± 143 s and 277 ± 117 s respectively. Recovery times were not significantly different between concentrations with an average time of 689 ± 39 s. Ovigerous (berried) and freshly captured females took longer to reach full anaesthesia and were quicker to recover compared to non-berried and captive females. Eugenol can be used as an effective anaesthetic for this species.

Abbreviations:

LS = Light sedation; HS= Heavy sedation; FA = Fully anaesthetised; PR = Partial recovery; FR = Full recovery

Cowing et al., 2015. Evaluation of different concentration doses of eugenol on the behaviour of *Nephrops norvegicus*. Aquaculture 442, 78-85. doi:10.1016/j.aquaculture.2015.02.039.

4.1. Introduction

Nephrops norvegicus (referred to as *Nephrops* from here on) is a benthic decapod crustacean that inhabits burrows in muddy substrate at depths of between 20 – 800 m (Farmer, 1975). The species is of significant value to the UK with a total catch of around 31,000 tonnes in 2016, the majority of which are caught in Scotland, and the fishery is worth around £80 million (MMO, 2012; Ungfors et al., 2013). Of the total catch (creel and trawl caught individuals) in the UK, around half is exported to Spain, France and Italy using live transport methods (Ungfors et al., 2013). Whilst mortality during transport is relatively low, it is estimated that the stress caused by capture, handling and aerial exposure increases mortality by over 30% in the stock once received at the holding facilities (Coyle et al., 2004; Ridgway et al., 2006; Lund et al., 2009; Powel et al., 2017). This has considerable implications for the transport of broodstock, the development of hatcheries, aquaculture techniques and optimising the economic gains for the lucrative trade in live animals (Neil, 2012).

The commercial aquaculture sector uses various methods to reduce stress whilst transporting broodstock and one such method is sedation (Ross and Ross, 2008; Harmon, 2009). Sedation is a preliminary state to anaesthesia, defined by reduced sensory perception and possible analgesia. Anaesthesia can be described as a 'loss of sensation and perception' and both states assume that full recovery will occur (Ross and Ross, 2008). Use of anaesthesia or sedation in several species of fish and crustaceans has shown that stress can be reduced and survival enhanced during and after transportation (Coyle et al., 2005; Saydmohammed and Pal, 2009). Therefore sedation or anaesthesia have the potential to reduce mortality during and after transport of *Nephrops* (Pramod et al., 2010; Barrento et al., 2011).

Sedation techniques and procedures for crustaceans are not well developed compared to those for commercial fish species, as most operations do not require the use of anaesthesia or sedation, nor have the legislative requirement to do so. Generally other methods are used such as cooling and distraction (allowing the individual to grasp onto an object and therefore immobilising them) (Ross and Ross, 2008). Investigations that have used sedation on crustaceans have concluded that there are varied results and success rates, (Coyle et al., 2005), and that for each species pilot studies should first be carried out to determine the relationship between dosages and lengths of exposure and their physiological and behavioural responses (Ross and Ross, 2008). Therefore, assessment of the sedation stage

can be derived from behavioural examination and a method to assess successive sedation stages for this species was also investigated by behavioural analysis. Since it has been observed in several species that behavioural changes occur with increased time in captivity (Hennig and Dunlap, 1978; Houlihan and Mathers, 1985; Araujo et al., 1995), and it may be necessary to hold *Nephrops* broodstock captive for long periods of time (overwintering), this factor will also be investigated within this study.

Eugenol has many uses including human dentistry and food flavouring (Akbari et al, 2010) and is the major constituent of clove oil (70-90%). Eugenol has been successfully used as an anaesthetic for a number of crustaceans (Coyle et al., 2005; Waterstrat and Pinkham, 2005; Saydmohammed and Pal, 2009; Akbari et al., 2010; Huntsberger, 2012; Parodi et al., 2012) and is relatively inexpensive, non-toxic and easy to administer making it ideal for use in industry. Other anaesthetics such as MS-222, CO_2, AQUI-S® are routinely used but many are difficult to acquire, more expensive, and have yielded varied results or have not been trialled with decapods (Coyle et al., 2005; Akbari et al., 2010). Several methods can be used to administer the sedative, including inhalation, parenteral and oral routes (Ross and Ross, 2008). Inhalation via addition to transport water is the most common delivery method for use with transportation of aquatic culture species and was therefore the method used for this study. Eugenol is absorbed through the gills and inhibits neural signal transmission by effects on sodium, potassium and calcium channels and *N*-methyl-D-aspartate, gamma-aminobutyric acid type A receptors (Zahl et al., 2011).

The aim of this study was to assess whether eugenol can induce sedation and anaesthesia in *Nephrops*, for future use during transportation and extended handling operations such as tagging. The specific objectives were to determine the concentrations, doses and application times of eugenol for effective sedation of *Nephrops* to different depths, to relate the depth of sedation to behavioural responses and to investigate if other variables such as the length of time in captivity and being ovigerous (bearing eggs, or berried) influence the times for induction and recovery from full anaesthesia.

4.2. Methods

4.2.1. Ethical Consent

The work has been conducted with ethical consent granted from the University of Hull and the work carried out is in accordance with the EU Directive 2010/63/EU for animal experiments located at the University of Hull, Scarborough, UK.

4.2.2. Broodstock

Nephrops (average CL of 37.93 ± 4.15 mm) were obtained from creel fishermen in Eyemouth, Scotland (D.R. Collin & Son Ltd) and vivier transported (5-8°C) for a duration of 3 hours (see Appendix 1 for further details) to the University laboratories at CEMS (Centre for Environmental and Marine Sciences, University of Hull), Scarborough, UK. All individuals were female and wet weight and carapace length measurements were taken. They were held un-banded, communally in 100 L glass aquaria, with plastic pipe shelters within a recirculating aquaculture system (RAS) maintained at a temperature of 11.5°C, a salinity of 40 psu and pH 8. The animals were fed once a week with one cold water prawn (*Pandalus borealis*) per individual. Animals underwent a 13-day acclimation period prior to experimentation. Initial vitality was assessed by checking their response to stimuli and their ability to right themselves. The eggs of berried females (n=44) were examined and their stage determined by the egg mass colour as described in Powell & Eriksson (2013). Individuals that remained within the aquaria for 40 days or more were considered "captive" (n=39) and were compared to individuals that were considered 'fresh' (held captive less than 40 days, n=36) the majority of which were experimented on between 14 and 29 days after receiving at the University. All experiments were conducted under dim red light and each randomly selected individual was only used once. No soft (recently moulted) females were used but non ovigerous females were used (n=38).

4.2.3. Experimental Procedure

Eugenol, ReagentPlus 99% 100G (Sigma Aldrich, E51791) was mixed 1:9 with ethanol >99.8% (Sigma Aldrich, 02883) using a magnetic stirrer for 10 minutes and was used as the test sedative. Ethanol was added to make the eugenol more soluble in low water temperatures (Anderson et al., 1997). Three concentrations were trialled; 300, 600, 900 µL/L (simply referred to as groups 300, 600, 900) and were added to an aerated 6 litre experimental tank

containing 4 litres of seawater, and further stirred with the magnetic stirrer for 10 minutes. After each experiment the tanks were emptied and rinsed with fresh water. The highest working concentration of ethanol alone did not induce anaesthesia and had no observable effect compared to the control group.

Preliminary trails were used to establish an ethogram (see Appendix 4A). Individual *Nephrops* were placed into the experimental tank and their behaviour was observed. The frequency of displayed behaviours was recorded for the duration of each experiment. The behaviours were broken down into two groups; Normal behaviour (termed "passive behaviour" from here on) where the individual was not stimulated, and "stimulated behaviour" referring to the response to direct contact. Direct stimulation was delivered using a plastic pipette by touching the rostrum or antennae. Control behaviour refers to non-sedated individuals.

During induction direct stimulation was given every 30 seconds for up to 15 minutes or until full anaesthesia had been reached, whichever came first. If there was no observed response the individual was placed in lateral/dorsal recumbency and further stimulated. The act of placing the individual in lateral/dorsal recumbency has been shown as an appropriate means to assess whether full anaesthesia has been reached in other crustacean species (Waterstrat and Pinkham, 2005; Huntsberger, 2012). Once full anaesthesia was reached, where by the individual had no response to stimuli in the lateral recumbency, the individual was placed in a recovery tank with fresh aerated seawater and monitored until revival and 'normal' behaviour resumed with direct stimuli given every minute. Once an individual started to become mobile (walking) and responded to direct stimuli, it was placed again in lateral recumbency until it righted itself and the experiment ended. If an individual did not reach full anaesthesia within 15 minutes, then it was removed and placed in the recovery tank. The time taken for each sedation stage and recovery was noted (Table 4.1). Following recovery, the *Nephrops* were placed into communal holding tanks and monitored for 7 days for any side-effects or post-experiment mortality. A control group was also analysed using the same experimental procedure and direct stimulation but with no eugenol or ethanol administered to the water for behavioural comparison.

A number of water quality parameters were collected before and after each trial referring to temperature (glass immersion thermometer), dissolved oxygen (YSI 550A handheld dissolved

oxygen meter) and pH (Ultrameter 2™ $6P_{si}$). Ammonia samples were also taken pre and post trials and immediately frozen. Randomly selected samples were chosen for later analysis. Ammonia concentration was determined using a Palintest photometer®.

Table 4.1. Stages of induction and recovery used for *Nephrops* in this study (adapted from Waterstrat and Pinkham, 2005; Vartak and Singh, 2006).

	Stage	Passive Behaviour	Reaction to Stimuli
Induction	Normal	Normal behaviour	Strong reaction.
	Light Sedation (LS)	Mobility is reduced.	Responsive.
	Heavy Sedation (HS)	Not mobile.	Weak response.
	Fully Anaesthetised (FA)	Immobilization or inactive movement.	No response.
Recovery	Partial Revival (PR)	Sporadic movement of walking legs, chelae, antennae. Not fully mobile.	Responsive may be delayed.
	Full Recovery (FR)	Regular movement of appendages. Mobile.	Strong response.
	Normal	Normal behaviour.	Turn to normal orientation from lateral recumbency.

4.2.4. Statistical Analysis

Data were analysed and developed using the R statistics programme, version 3.1.1 (R Core Team 3.1.2, 2015). The water parameter data were examined using the Wilcoxon signed rank test to test for differences in water samples before and after each trial. Sedation stage timings (induction and recovery) and categorised damaged data were analysed using a Kruskal-Wallis test and a post hoc multi comparison analysis. Analysis for differences in captive, berried and damage females focussed only on two stages; full anaesthesia and full recovery as these were deemed most important to analyse any differences. Differences in behaviour between each sedation stage were analysed using ANOSIM and SIMPER (Lehner, 1996) using PRIMER software (Clarke and Gorley, 2006).

Data comparing “berried and non-berried” and “captive and fresh” were analysed using one-way ANOVA and post hoc Tukey’s test. Data were tested for normality using the Shapiro test and equal variances using the Levene’s test. Data that did not conform to the assumptions of the ANOVA test were Log_{10} or square root transformed.

4.3. Results

4.3.1. Mortality and Water Parameters

No individuals died during the experiment but two perished within the 7 days post experiment period, both from the control group. No mortality occurred during any experiments and no post experiment side effects were observed such as difficulty in movement, and response to stimuli. There was no significant difference ($p>0.05$ in all cases) in all water parameters between pre and post experiment except for ammonia concentration ($\mu L\ L^{-1}$) ($W=1$, $Z=-3.4211$, $P<0.05$, $r=-0.570$) for which there was an increase of 0.073 mg L^{-1} ± 0.068.

4.3.2. Behavioural Analysis

4.3.2.1. General Behaviour

The behaviour of each individual was analysed to quantify a method that can be used to assess the different sedative stages for this species and identify how eugenol affects displayed behaviour (Table 4.2 and Table 4.3). There was a significant difference in the frequency and type of behavioural elements between each sedation stage for both the passive behaviours (ANOSIM, Global R = 0.392, P < 0.001) and stimulation behaviours (ANOSIM, Global R = 0.267, P < 0.001). Pairwise comparison of the sedation stages revealed that all stages were significantly different (P = 0.001) apart from the ‘control and normal’ and ‘control and light sedation’ stages. An indexed table (Table 4.7) is provided for quick assessment of sedation stage, and approximate times based from the results of this experiment at a temperature of 11.5˚C.

Mobile behavioural elements declined with increasing sedation stage. Mobile behaviours became apparent again during partial recovery. No movement was displayed during the anaesthetised stage. Movement was less in the full recovery stage compared to the control and normal stages. Captive individuals displayed less active movement and increased aggression compared to fresh individuals. Stationary behaviours increased in frequency as

sedation stages increased until at the highest when fully anaesthetised (Table 4.2). Submissive behaviours increased in frequency with increasing sedation stage whilst aggressive behaviours were reduced and remained low during recovery. Sedated movement was apparent at lower frequencies in both the control and normal stage, but increased to the highest frequency during heavy sedation. Sedated movement then decreased in frequency replaced by increasing stationary behaviours as recovery progressed.

Table 4.2 The proportion of the behaviours displayed per minute and response to stimuli for each of the sedation stages. Note the bold numbers are passive behaviours and the numbers in the captions are response to stimuli. ± denotes standard deviation. Downward arrows indicate a decrease in displayed behaviours and upward arrows an increase in behaviours with increasing sedation. Solid arrows are in relation to control behaviours (induction) and dashed arrows to full anaesthesia (recovery). N = 30 control and 75 for the induction and recovery stages.

	Stage	Movement	Stationary	Submission	Aggression	Sedated Movement	No Response
Induction	Control	**0.35 ± 0.18** [0.00 ± 0.00]	**0.28 ± 0.14** [0.04 ± 0.04]	**0.01 ± 0.03** [0.51 ± 0.11]	**0.35 ± 0.22** [0.44 ± 0.13]	**0.02 ± 0.03** [0.00 ± 0.00]	[0.01 ± 0.01]
	Normal	**0.34 ± 0.22** [0.02 ± 0.03]	**0.33 ± 0.17** [0.09 ± 0.07]	**0.06 ± 0.15** [0.53 ± 0.14]	**0.24 ± 0.36** [0.28 ± 0.15]	**0.03 ± 0.06** [0.00 ± 0.01]	[0.08 ± 0.24]
	Light Sedation	**0.25 ± 0.24** [0.02 ± 0.03]	**0.40 ±0.16** [0.27 ± 0.13]	**0.11 ± 0.17** [0.18 ± 0.12]	**0.16 ± 0.25** [0.21 ± 0.12]	**0.09 ± 0.09** [0.04 ± 0.05]	[0.28 ± 0.26]
	Heavy Sedation	**0.10 ± 0.08** [0.00 ± 0.02]	**0.43 ± 0.11** [0.06 ± 0.08]	**0.13 ± 0.13** [0.05 ± 0.08]	**0.01 ± 0.03** [0.16 ± 0.13]	**0.33 ± 0.10** [0.13 ± 0.10]	[0.60 ± 0.53]
	Anaesthetised	**0.00 ± 0.00** [0.00 ± 0.00]	**0.85 ± 0.05** [0.06 ± 0.07]	**0.04 ± 0.05** [0.01 ± 0.02]	**0.00 ± 0.00** [0.02 ± 0.04]	**0.11 ± 0.04** [0.08 ± 0.06]	[0.83 ± 0.42]
Recovery	Partial Recovery	**0.12 ± 0.09** [0.03 ± 0.03]	**0.60 ± 0.11** [0.29 ± 0.07]	**0.04 ± 0.05** [0.08 ± 0.04]	**0.07 ± 0.07** [0.25 ± 0.07]	**0.18 ± 0.06** [0.11 ± 0.04]	[0.23 ± 0.15]
	Full Recovery	**0.26 ± 0.10** [0.03 ± 0.02]	**0.38 ± 0.08** [0.15 ±0.04]	**0.07 ± 0.05** [0.22 ± 0.06]	**0.06 ± 0.07** [0.39 ± 0.08]	**0.23 ± 0.07** [0.19 ± 0.05]	[0.02 ± 0.02]

4.3.2.2. Key behaviours

Reactions to stimulation showed clear differences between all the sedation stages (Table 4.3). The control group typically reacted strongly with either aggressive or submissive behaviours such as chelae raising and tail flipping and this was similar for the normal stage. Passive

behaviours were very mobile with walking around the tank intermittent with standing and full antennae sweeping and antennule flicking.

During light sedation the reactions were varied but mostly stationary reactions such as moving the antennae backwards and delayed aggressive responses. Passive behaviour displayed was similar to normal but less mobile and included increased leg movement.

During heavy sedation the reaction was a mixture of stationary and sedated movement such as leg and chelae movement. The individual remained in the sitting position and all mobile movement had ceased. The individuals that had no mobile behaviours and delayed reactions were placed in lateral recumbency and remained in this position with some leg movement and antennae flicking. There was also a high frequency of no responses during this stage. During full anaesthesia the common reaction was no response.

During recovery the first most common behaviour was stationary such as sitting and pleopod beating with no response to stimuli. During partial recovery the common reactions were stationary such as antennae back, sedation movement (leg movement) and delayed aggressive responses (chelae raise). Passive behaviour was also mostly stationary, with large amounts of sitting with leg movement and intermittent standing and sitting positions.

During full recovery the reactions to stimuli were mostly sedated movement (leg movement) and trying to walk with chelae movement and a strong submissive response (tail flipping) which usually turned the individual from lateral recumbency to a standing position. Passive behaviour was similar to partial recovery, but movement of the antennae and/or antennule has recommenced and stronger standing position with leg movement and increased mobility.

There were few ‘no response’ behaviours for the control group indicating that the technique of stimulating the rostrum was sufficient in assessing sedation stage and that individuals did not habituate to the stimulation.

Mandible movement was observed during throughout the experiment. It was most frequently displayed when first placed into the induction tank with 28% displaying this behaviour within the first 30 seconds. It was also a frequent behaviour during recovery in the early stages of partial recovery although there seems to be no predictable trend or pattern. This was also true for cleaning behaviour which seemed to be loosely associated with recovery. This may

be because cleaning is a more complex activity and an indication of partial recovery for individuals.

Table 4.3. Visual representation of the most frequently displayed behaviours for each sedation stage and response to stimuli. Behaviours are in order (left to right) of highest frequency.

Sedation Stage	Passive Behaviours			Reaction to Stimulation		
Control	 Walking	 Antennae	 Chelae Raise	 Tail Flipping	 Chelae Raise	 Walking Backwards
Normal	 Walking	 Antennae	 Standing	 Walking Backwards	 Chelae Raise	 Tail Flipping
Light Sedation	 Standing	 Antennae	 Leg Movement	 Antennae Back	 Delayed Chelae Raise	 Tail Flipping
Heavy Sedation	 Lateral Recumbency	 Leg Movement	 Antennae Movement	 Chelae Movement	 Leg Movement	 No Response
Full Anaesthesia	 Sitting	 Pleopod Beating	 Leg Movement	 No Response	 Leg Movement	 Antennae Back
Partial Recovery	 Sitting	 Standing	 Leg Movement	 Antennae Back	 Leg Movement	 Delayed Chelae Raise
Full Recovery	 Leg Movement	 Antennae	 Standing	 Leg Movement	Chelae Movement	Tail Flipping

4.3.3. Induction Times

The sedative induction times from lightly sedated to fully anaesthetised are presented in Figure 4.1 for all eugenol concentrations. A Eugenol concentration of 300 µL/L caused light sedation and heavy sedation but only 56% of individuals reached full anaesthesia. Concentrations of 600 µL/L and 900 µL/L eugenol caused light sedation, heavy sedation and full anaesthesia.

There was a significant difference in the time taken to reach each induction sedation stage between concentrations (Table 4.4). Concentration 900 µL/L induced full anaesthesia in the least amount of time (277 ± 117 s) and was significantly different to 600 µL/L and 300 µL/L (Table 4.4).

Carapace length ranged from 31-49 mm, with no significant differences between the three concentration treatments (Kruskal Wallis test, Chi squared=1.4364, df=2, p=0.4876) (Appendix 4A; Figure 4.5).

There was no significant correlation between the time to induce each sedation stage and carapace length or weight ($p>0.05$). There was also no significant correlation between the length of sedation (from light sedation to partial recovery) and time to reach full recovery.

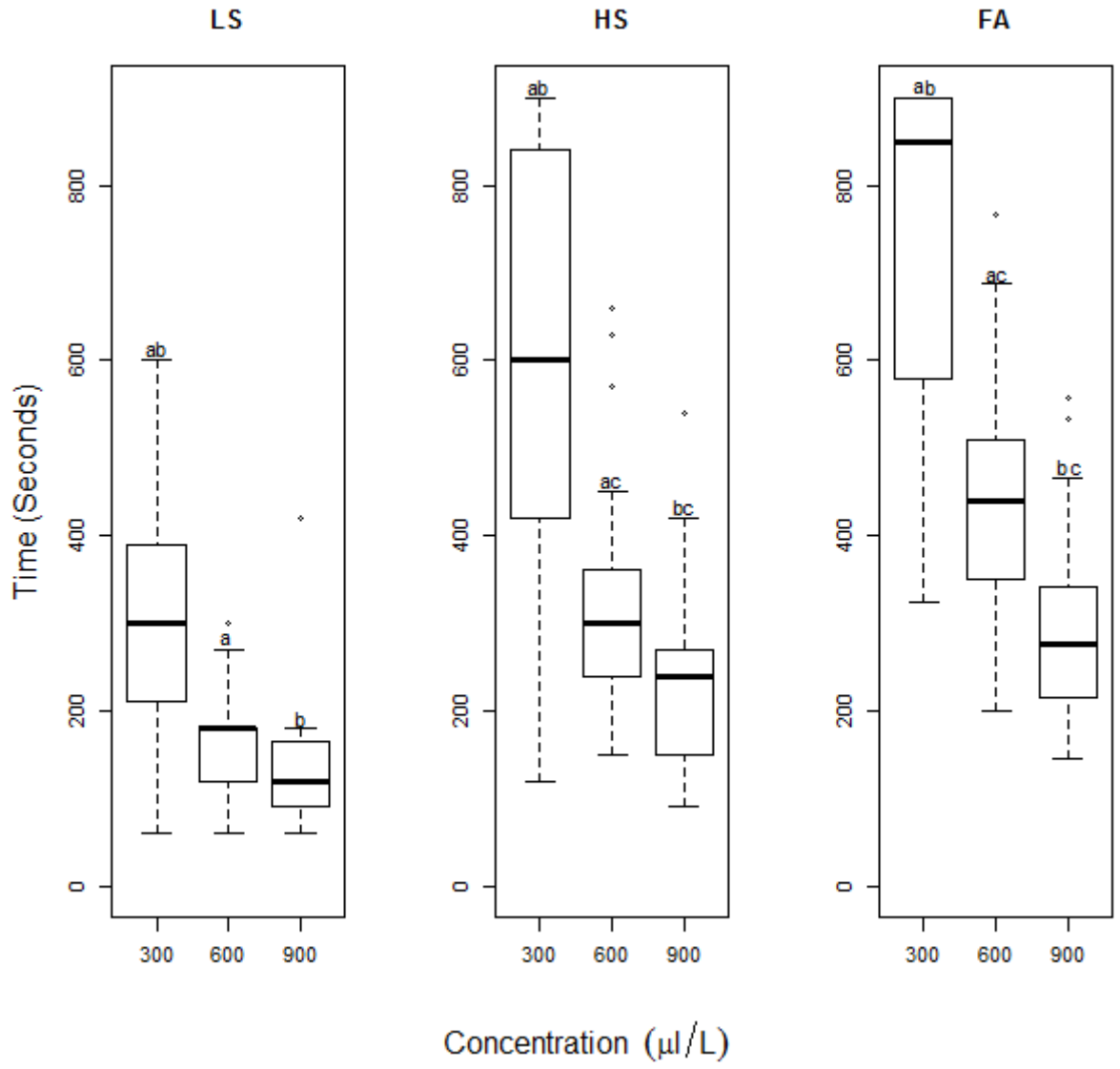

Figure 4.1. Sedative induction times to full anaesthesia between eugenol concentrations. LS= light sedation; HS= heavy sedation; FA= Full anaesthesia. Letters above the 95% confidence interval bars indicate where significant differences lie between concentrations. 300 n=27; 600 n=21; 900 n=27.

4.3.4. Recovery Times

The 300 µL/L concentration induced partial recovery and full recovery in the most rapid time at an average of 60 ± 181 and 641 ± 298 s respectively (Figure 4.2). This compared with full recovery times of 695 ± 296 s at 600 µL/L and 730 ± 364 s at 900 µL/L. However there was no significant difference in time taken to reach both recovery stages (partial and full recovery) among all three eugenol concentrations (Table 4.4). On average, including all concentrations, full recovery took 689 ± 39 s.

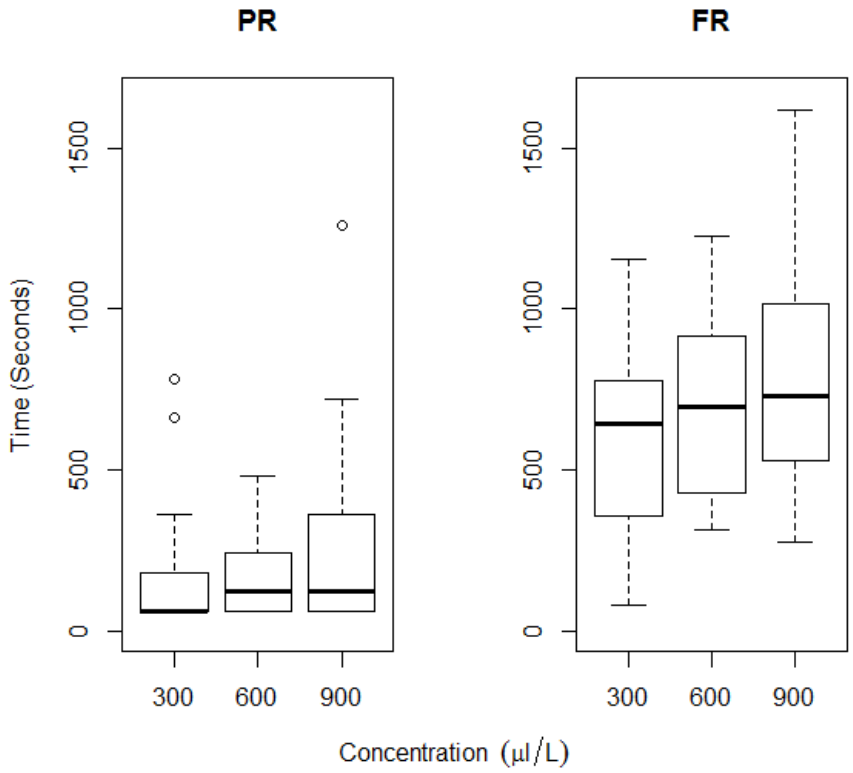

Figure 4.2. Recovery times between concentrations. PR= Partial Recovery; FR = Full Recovery. 300 n=27; 600 n=21; 900 n=27.

Table 4.4. Times of induction and recovery, standard deviation and the letters denote where significant differences are between concentrations (Kruskal Wallis, P<0.001).

Sedation Stage	Concentration µL/L	Median Time in seconds	Standard dev	Significant difference	H value / P value
Light Sedation (LS)	300	300	145	ab	H= 25.1545
	600	180	61	a	P= 3.45e-06
	900	120	72	b	
Heavy Sedation (HS)	300	600	261	ab	H= 28.4637
	600	300	143	ac	P= 6.595e-07
	900	240	97	bc	
Fully Anaesthetised (FA)	300	849	200	ab	H= 42.3249
	600	439	143	ac	P= 6.445e-10
	900	277	117	bc	
Partial Recovery (PR)	300	60	181	None	H= 3.599
	600	120	123	None	P= 0.1654
	900	120	268	None	
Full Recovery (FR)	300	641	298	None	H= 3.1293
	600	695	296	None	P= 0.2092
	900	730	364	None	

4.3.5. Egg Presence

The times to reach full anaesthesia and full recovery between the females that were carrying eggs (berried) and those that were not are shown in Figure 4.3.

Generally for all eugenol concentrations berried females took longer to reach full anaesthesia, but the least amount of time to recover fully when compared to non-berried females. Overall there was a significant difference of 133 ± 22 s to reach full anaesthesia (ANOVA, $F_{1,73}$=6.331, P<0.05) and a 328 ± 95 s difference to reach full recovery between berried and non-berried individuals (ANOVA, $F_{1,73}$= 20.43, P<0.001). Among different concentrations the 300 µL/L group showed a significant difference between berried and non-berried groups to reach full anaesthesia, and both the 300 and 900 µL/L groups for time to reach full recovery.

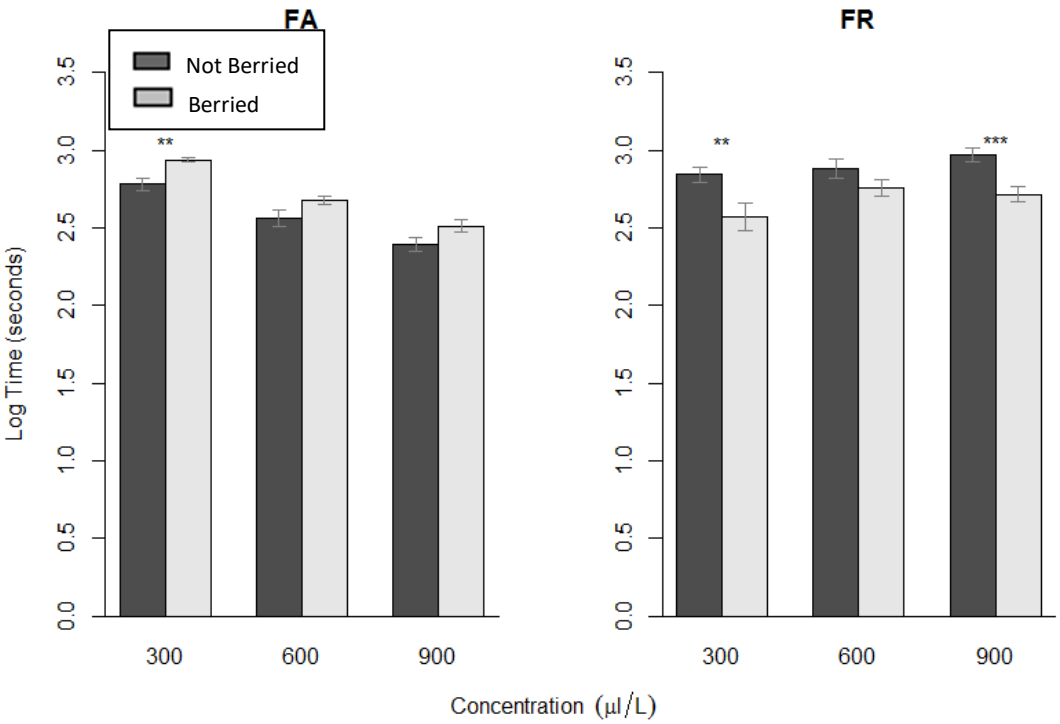

Figure 4.3. The time to reach full anaesthesia (FA) and Full Recovery (FR) between berried and non-berried females for each concentration. Error bars denote standard error. Asterisks (*) denote significant differences within discrete concentrations between berried and non-berried females (ANOVA, P≤0.05). Not berried 300 n=12; 600 n=11; 900 n=15. Berried 300 n=15; 600 n=10; and 900 n=19.

4.3.6. Captivity

Captive females were held in the aquaria facilities for over 40 days and were compared to freshly acquired females (held in the aquaria for less than 40 days). Qualitative assessment of each individual indicated that both fresh and captive females were vigorous, however fresh females had a stronger reaction to the touch stimulus than the captive held females. There was a significant difference in time to induce full anaesthesia between captive and fresh females (ANOVA, $F_{3,73}$=13.03, P<0.001) and full recovery (ANOVA, $F_{3,73}$=14.62, P<0.001). Overall fresh females took 191 ± 43 s longer to reach full anaesthesia and the least amount of time to fully recover (304 ± 122 s) compared to captive females as illustrated in Figure 4.4. It was observed that the average time remaining in lateral recumbency for captive females was significantly longer (558 ± 323 s) than the fresh individuals (186 ± 130 s) (Welch Two Sample *t*-test, t = 3.1345, df = 54, P = 0.002). To account for potential bias in the results from females with major damage (which can cause delayed turning times) the analysis was performed only on females that had no observed damage. Females that had gone through

ecdysis whilst in captivity took significantly longer to fully recovery than those that had not (Wilcox Test, P<0.05).

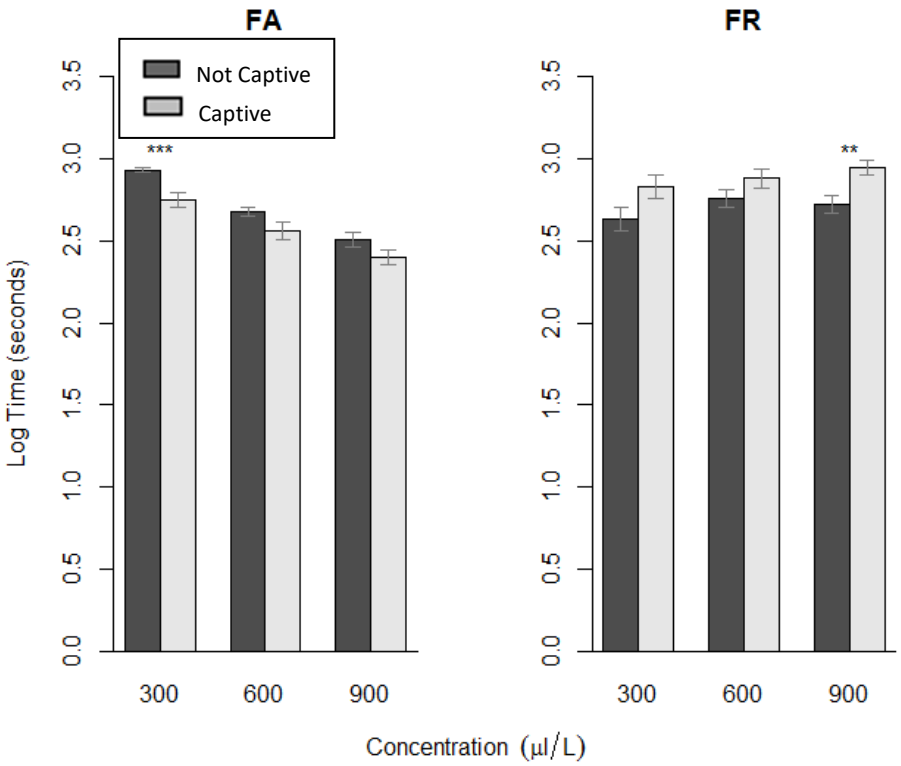

Figure 4.4. Time to reach full anaesthesia (FA) and full recovery (FR) between captive and freshly caught females. Error bars denote standard error. Asterisks (*) denote significant differences within discrete concentrations between captive and freshly caught females (ANOVA, P<0.05). Not captive 300 n=15; 600 n=10; 900 n=11; Captive 300 n=12; 600 n=11; 900 n=16.

4.4. Discussion

4.4.1. Times and Concentration Doses

Time to reach each successive sedation stage was dose dependent, similar to findings for other species (Coyle et al., 2005). The highest dose (900 µL/L) induced anaesthesia in the shortest time; whilst the 300 µL/L dose did not successfully induce full anaesthesia within 15 minutes for any of the individuals. Recovery for all concentrations followed a similar pattern; the higher concentration had the longest recovery time, although the total time to full recovery was similar for all three concentrations. There was a shorter time to partial recovery

for the 300 concentration group, possibly due to the large number of individuals that only reached heavy sedation and not full anaesthesia.

The established concentrations from this study can be used as anchor point for further evaluation and potential applications. For instance, lower concentrations that allow the individuals to be lightly-heavily sedated for longer periods of time could be considered for transportation (see appendix 4A, table 4.8 for pilot trial over a 24-hour period). A withdrawal period may need to be incorporated prior to sale to abolish tainting of the flesh, and guidance from the relevant authorities should be taken (Ross and Ross, 2008). Concentrations below that which induces anaesthesia within 30 minutes were used for *Litopenaeus vannamei* (Parodi et al., 2012) and equivalent experimentation with *Nephrops* should now be conducted with the results from this study *i.e.* below 300 µL/L.

Higher concentrations (*i.e.* 900 µL/L) could be used for short term sedation where full anaesthesia is required. Such applications could be during excessive handling, surgical techniques, and tag application (Ross and Ross, 2008; Saydmohammed and Pal, 2009).

Huntsberger (2012) found minimal damage within the adjacent muscle when injected with eugenol for anaesthesia but showed a reparative inflammatory response within one week. Therefore eugenol could potentially have a harmful effect when given in large quantities or multiple doses and more research needs to be conducted. If eugenol is to be used as an anaesthetic for transport of catches to market then injection of *Nephrops* individually is not feasible, whereas immersion in a eugenol solution, so that the sedative is absorbed through the gills, is a practical alternative. Since in most cases *Nephrops* that destined for human consumption, then depuration time may be needed to allow the accumulated eugenol to diffuse out of the flesh. Eugenol accumulation and clearance in *Macrobrachium rosenbergii* indicate purging for 24 hours completely removed residues from the tissues (Saydmohammed and Pal, 2009). Currently the US requires a 3-day withdrawal period for fish that have been sedated with eugenol before human consumption (Trushenski et al., 2013).

4.4.2. Behaviour

Both passive and stimulated behaviours differ between each sedation stage and therefore could be used as a guide to define each level of sedation. Overall more complex behaviours such as movement decreased, and less complex stationary behaviours increased with

increasing sedation stage (Stoner, 2012). Interestingly, aggressive behaviours were reduced in frequency and submissive behaviours increased. This may have potential use not only for transportation but also for the activities after arrival at the holding facilities. For example it could be used to lower the short term aggressive responses of the established inhabitants in a communal holding tank to the introduction of new individuals (Katoh et al., 2008), thus potentially minimising further stress and damage to them.

During the anaesthetised stage there was no response or movement, which would allow the individual to be easily manipulated and handled. This does not assume that the stress levels are reduced and therefore needs to be assessed in future studies. A reduction in handling stress, such as lower levels of glucose, glycogen and stress related enzymes was recorded with the use of eugenol as a sedative in other crustacean studies (Saydmohammed and Pal, 2009). The results do not establish whether the procedure itself is more 'stressful' than handling or transportation alone, and if stressed individuals will behave differently to "non-stressed" individuals, again this will need to be verified in further research. The common behaviour of pleopod beating during anaesthesia has been associated with high levels of hypoxia in *Nephrops* (Gerhardt and Baden, 1998) and increases irrigation over the respiratory surfaces and is increased whilst carrying eggs (Eriksson et al., 2012). Thus, it is unclear whether total neural inhibition has taken place and adds to the general uncertainty of using sedatives for analgesia within crustaceans.

4.4.3. Ovigerous Differences

The reasons for the differences in induction and recovery times between berried and non-berried females are not clear, but it may be due to the absorption of the eugenol by the tissues of the female or the lipid rich eggs. Huntsberger, 2012 identified that both *Carcinus maenas* and *Homarus americanus* had reacted differently at different times in the season to the same eugenol concentrations, and was assumed to be caused by differences in lipid content within the gonads caused by the physiological changes brought on by the breeding season. This implies that females not in the mating season (not berried) need less contact time with the anaesthetic than those that are berried. Further studies will examine whether the sedative at these concentrations will impact upon egg and larval development and subsequent post hatch survival, an important aspect if this is to be used for hatchery transport of broodstock. Several fin fish studies found that clove oil is toxic to egg development and

impacts upon survival if used at high concentrations (>1000 mg L^{-1}) (Usta et al., 2002; Wagner et al., 2002; Hoskonen et al., 2013). Since only females were used in this investigation, any differences in the effects of eugenol on males still needs to be determined.

4.4.4. Captivity Impacts

Captive females took a shorter time to reach full anaesthesia and an increased time to fully recover. Although captive individuals displayed behaviours that were associated with being fully recovered, *i.e.* response to stimuli and full range of appendage movement, captive individuals remained in lateral recumbency for longer periods than fresh females, which would either turn or tail flip to return to a normal position. The affect of captivity has been demonstrated in a range of crustaceans and it has been known to effect the activity levels, exercise performance, reduced muscle fibre, reproduction efficiency, energetics, metabolism and immunology (Houlihan and Mathers, 1985; Marsden et al., 2007; Taylor et al., 2009; Gear et al., 2010). This study now provides an example of how individuals held in captivity for long periods also alter their behaviour

In conclusion, using a suite of behaviours and states it has been found that eugenol is effective in anaesthetising *Nephrops*. Moreover, it is suggested that behavioural analysis can be used to assess the sedation stage. However other variables such as the stage in breeding cycle and length of time in captivity also affect sedation times and therefore dosages may have to be adapted accordingly.

4.5. Acknowledgements

I am grateful to Jessica Haigh, Jimi Crane, Abigail Weston, Nicola Dobson, Barry Penrose, Dennis Gowland, David McKenzie and Dawson Shearer for their support and guidance. Paul Virtue and colleagues at D.R. Collin and Son Ltd for their continued supply of quality *Nephrops*. This study has been in conjunction with the ongoing research conducted under the *Nephrops* project which has received funding from the European Community's Seventh Framework Programme under Grant Agreement No: FP7-SME-2011-286903.

Appendix 4A. Supplementary Data

Table 4.5. Ethogram used to describe the passive and stimulated behaviours observed, and how they were grouped.

	Key	Description	Detailed Description
Movement	W	Walking	Use of the walking legs (pereopods) to propel the individual in any direction whilst the underside of the body is not touching the floor.
	W-PL	Walking and pleopod beating	The behaviour of walking with pleopods beating.
	TUF	Climbing	Anterior is raised up against the tank side with the use of the walking legs with chelipeds raised above. May include pleopod beating also.
	T	Turning	The individual rights itself from lateral/dorsal recumbence.
Stationary	S	Sitting	When the coxa is touching the bottom of the tank floor and legs remain motionless.
	ST	Standing	Body held high supported by the pereopods with abdomen raised off the ground.
	A	Antennule/ Antennae Movement	Movement being either flicking or waving of either the antennae and/or antennules.
	AB*	Antennae Backwards	Antennae flick backwards and remain pointing backwards for more than 2 seconds.
	M	Mandible Movement	Mouthpart activity. Movement associated with the mouthparts except for 'eating' and 'cleaning' behaviours.
	CL	Cleaning	Use of mouthparts combing or rubbing another appendage (usually the antennae or chelae). Or using pereopods to clean the eyes, or dorsal side of the individual.
	CL-PL	Cleaning Pleopods	Use of pereopods reaching towards the pleopod region and agitation of the egg sack or pleopods.
	E	Eating	Repeated pereopod and chelae movement from the side or upwards motion towards the mouthparts and mouthpart movement.
	PL	Pleopod Beating	Pleopod activity typically beating, stroking or waving.
	D	Digging	1st and 2nd pereopod movement pushing the bottom of the tank to the side motion and/or the chelae opening and closing.
Aggressive	C	Chelae Display	One or both chelae horizontally spread wide and/or raised.
	G	Grab	Chelae hold onto airstone or pipette.
	2*	Attack Response	Rapid movement of the chelae towards the stimulant.
	2D*	Attack Response Delayed	Movement of the chelae towards the stimulant in a slower motion or in a delayed response.

	TU	Tail Up	Posterior (tail) is raised or pressed against the corner of the tank.
	UP OWN	Lateral / Dorsal Recumbency	The individual rotates to be in lateral / dorsal recumbency by their own movement and not from the experimenters' direct influence.
	1*	Escape Response	Rapid movement in the opposite direction of the stimulant by walking. Typically walking backwards.
	1D*	Escape Response Delayed	Delayed movement in the opposite direction of the stimulant by walking. Typically walking backwards.
	TF*	Tail Flipping	Rapid movement of tail to propel the individual backwards.
	TFD*	Tail Flipping Delayed	Tail Flipping response but delayed from time of direct stimulation.
Submissive	LM	Leg Movement	Pereopod movement but not walking.
	CM*	Chelae Movement	Slight chelae movement.
	AD*	Antennule Movement	Slight movement of either the antennae and/or antennules but in a slower motion not flicking or waving.
Sedated Movement	TUCK/ CURL	Tail Tuck	Tail is curled under and the telson in underneath the body.
	PUSH	Push Up	In the sitting position the use of the chelae are used to lift the anterior off the tank bottom. The telson remains on the bottom.
	ARCH	Arching	Whilst in dorsal recumbency the individual uses the chelae to push off from the bottom of the tank causing the abdomen to lift off the bottom of the tank. The abdomen is flexed in an arch shape.
	PL-ST*	Pleopod Beating Stops	Pleopod beating ceases simultaneously as direct stimulation is given.
Other	0	No Observed Response	No observed response to stimulation.
	UP	Lateral Recumbency	The individual is manually placed upside down (ventral side is facing upwards) or rotated on the side.

Table 4.6. Differences from pre and post samples of several water parameters for each sedative concentration.

Concentration (µL L^{-1})	Ammonia (mg L^{-1})	Dissolved Oxygen (%)	Temperature (°C)	pH
0 (Control)	+0.007 (n=4)	-2.06 (n=30)	+0.4(n=30)	+0.01(n=30)
300	+0.132 (n=5)	-2.61(n=26)	+0.6(n=27)	-0.04(n=27)
600	+0.066(n=5)	-1.34(n=15)	+0.4(n=21)	-0.02(n=15)
900	+0.073(n=4)	-0.54(n=22)	+0.4(n=28)	-0.04(n=28)

Table 4.7. Indexed scale for behavioural assessment of each sedation stage with median time (seconds).

Stage	Movement	Response	Time (seconds)		
			300 µL/L	600 µL/L	900 µL/L
Normal	5	5	N/A	N/A	N/A
Light Sedation	4	4	300	180	120
Heavy Sedation	3 / 2	3 / 2	600	300	240
Full Anaesthesia	1	1	849	439	277
Partial Recovery	1 - 3	2 – 4	60	120	120
Full Recovery	4 / 5	5	641	695	730

KEY

Movement	Response
5 - Walking	5 – Tail flipping
4 - Standing	4 – Delayed response
3 – Leg movement	3 – Slight movement of Chelae or legs
2 – Antennae movement	2 – Antennae movement
1 - Sitting	1 – No response

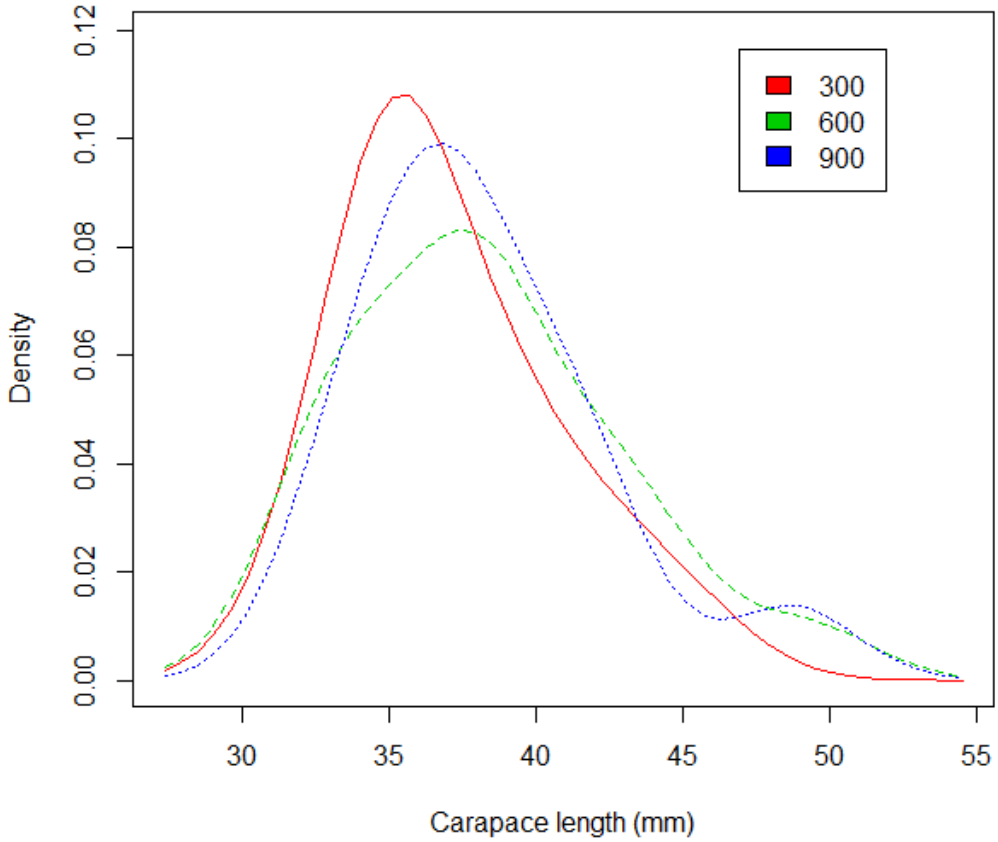

Figure 4.5. Kernal density graph to indicate carapace length (mm) density distribution between the three concentrations.

Table 4.8. Pilot scale investigation on using lower concentrations of eugenol over a longer treatment period (24 hours).

Concentration ($\mu L\ L^{-1}$)	CL (mm)	Time (seconds) to reach sedation stage			
		Light Sedation	Heavy Sedation	Full Anaesthesia	Full Recovery
0 (n=1)	30.3	N/A	N/A	N/A	1
18 (n=1)	42.1	10800 (3 hr)	43200 (12 hr)	N/A	1
40 (n=1)	49.2	900 (15 min)	N/A	N/A	5
60 (n=1)	49.7	900 (15 min)	86400 (24 hr)	N/A	2
80 (n=1)	36.7	900 (15 min)	21600 (6 hr)	N/A	213

5. Primary Investigations of the Effects of Feed Types and Regimes on the Growth and Survival of *Nephrops norvegicus* Larvae

Abstract

Nephrops norvegicus (Norway Lobster) larvae have proven challenging to culture through to the postlarval stage, with research efforts typically resulting in poor survival. Optimisation of feeding techniques may assist with the development of successful larval rearing and hatchery strategies for the species. In this study, three diets consisting of enriched *Artemia*, enriched *Artemia* with added probiotics and wild zooplankton were tested to investigate any differences in growth, development time and survival for Zoea 1 to Zoea 3 larvae. In addition, two feeding frequencies were analysed: batch (i.e. once per day) and continuous feeding. The results indicate that there were no significant differences in survival between diets, but the time to develop to zoeal stage 3 was less and growth was significantly higher in the enriched *Artemia* with probiotic treatment than the wild zooplankton diet. The enriched *Artemia* and enriched *Artemia* with probiotic treatments showed comparable results. Finally, continuous feeding resulted in significantly higher survival (*ca.* 8%) and significantly faster development (*ca.* 4 days) than batch feeding. Overall the larval development time was significantly reduced and survival rate was increased compared to the results of previous larviculture studies, which could be a promising development for research and future aquaculture developments.

Abbreviations:

EA, enriched *Artemia*; PA, enriched *Artemia* with probiotic; WP, wild caught zooplankton; BA, batch feeding frequency; CO, continuous feeding frequency; Z1, Zoea stage 1, Z2, Zoea stage 2; Z3, Zoea stage 3; PL, Post larvae; EFA, essential fatty acid; PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; HUFA, highly unsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid; LNA, linolenic acid; LOA, linoleic acid.

5.1. Introduction

Nephrops norvegicus (Norway Lobster, Langoustine, Dublin Bay Prawn or Scampi, referred to as *Nephrops* from here on) are a highly valued seafood and an important fishery particularly within the UK (value *ca.* £80 million), Ireland (value €33 million) and Scandinavian countries (Ungfors et al., 2013; Marine Institute, 2014; Marine Management Organsation, 2014).

Whilst initial research into the culturing of *Nephrops* started in the early 1970's, *Nephrops* can still be considered an emerging candidate aquaculture species with many areas of development required to increase survival and growth during the zoeal stages (Cowing et al., 2015). Powell and Eriksson, (2013) provide a review of the larviculture techniques used for this species to date. However, low survival rates, and lower economic yield have reduced the development of larviculture of this species. In comparison, the larviculture techniques for *Homarus* spp are now technically advanced with ongoing remediation work, driven by the principle aim of stock enhancement (Nicosia and Lavalli, 1999). Therefore, there is a need for improving the survival from current estimates which would be interesting for use in academia, fisheries and aquaculture sectors.

Nephrops larvae have three planktonic zoeal larval stages (referred to as Z1; Z2; Z3) with the fourth considered to be the first post larval (PL) stage following metamorphosis (Jorgensen, 1923). Survival from experimental studies has shown this species to be difficult to culture with high mortalities between each successive larval stage (Figueiredo and Vilela, 1972; Hills, 1975; Smith, 1987; Thompson and Ayers, 1989; Eriksson and Baden, 1997; Dickey-Collas et al., 2000; Rotllant et al., 2001). It is critical for the success and development of *Nephrops* larviculture that adequate feeding protocols are developed that increase survival, growth rates and larval resilience to abiotic stress (Rotllant et al., 2001; Wood et al., 2015). To achieve this several diets and feed schedules must be initially trialled and to determine their nutritional values and their suitability for potential use in aquaculture. .

Previous studies have used *Artemia* spp. (referred to as *Artemia*) as the major feed, in conjunction with other feeds such as shrimp, *Crangon crangon* eggs, *Mytilus edulis*, *Alloteuthis* sp. various microalgae species, *Fucus spiralis*, commercial microencapsulated feeds and shrimp feed (Figueiredo and Vilela, 1972; Hills, 1975; Thompson and Ayers, 1989; Smith, 1987; Eriksson and Baden, 1997; Dickey-Collas et al., 2000; Rotllant et al., 2001; Powell

and Eriksson, 2013). *Artemia* is favoured as a food source because the eggs can be stored dormant for long periods of time, they are quick to culture and offer benefits that only live food can offer for larviculture (Léger et al., 1986). Early feeding of *Nephrops* larvae and other marine species has shown that *Artemia* alone as a feed may not be adequate for survival and growth, and could be improved if the correct amount of essential nutrients are met for each growth stage (Rotllant et al., 2001; Pochelon et al., 2009; Palma et al., 2014). It is known that the nutritional quality of *Artemia* for growth of marine larvae can be limiting due to their low essential fatty acid content (Léger et al., 1986). Several aquatic species (*Homarus americanus*, *Hippoglossus hippoglossus* and *Hippocampus guttulatus*) fed on *Artemia* alone have been shown to experience deformities and malnourishment, which further affect growth and survival into later stages of development (Hamre et al., 2002; Fiore and Tlusty, 2005; Palma et al., 2014). To improve the nutritional composition of the feed, the *Artemia* can be enriched to improve the lipid, phospholipid, protein and vitamin composition, which has been shown to improve the survival in several cultured crustaceans (Léger et al., 1986; Nelson et al., 2002; Dhont and Van Stappen, 2003; Chakraborty et al., 2010).

In addition, probiotics are also believed to confer health benefits to the host. Probiotics are live microbial bacteria that can be added directly to the culture water, to the dry feed or incorporated into the tissues of the live prey (Dagá et al., 2012). Probiotics enhance the host's immune and digestive systems when ingested (Liu et al., 2009; Dagá et al., 2012). This enhancement can impact upon the nutritional reserves and immune suppression, therefore potentially increasing tolerance and survival during the acute stress that they may be subject to in culture vessels (Rollo et al., 2006; Avella et al., 2010; Daniels et al., 2013). Probiotics in feed supplements have received extensive attention within the last decade with regard to improving the survival, growth and immunity of several crustaceans, including lobsters, crabs, penaeid shrimp and prawns (Daniels et al., 2010, 2013; Hai et al., 2009; Liu et al., 2009; Zhou et al., 2009; Zhang et al., 2011; De Souza et al., 2012; Middlemiss et al., 2015). *Bacillus* spp. are found in the marine environment and are spore forming bacteria that can be easily produced and stored as a probiotic, and have been used in several crustacean studies with promising results regarding survival and growth (Wang, 2007; Liu et al., 2009; Daniels et al., 2010, 2013; Zhang et al., 2011 De Souza et al., 2012; Middlemiss et al., 2015).

Several studies have compared the effectiveness of feeding marine larvae with traditional aquaculture diets (*i.e. Artemia*) with an alternative such as wild zooplankton (Gendron et al., 2013; Hamre et al., 2002; Katan et al., 2016; Naess et al., 1995; Rocha et al., 2017). Wild zooplankton constitutes the base of the marine food chain, linking primary producers to carnivorous larvae. Offering cultured larvae prey in the form of wild zooplankton may optimize the composition of macronutrients, amino and fatty acids, vitamins and minerals available and therefore increase growth and survival in each stage of development (Hamre et al., 2002). However, this may be offset by the fact that collecting fresh zooplankton can be labour and cost intensive. Many marine fish have shown improved survival, growth and aesthetics when on a diet of either wild caught or cultured copepods (Støttrup, 2003). Only one study has used 'wild plankton' (in conjunction with *Artemia)* as a feed for *Nephrops* larvae, and this resulted in ~5% survival to Z3 (Thompson and Ayers, 1989). Gendron et al. (2013) indicates although the growth rate and condition index of *H. americanus* were improved compared to the *Artemia* diet, survival was not. This study therefore aims to provide fundamental knowledge of the appropriateness of using this diet for *Nephrops* larviculture.

It is not only the quality of the feed provided but also its quantity that can influence the development of cultured species. The feed density currently used by commercial *Homarus gammarus* hatcheries is high (5-25 ml^{-1}) and sometimes provided two or three times per day (Arnold et al., 2009; Browne et al., 2009; Burton, 2003). The shrimp *Penaeus vannamei* has shown significant improvements in growth when the feeding frequency is two and four times per 24 hours (Robertson et al., 1993). In contrast *Nephrops* are typically fed once per day and at a much lower feed density (3-5 ml^{-1}) (Powell and Eriksson, 2013). Pochelon *et al*. (2009) found that larvae increased the consumption of feed with the density of feed available, and suggested that the larvae were never fed to satiation even at a feed density of 5 ml^{-1}. *Nephrops* larvae may therefore benefit from an increased feeding frequency of more than once per day.

The objectives of this study are to compare diets that are being used in commercial operations (such as *Artemia*), and also compare these with wild zooplankton. In addition, the effects of different feeding frequencies on the survival, growth, and development time of *Nephrops* larvae will also be investigated. The results are discussed along with nutritional information

(provided by a review) and the possible implications this may have upon further nutritional research and the development and aquaculture of this species are considered.

5.2. Methods

5.2.1. Animals and Larval Production

Ovigerous females were creel-caught between March and May 2014 in the Gullmarsfjord, Sweden, and brought into the Sven Lovén Centre for Marine Sciences culture facility (Kristineberg), where they were housed in separate compartments and fed *Pandalus borealis* once per week. The hatching of larvae occurred over three days in June and came from a total of 18 females. Zoea Stage 1 (Z1) larvae (Jorgensen, 1923) were collected shortly after hatching using plastic beakers and randomly distributed between ten 10 litre cylindro-conical culture tanks (50 larvae per culture tank) so that each culture tank comprised of mixed batch from at least 3 different females. The experimental tanks were maintained at a temperature of 15.0 ± 1.0^{o}C, salinity of 33 ± 0.9 ‰ and set to a 16:8 (light:dark) cycle with a water flow through system, taken from a depth of around 32 m in the Gullmarsfjord. The flow rate was initially set at 1 litre min^{-1} and increased to 2 litre min^{-1} during later developmental stages as pelagic larvae became increasingly benthic in habit. Tanks were cleaned every three days (after gently transferring larvae for a short period of time into a new hopper).

5.2.2. Experimental Design

5.2.2.1. Measured Parameters

Larvae were removed from the culture tank every three days to assess survival, developmental stage and growth. Carapace length (CL, to an accuracy of 0.01 mm) of the larvae was assessed on days 9, 12 and 19 from randomly selected larvae (n=6) by gently removing the larvae using a water jug and then placing the larvae into a culture cell (Costar 3516, Corning Incorporated, USA). The larvae were photographed using a Dino-Eye Microscope Eyepiece camera (AM4023B, Dino-Lite, Taiwan) in a stereomicroscope (Leica MZ95, Leica Microsystems Ltd, Switzerland 0.63 x 1.0x magnification) and the carapace length measured from the posterior margin of the orbit to the posterior dorsal carapace margin (Farmer, 1975; Daniels et al., 2010).

Survival (%) and the mortality rate (%) were calculated using the following formulae;

$$Survival\ (\%) = 100 \times (Lt_x \div Lt_0)$$

$$Mortality\ rate\ (\%) = 100 \times (Lt_x - Lt_{x+1}) \div Lt_x \div (t_{x+1} - t_x)$$

Where; *L* = number of larvae, t = time (days).

Developmental data indicate relative development stage (% of the surviving larvae at each zoeal stage).

On day 19 all the remaining larvae were counted, staged and the carapace length measured. They were subsequently blot dried and frozen (-80°C), freeze dried and then weighed (0.00001 g).

Mortality was also compared by computing the day at which 50% mortality was reached.

5.2.3. Experiment 1 (Diets)

Three diet treatments were provided, enriched *Artemia* (EA), enriched *Artemia* with added probiotic (PA) and wild-caught zooplankton (WP). Each diet treatment had 3 replicates with 50 larvae in each. The diet treatments were evaluated against survival, growth and development time to zoea stage 3 (Z3). The density of feed for both the *Artemia* and WP diets was 5 (± 2) individuals per ml which were retained in the tank by the use of smaller filter sizes (100 µm). After seven hours of feeding the filters were changed to ones with larger sized mesh (300 µm), and the feed flowed out from the tank until the next days feed.

5.2.3.1. Artemia Diets

Artemia cysts (Great Salt Lake Premium *Artemia* Cysts, Sanders Inc., USA) were placed into 15 L containers under high light intensity and left to hatch over 24 hours at 28°C. The *Artemia* were then harvested, washed and then placed into a 5L flask for a further 18 hours with a liquid enrichment product that contains proteins, lipids, minerals and vitamins (SELCO® S.presso, INVE Aquaculture, Belgium). The *Artemia* were then harvested once again, rinsed and a sub sample taken for calculation of the concentration needed.

For the enriched *Artemia* with probiotic diet (PA), the *Artemia* were additionally enriched by adding probiotic powder (Sanolife MIC-F - INVE Aquaculture, Belgium) at 50 g per 100 L for 3 hours prior to being harvested. This ensured that both *Artemia* treatments were enriched for the same duration of time. The *Artemia* were subsequently washed and then fed to the

Nephrops larvae immediately and at the same time as the other diets. For all treatments the feed was sub-divided in advance so that the correct concentration was delivered for each tank.

5.2.3.2. Wild Zooplankton Diet

Wild zooplankton (WP) were collected every third day (May – June, 2014) in the Gullmarsfjord using a WP-2 zooplankton tow with a 125 µm mesh pulled at approximately 3 knots for 5 minutes at a depth of around 20 m. The zooplankton were collected and passed through a 500 µm filter to remove larger zooplankton that may predate on the *Nephrops* larvae. The remaining zooplankton were passed through a 200 µm filter to capture the micro and meso-zooplankton that are of similar size to the *Artemia* (Sieburth et al., 1978) complementary to the methods used in Gendron et al. (2013). The zooplankton were rinsed and placed into a 5 L plastic container with aeration and fed with algae (Rotifer Diet®, Reed Mariculture Inc., CA, USA) every other day. The WP were stored at 3°C in darkness for no more than 48 hours to minimise metabolism and retain their nutritional profile (Harris et al., 2000). Replenishment with freshly caught zooplankton occurred every third day. A sub-sample was taken to determine the concentration of zooplankton. The final concentration of wild zooplankton used as feed for the larvae was the same as the concentration to be used for the *Artemia* feeds, so that the prey density was the same in all three feed treatments. The feed was poured into the tanks carefully so as not to disturb the larvae.

The biovolume (V mm^3) was derived by measuring the total length and width of each species present for each treatment and applying the following formulae according to the species or shape (Mauchline, 1998; Binggeli et al., 2011);

Ellipsoid $$V = \frac{\pi}{6(lw^2)}$$

Cylindrical $$V = \pi r^2 l$$

Spherical $$V = \frac{4}{3}\pi r^3$$

Arcatia clause and Oithona similis $$\log V = 3.1641 \log l - 10.690$$

Cladocera $$V = 4\pi l w^2$$

The volume was then multiplied by the average abundance of that species within each treatment. Coefficients given by Svetlichny (1983) were used to calculate the wet weight (*WW*) along with the dry weight (*DW*) (Cushing et al., 1958) and carbon content (*CC*) (Ara, 2001) using the following formulae;

$$WW = Klw^2$$

$$DW = 0.20WW$$

$$CC = 0.45DW$$

Where *V* = biovolume; *r* = radius; *l* = total length: *w*= width; *WW* is the wet weight, and *K* is a coefficient for each species. If the species wasn't present, then a species of similar size and shape was used instead.

5.2.4. Experiment 2 (Feeding Frequencies)

The two feeding frequencies that were investigated were batch feeding (BA), where feed was delivered once per day, as is typical aquaculture practice for *Nephrops* and *H. gammarus*, and continuous feeding (CO) where feed was delivered several times throughout the day. Each feeding treatment had 5 replicate tanks with an initial stocking density of 50 larvae in each tank. Both treatments used *Artemia* prepared as in experiment 1 for the EA treatment, using freshly hatched *Artemia* nauplii enriched in SELCO.

Within the BA treatment, *Artemia* were delivered to the tanks at approximately 15:00 h and 100 µm filters were placed over the outflow to stop any feed flowing out from the tank. After approximately seven hours of feeding the filters were changed to 500 µm to ensure the *Artemia* flowed from the tank and feeding of the larvae was stopped. The initial feed density provided was at 6 *Artemia*/ml.

Within the CO treatment fresh *Artemia* (stored on ice) were delivered using a peristaltic pump (Watson Marlow 504S, Stockholm, Sweden) with an array of no more than four extension pump heads (3 roller, 1.6mm WT tube). The feeding commenced at the hours of 06:00, 12:00, 18:00, 00:00 h. A 300 µm mesh filter remained over the filter to allow a steady flow of live feed to go from the culture tank. This ensured that the concentration of *Artemia* was the same within both treatments when each feed was initiated. The density of feed within the CO

treatment would steadily drop over the 5-6 hours to nothing before the next pulse of feed was delivered (residency time of the *Artemia* was 5-6 hours). The flow rate was set to pump at 540ml/min. This flow rate equated provided a density of *Artemia* within the tank of 6/ml for the start of each pulse. This feed density has been previously proven as an adequate density of *Artemia* prey for *Nephrops* larvae development (summarised by Powell and Eriksson, 2013).

5.2.5. Statistical Analysis

All statistical data and graphical outputs were produced with Excel and the R statistics programme (R Core Team 3.1.2, 2015). All data were tested for normality (Shapiro test) and equal variances (Levene's test). If the data were not normally distributed and transformed data did not meet the assumptions of the tests, analysis was carried out using a non-parametric test.

In experiment 1, survival (%), mortality rate, moult stage and dry weight (mg) data were analysed using the Kruskal-Wallis test. Where significant differences did occur ($P<0.05$) then data were further analysed with a post hoc test (Dunn's test). Length (CL) data were analysed using a one-way ANOVA test.

In experiment 2, survival, moult stage, length and weight data were analysed by using a T-test. Daily mortality rate was analysed using a Mann-Whitney U test. Weight data were square root transformed prior to statistical analysis to conform to normal distribution.

5.3. Results

5.3.1. Survival

5.3.1.1. Experiment 1

There was no significant difference found in total survival (%) among diet treatments (Figure 5.1A) (Kruskal-Wallis, chi squared=3.47, df=3, $P>0.05$). At day 19, larvae fed on the PA and EA treatments showed the highest survival (26% ± 3; 25% ± 14 respectively) compared to the WP (17% ± 6) treatment (Figure 5.1A). The WP fed larvae had a higher mortality rate within the first 6 days (36% ± 16) compared to larvae in the EA (17% ± 5) and PA (12% ± 3) treatments. After day 8 the mortality rate for WP fed larvae was similar to the other treatments (Figure 5.1C).

By day 16 all diet treatments had a survival of less than 50% (Figure 5.1A). Variance of the replicates for the different diets was lower in the WP diet and is shown in Appendix 5A.

5.3.1.2. Experiment 2

There was no significant difference in the survival at the end of the experiment between the two feeding frequencies (t-test, t=-0.90, df=57.781, P>0.05). There was however a significant difference in survival between the two treatments between days 9 and 12 (t-test, t=-2.37, df=7.09, P<0.05). CO fed larvae had higher percentage survival than BA fed larvae on day 19, with 36.0% ± 3.2 and 28.4 % ± 4.2 respectively (Figure 5.1B).

The mortality rate of larvae was highest between days 15 and 19 at 21.3% and 21.2% within the CO and BA treatments respectively (Figure 5.1). The mortality rate was significantly higher for larvae within the BA treatment (10.3% ± 2.2) on day 12 than CO fed larvae (3.6% ± 0.8; Mann-Whitney U Test, W=22, P<0.05). By day 17, 50% total mortality occurred within the BA (day 15 ± 1) and the CO treatment (day 17 ± 2; Figure 5.1B). However, there was no clear trend differences between the two treatments.

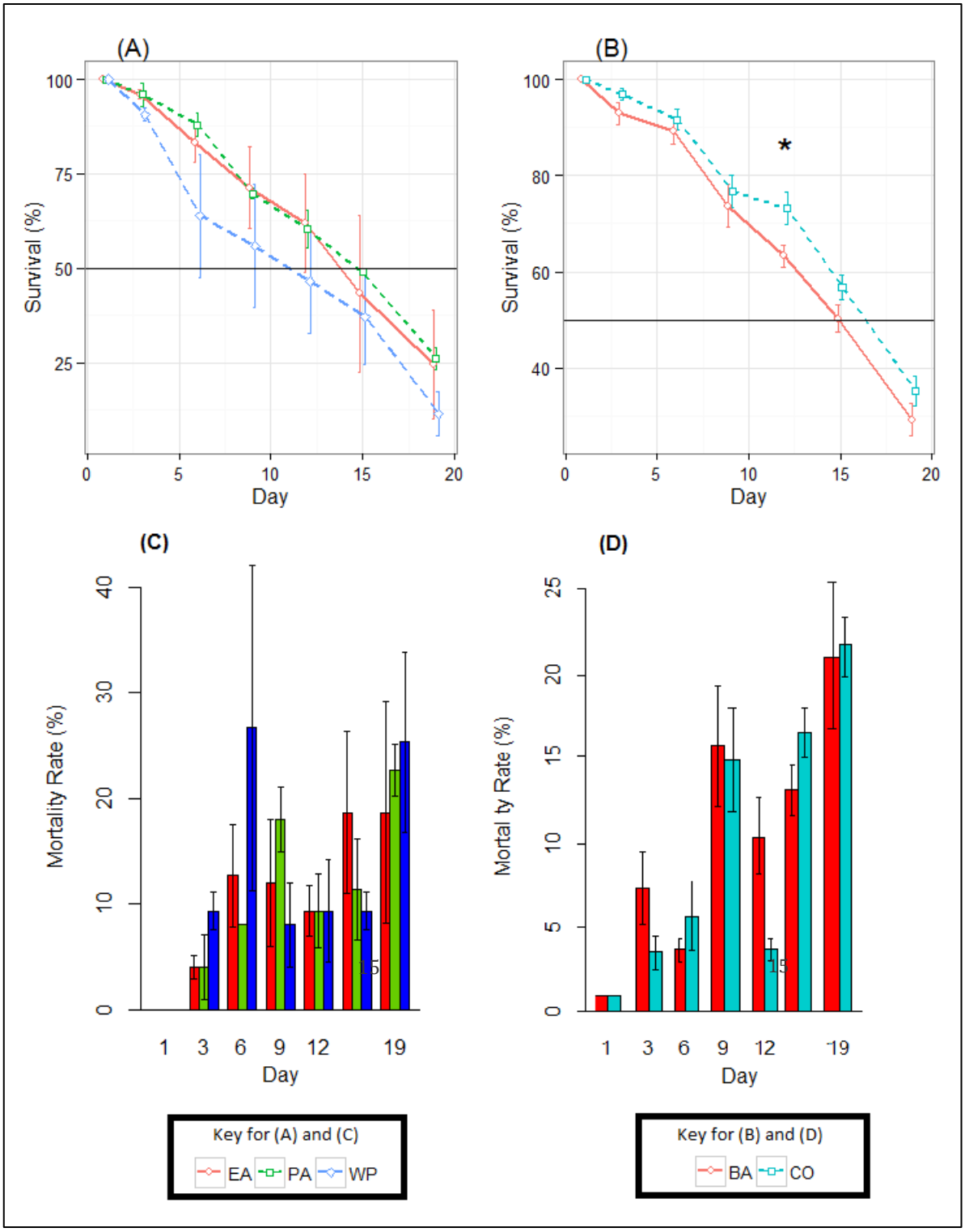

Figure 5.1. (A & B) Survival (%) and (C & D) mortality rate (%) between subsequent sampling days of *Nephrops* larvae fed on different types of feed (A & C) and different feed frequencies (B & D) over 19 days (mean ± SEM). The solid black line indicates 50% total mortality (A & B). EA; Red=Enriched *Artemia* (n = 3); PA; Green = Enriched *Artemia* with probiotic (n = 3); WP; Blue= Wild zooplankton (n = 3); BA; Red= Batch feeding frequency (n = 5); CO; Cyan= Continuous feeding frequency (n = 5). * indicates where significant differences lie between the treatments for each discreet day (T-test, P<0.05).

5.3.2. Zoeal Stages (Development)

5.3.2.1. Experiment 1

All treatments successfully yielded zoea stage 3 (Z3) larvae before day 19. There were significantly more zoea stage 2 (Z2) larvae in the EA and PA treatments compared to the WP treatment by day 9 (Kruskal-Wallis, chi squared=12.66, df=2, $P<0.05$). The majority of larvae had reached Z2 by day 12 except for 2% within the WP treatment (Figure 5.2).

By day 15 the PA treatment yielded a higher percentage of Z3 larvae (58% ± 3) which was significantly higher than both the EA and WP fed larvae at 22% ± 11 and 6% ± 4 respectively (Kruskal-Wallis chi-squared=12.33, df=2, $P<0.05$). This remained the case until day 19 with 98% ± 2 of the PA fed larvae at Z3 which was significantly higher than the EA (82% ± 15) and WP (88% ± 1) fed larvae (Kruskal-Wallis chi-squared=6.39, df=2, $P<0.05$).

The average time taken for the larvae fed PA to reach Z2 and Z3 was 11.6 ± 0.4 and 19.9 ± 1.0 days respectively. This was significantly shorter than the WP fed larvae (15.1 ± 2.1 and 22.2 ± 0.1; Kruskal-Wallis chi-squared=11.52, df=2, $P<0.05$) but not the EA fed larvae ($P>0.05$; Table 5.1).

5.3.2.2. Experiment 2

Both feeding frequencies produced Z3 larvae (Figure 5.2). There were significantly more Z2 larvae by day 6 within the CO treatment (61%) compared to BA feed larvae (18%) with the rest of the larvae remaining at Z1 (t-test, t=-3.00, df=7.98, P=0.017). Similarly, there were significantly more Z3 larvae by day 15 in the CO treatment (62%) compared to BA treatment (25%) (t=5.36, P=0.009). There was no significant difference in the number of Z3 larvae by day 19 (t-test, $P>0.05$).

The average time taken to reach Z2 and Z3 was significantly shorter for the CO treatment (day 9.15 ± 0.70 and 19.32 ± 0.70) than the BA treatment (day 12.19 ± 1.60 and 22.68 ± 2.36 respectively; Table 5.1). The intermoult duration for each zoeal stage was less for the CO treatment than the BA treatment.

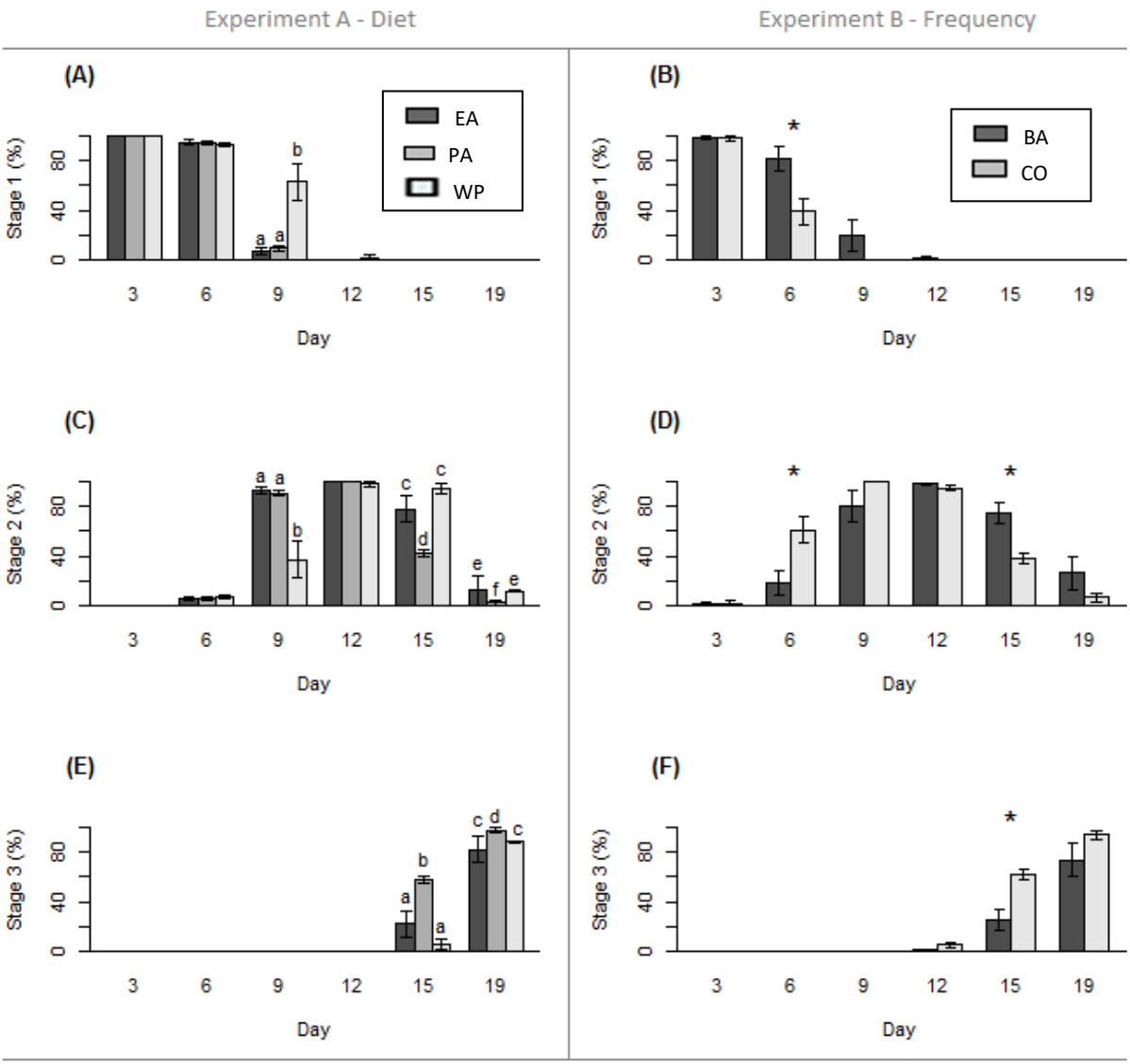

Figure 5.2. The proportion of larvae at each developmental stage (Z1-Z3) between different diets (A, C, E) and feeding frequencies (B, D, F). Superscript letters indicate where significant differences are between the diet treatments within discreet sampling days (Kruskal Wallis P<0.05). Asterisk denotes where a significant difference lie between the two feeding frequencies (t-test P<0.05). EA=enriched *Artemia* (n = 3); PA = Enriched *Artemia* with probiotic (n = 3); WP = Wild zooplankton (n = 3); BA= Batch feeding frequency (n = 5); CO = Continuous feeding frequency (n = 5). N.B. that data was arcsine transformed for analysis for experiment 2 but % data is shown.

5.3.3. Growth

5.3.3.1. Experiment 1

Larvae fed the PA diet had the largest CL by day 19 (3.11 mm ± 0.12), which was significantly larger than larvae from the WP diet (2.70 mm ± 0.25) but not compared to EA diet (2.94 mm

± 0.26) (ANOVA, P<0.05; Figure 5.3). The larvae, on day 9, 15 and 19 were significantly smaller in the WP diet treatment (2.07 mm ± 0.25; 2.47 mm ± 0.36) than the EA (2.52 mm ± 0.02; 2.86 mm ± 0.26) and PA diet treatments (2.81 mm ± 0.02; 2.92 mm ± 0.12; ANOVA, P<0.05), but had the highest total CL growth (mm) by day 19 of 0.63 mm ± 0.27 (Table 5.1).

There was a significant difference in final dry weight (mg) between the three feed treatments (Kruskal Wallis, chi-squared=6.98, df=2, P=0.03). Larvae fed EA weighed significantly more (4.03 x 10^{-3}mg) than larvae fed on WP (3.14 x 10^{-3}mg) (Figure 5.3).

The biovolume, wet weight and carbon content of the feed was higher in the WP when compared to the *Artemia* treatments (see Appendix 5D).

5.3.3.2. Experiment 2

By day 19 the CL of the larvae in the CO treatment (3.01 mm ± 0.09) was not significantly different to the BA treatment (2.82mm ± 0.17; t-test, P>0.05). The only significant difference in size between the two treatments was on day 15 where the CO larvae (2.86 mm ± 0.14) were significantly larger than the BA larvae (2.59mm ± 0.19) (t=-2.48, P<0.05; Figure 5.3). The increase in CL was largest within the CO treatment at a total of 0.66 mm ± 0.08, with a large amount of growth between day 9 and 15 (0.50 ± 0.11).

There was no significant difference in final weight between the two feeding frequencies (t=-, df= 63.71, P=0.32) (Figure 5.3).

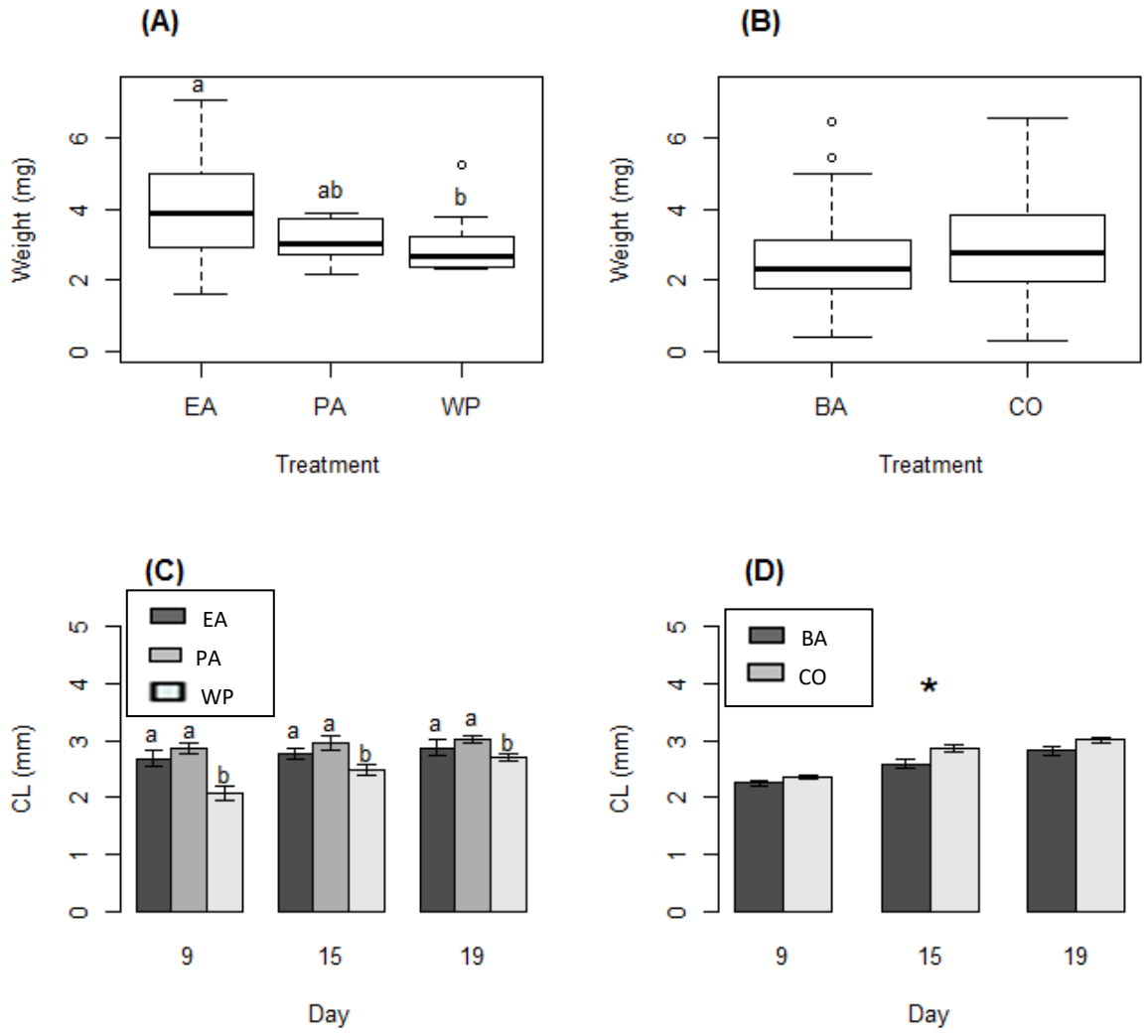

Figure 5.3. Final dry weight (mg) of *Nephrops* larvae from experiment 1 (A) and experiment 2 (B). Superscript letters denote where significant differences lie between A: treatments (Kruskal Wallis; P<0.05), C: treatments within discreet day groups (C = ANOVA, P<0.05). Mean carapace length (CL) (mm) ± SEM of larvae between days from experiment 1 (C) and experiment 2 (D). Asterisks denote where significant differences lie between treatments within discreet day groups (t-test; P<0.05). EA=enriched *Artemia* (n = 3); PA = Enriched *Artemia* with probiotic (n = 3); WP = Wild zooplankton (n = 3); BA= Batch feeding frequency (n = 5); CO = Continuous feeding frequency (n = 5).

Table 5.1. Survival and development parameters of *Nephrops* larvae over different zoeal stages for diet (1) and feed frequency (2) experiments. EA=Enriched *Artemia* (n = 3); PA = Enriched *Artemia* with probiotic (n = 3); WP = Wild zooplankton (n = 3); BA= Batch feeding frequency (n = 5); CO = Continuous feeding frequency (n = 5). Values are given as mean ± SEM. Means with different superscripts are significantly different against other treatments within each zoeal stage (P<0.05).

Experiment	Zoeal Stage	Treatment	% Survival*[1]	Moult Day to subsequent Zoeal stage*[2]
1	1	EA	71 ± 5.46	8.30 ± 0.12[a]
		PA	70 ± 0.88	8.34 ± 0.14[a]
		WP	17 ± 0.41	10.34 ± 1.40[b]
	2	EA	62 ± 6.56	11.86 ± 0.16[a]
		PA	60 ± 2.65	11.61 ± 0.41[a]
		WP	37 ± 6.01	15.13 ± 2.12[b]
	3	EA	25 ± 7.22	21.56 ± 2.05[a,b]
		PA	26 ± 1.53	19.86 ± 1.00[a]
		WP	17 ± 0.41	22.21 ± 0.09[b]
2	1	BA	73 ± 5.32	7.35 ± 1.20[a]
		CO	92 ± 2.79	5.46 ± 0.46[b]
	2	BA	63 ± 2.97	12.19 ± 1.60[a]
		CO	77 ± 4.64	9.15 ± 0.70[b]
	3	BA	28 ± 4.21	22.68 ± 2.36[a]
		CO	36 ± 3.19	19.32 ± 0.70[b]

***1= Survival figures refer to each stage; *2= Z3 estimated moult day to PL stage using the regression coefficients.**

5.3.4. Costs

The approximate cost per day was lowest for the EA and BA treatments, but it is dependent upon the size of the hatchery, the fluctuating price of the A*rtemia* cysts and the non-fixed price of electricity (see Appendix 5C for calculations). Overall the PA treatment had the lowest cost per batch and per Z3 larvae (table 5.2). The WP treatment had the highest costs per day, batch and Z3 larvae and slight increase in time for preparation although the majority of work

was done every other day. The CO treatment had higher costs when compared to batch feeding. The preparation time was similar to the batch feed plus the added time for maintaining the pumps. These prices reflect the costs associated with feeds and does not include capital, filtration, pumping, labour and rental costs. The lowest cost to produce per PL was at £2.19, which is currently higher than the prices paid (average £ ± SD) for warm water PL (£0.05 ± 0.17 per PL) and to produce *Homarus* sp. PL (£1.44 ± 1.24; Figure 5.4).

Table 5.2. The cost factors of the feeding treatments in this study. N.B. All costs and times are approximate and calculated using average prices at the time of writing (May, 2015).

Treatment	Cost (£) of feed production per day	Cost (£) per batch	Cost (£) per PL	Labour (hours per week)
EA	1.40	30.16	2.51	21
PA	1.43	28.41	2.19	21
WP	3.68	81.70	9.08	23.5
BA	1.40	31.73	2.27	21
CO	4.95	95.67	5.31	24

EA= Enriched Artemia; PA= Enriched Artemia with probiotic; WP= Wild Zooplankton; BA= Batch feeding frequency; CO= Continuous feeding frequency

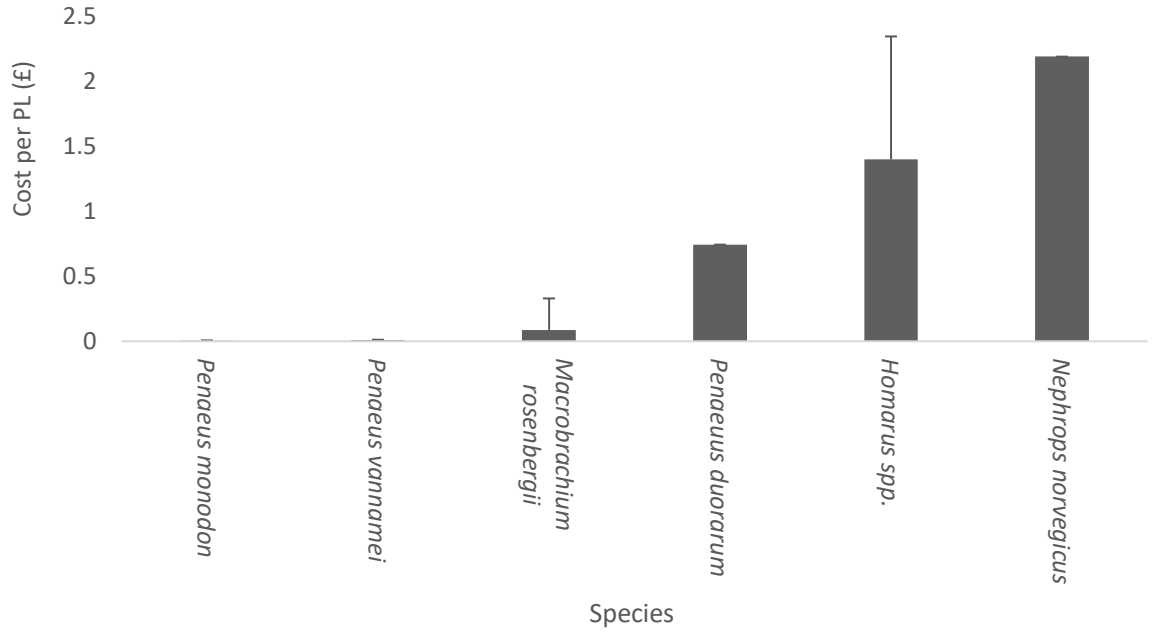

Figure 5.4. Price (£) comparison of PL between species. Data taken from Burton and Adamson, 2002; Haché et al. 2014; Miami Aqua-culture, 2018; Moksness et al. 1998; KT Prawns, 2018; Telsnig, 2011; Wickins and Lee 2002.

5.4. Discussion

In experiment 1 the survival of *Nephrops* larvae was highest within the PA and the EA treatments. There was large variation in survival between the replicates for EA and WP feed treatments but relatively small for the PA treatment. Different bacterial loading may develop amongst an array of small hoppers at high temperature with medium water turnover and this could explain high variability within the EA and WP treatments. Probiotics improve the immune system by physiological changes and colonization in the gastrointestinal environment for many species including *Homarus gammarus* (Merrifield et al., 2009; Daniels et al., 2010). The probiotics exclude or reduce (by competition) harmful pathogens such as *Vibrio* sp. within the gastrointestinal environment and could reduce variable mortality due to infections (Wang et al., 2005; Dagá et al., 2012; De Souza et al., 2012; Daniels et al., 2013). This may be one reason why we see a difference in the improved development time within the PA fed larvae. Therefore, the addition of probiotic to feed diet is beneficial, as it indicates less variability between replicates in survival rates, which is an important factor for production planning in hatchery management.

The suitability of the diets depends upon different factors such as the density, the size, the ability of the larvae to catch the prey at each development stage and the nutritional value for sufficient survival and growth. *Nephrops* larvae display an ontogenetic shift, selecting larger prey items as they develop (Pochelon et al., 2009). Z1 larvae have shown to have a preference for *Artemia nauplii* size range from 400 to 500 µm which increases in Z2-Z3 larvae (Vanhaecke and Sorgeloos, 1980; Pochelon et al., 2009). Wild zooplankton on the other hand could offer a range of sizes available for predation by different larval stages (Hamre et al., 2002; Pochelon et al., 2009). The WP in this study largely consisted of copepods (>30%), dominated by *Acartia clausi* and to some degree *Oithona similis*, and *Evadne spinifera*. (24%). Species such as *Acartia* spp. have been shown to have adequate suitability as live feed for several marine fish species (Rajkumar and Kumaraguru vasagam, 2006; Wilcox et al., 2006; Øie et al., 2015). Furthermore, the total biovolume and carbon content was higher within the WP treatment when compared to the *Artemia* treatments (Appendix 5D), even though the amount of feed provided was the same between the treatments.

In addition, a review of the literature that compares the nutritional values of *Artemia* and wild zooplankton is provided in Appendix 5B and is used for interpretation and discussion. Many of the studies indicate that zooplankton has a higher nutritional value compared to EA. The accumulation of fatty acids and amino acids are essential for survival and development through to PL stages (Wang et al., 2014). The review of the amino acid profiles suggests that there is little difference between the diets, with possibly the exception of higher levels of arginine found in WP. According to Mente (2010) the most important amino acids for *Nephrops* are arginine, lysine, leucine, with levels of methionine becoming the first component to become limited in both *Artemia* and WP. All 10 essential amino acids are present at sufficient levels (derived from the muscle of juvenile *Nephrops*) in both diets (Rosa and Nunes, 2003).

It is suggested that temperate marine species are unable to sufficiently synthesize long chain polyunsaturated fatty acids (PUFA), docosahexaenoic acid (22:6n-3, DHA), eicosapentaenoic acid (20:5n-3, EPA) and arachidonic acid (20:4n-6, ARA), and therefore these must be provided within the diet (Lovell, 1989; Goddard, 1996). The preponderance of the correct EPA:DHA:ARA ratios that mimic that of their natural prey is assumed to be the superior larval diet (Rainuzzo et al., 1995; Bell et al., 2003; Bell and Sargent, 2003). A ratio imbalance

(EPA:DHA:ARA) can cause lower growth and an insufficient production in eicosanoids which can impact upon stress responses (Sargent et al., 1999a). From the literature reviewed the lipid components 14:00, EPA, DHA, ∑PUFA and ∑n-3 were higher in WP when compared to the *Artemia* diets within each respective study.

Research suggests that there is a large consumption of DHA during embryonic development indicating that a diet rich in DHA could be an important requirement for *Nephrops* larvae (Rosa et al., 2003). Identification of the essential fatty acid (EFA) profile can be obtained by analysing the embryo lipid profiles (Sargent et al., 1999b) and is also provided in Appendix 5B. However, the results indicate high variation between broods and further investigation of wild larvae would be advantages (Sargent, 1995). Previous studies conclude that WP diets are rich with PUFA and free amino acids, being almost double that in enriched *Artemia* (Naess et al., 1995).

It is surprising then that WP fed larvae had a large decline in survival within the first seven days compared to the PA and EA fed larvae. This trend is similar to that of starved larvae (personal observation). This may suggest that the larvae in the WP treatment were either at risk from infected or contaminated WP, or were not effectively feeding upon the prey. For example, copepods have highly developed skills to evade capture, and *Cladoceran* sp. have been shown to evade capture due to the high locomotor behaviour. Therefore, the *Nephrops* larvae could have consumed only less nutritious prey, or even starved (Naess et al., 1995; Pochelon et al., 2009; Drillet et al., 2011; Gendron et al., 2013). Thus, the species composition in the WP may not be suitable as feed for *Nephrops* larvae, and more investigation, are required into *Nephrops* larval feeding behaviours and the techniques for capturing prey (Gogoi et al., 2016).

Starvation impacts upon the integrity of the cellular membranes and the ability of the larvae to successfully moult into subsequent stages (Ritar et al., 2003; Sánchez-Paz et al., 2006). If starvation from hatching is over a prolonged period (such as seven days) this will impact upon later survival and prolong the intermoult duration (Mikami, 1993). Starvation may then explain the high mortality for WP feed larvae up to day seven and the large percentage of Z1 larvae remaining by day 9 when compared to EA and PA fed larvae. The WP diet has been an inferior diet during the first stages, but could be improved with live microalgae and

supplemented to the EA/PA diet from day 12 when they show similar mortality rates to the *Artemia* diets.

The preparation differences between the WP and the *Artemia* diets may have also influenced the results. For example, the lipid profile within WP is dependent upon the surrounding environment and the nutrients (wild algae) that the zooplankton consume prior to being caught (Goddard, 1996). Therefore, a diet dependent upon uncontrollable environmental conditions could have variable nutritional benefits and therefore be troublesome for commercial larval rearing. It has been shown that the condition of *H. gammarus* larvae is affected by the nutritional qualities of the primary producers and consumers (Schoo et al., 2014). In the present study, WP was further enriched with off the shelf microalgae paste (not live algae) every other day from being captured and the WP were refrigerated in darkness, albeit for only a maximum of two days. This may have caused increased mortality, lowering the diversity of species within the sample. As *Nephrops* Z1 larvae are positively phototaxic, and any dead, sessile prey that become negatively buoyant would become less available, therefore the feed density would potentially be lower than anticipated. Variable WP populations could also be a future concern for commercial larviculture, as the community structure will change depending upon the season and environmental parameters (Maar et al., 2004; Tönnesson et al., 2006; Calliari and Tiselius, 2009). In contrast, the EA and PA diets were enriched with a synthetic mixture, specifically engineered to improve the nutritional quality of the prey and fed to the larvae immediately after enrichment. Differences between the two enrichment procedures in terms of fatty acid profiles and protein content are not clear, although Aragão et al. (2004) indicate that the different enrichment procedures influence the amino acid composition and total protein content.

The literature suggests an increase in EPA, DHA, LNA (linolenic acid, 18:3n-3) levels in *Artemia* due to enrichment. The lipid profiles of EA indicate higher levels of 18:00, 18:1n-9, 18:1n-7, ∑monounsaturated fatty acids (MUFA), linoleic acid (18:2n-6, LOA), LNA, ARA and n-6 (position of the first double bond from the methyl end). The fatty acids with n-6 position have shown to be an important requirement for larval growth (Goddard, 1996; Lovell, 1989). Although this is usually found in homeothermic species, the ratio of n-3 and n-6 is also important for improved growth and development in some shrimp species (D`Abramo, 1989; Lovell, 1989). The LOA and LNA acids can be chain elongated, desaturated and synthesized in

their respective highly unsaturated fatty acids (HUFA; C20 and C22) (Goddard, 1996). However, there is no evidence from the literature whether *Nephrops* larvae can or cannot synthesize essential PUFA from LOA and LNA and therefore it is uncertain whether these profiles are needed within the diet. Therefore the provision of HUFA within the diets would provide better growth (D`Abramo, 1989). Literature suggests that ARA profiles are higher within the enriched *Artemia* diets than within WP (Bell and Sargent, 2003), and could explain the better survival and development results within this study. However, ARA ratio imbalance with EPA can negatively influence the genes involved with stress response (Sargent, 1995). In addition, both diets show higher levels of DHA indicating that this is species specific. DHA levels can become reduced in enriched *Artemia* over time post enrichment (McEvoy et al., 1998; Dhont and Van Stappen, 2003). However DHA levels would remain high within the CO treatment due to the continual replenishment of fresh EA which had been held on ice (Dhont and Van Stappen, 2003).

The diet types were based upon previous investigations of feeds that are used commercially for crustacean species. Results from the preliminary studies indicate that an unsuitable diet can cause catastrophic mortality within a season (see Chapter 2 – Preliminary Broodstock and Larvae Trials). This initial study suggests that there are no benefits of acquiring wild zooplankton compared to enriched *Artemia* for the growth of *Nephrops* larvae. Further investigation could be made into the use of early hatched *Nephrops* larvae as feed for the later developed larvae, however ethical consideration should be given. Overall the *Artemia* treatments offered the best cost-effective feed treatment taking into consideration the survival and time requirements for preparation. In addition, the relative ease and control of securing *Artemia* feed of known supply and composition suggests this as a realistic and satisfactory diet regardless of enrichment type. In contrast the added resources in terms of time and money for capturing wild zooplankton did not yield significant benefits in terms of greater survival or growth.

Similar to Rotllant et al., (2001) freshly enriched nauplii of *Artemia* were considered to be the best diet to date for *Nephrops* larval rearing, with up to 41% of larvae reaching stage 3. This study indicates that survival and development time can be improved by supplying *Artemia* more than once per day. It is assumed that the consumption rate increases with each successive larval stage, and this rate is more likely to be met with a continuous feeding

frequency when the optimal density of feed is provided (Logan and Epifanio, 1978; Pochelon et al., 2009). *Nephrops* have an encounter feed mechanism (personal observation) which directly relates to the rate of prey capture (Cox and Bruce, 2003; Epelbaum and Borisov, 2006; Maciel et al., 2012). It is suggested that the capture rate in the CO treatment would be higher due to the continuous supply of feed over a 24-hour period. In addition, within the BA treatment the *Artemia* remain in the tank for an average of 7 hours. During this time the *Artemia* may become less attractive, less palatable and less nutritionally beneficial (i.e. lowering DHA levels), and their density per ml is continually declining. In comparison, larvae within the CO treatment are provided with fresh *Artemia* every six hours.

In addition, stomach fullness has been shown to affect the behaviour and physiology of other crustaceans (Tarling and Johnson, 2006). Though not as aggressive and cannibalistic as *Homarus* larvae, *Nephrops* may eat their conspecifics/siblings and the rate of this is likely to increase with starvation (Smith, 1987). *Homarus* larvae were seen to be more discriminatory when starved for over 6 hours (Kurmaly et al., 1990), which may indicate that hunger within the constantly fed larvae within our study was not reached. Therefore, more energy is available for growth and development rather than being expended in aggressive behaviour with the potential damage it may cause (Thomas et al., 2003; Nhan et al., 2010; Manley et al., 2015). In addition, the larvae within the CO treatment may have had more encounters with feed rather than with conspecifics.

The CO treatment showed significant improvements in the intermoult time taken to reach Z2 and Z3, with potentially more than five days difference when compared to batch feeding. This has important implications for hatchery operations, including lowered costs, fewer resources, less cannibalism and a larger total output over the short larval season. The results resemble findings for other crustaceans where increased feeding frequency reduced the rearing cycle and metamorphosis time (Vijayakumaran and Radhakrishnan, 1986; Nhan et al., 2010). However, providing continuous feeding requires additional resources such as a larger supply of *Artemia* cysts, additional ice and increased aeration for the feeding tanks, and these should be factored into any cost-benefit analysis.

The cost to produce each *Nephrops* PL is a 41% increase compared to lobsters and over a 900% increase to purchase warm-water shrimp and prawn PL. However, despite the market

value of the released and recaptured stock being lower or near to the production costs, several lobster stock enhancement programmes have been classed as successful based upon the results of increased stock abundance alone (Bannister and Addison, 1998; Burton, 2001; Ellis et al., 2015). To evaluate whether producing PL is viable depends upon the aim of the programme. For example, the social benefits shown from stock enhancement programmes were the enablement of fishers and managers to become more involved and have greater control of their fishing grounds, despite the monetary costs (Bannister and Addison, 1998). Another aim could be to use the PL for restocking, supplementation and re-introduction (defined in table 1.1), where economic viability is not the main objective. A continual decrease in production costs, such as lowering feed costs, lowering the electricity costs and capital equipment, as seen previously in warm-water species, whilst increasing survival would assist in the development of *Nephrops* aquaculture. In addition, developing the techniques to rear the larvae at mass-scale, improving tank hydrodynamics, improving transfer techniques, and lowering labour costs would make production more efficient and potentially economically viable. Therefore, a systematic approach is needed to determine the cost-benefit of producing hatchery reared *Nephrops* PL in accordance to the specific aims of that programme.

In conclusion, the PA diet and CO feeding frequency induced better survival and faster development time to reach Z3. The results from the PA diet was marginally improved from the EA fed larvae, although the latter had increased final weight. The WP fed larvae had the lowest survival and longest growth duration to reach Z3. The composition of WP and the nutritional value will also change dependent upon the time of year. These results indicate that the best feeding strategy for larval growth would be as follows. Continuous feeding of fresh PA during the first zoeal stages (to ~day 6/7) to develop the GI environment and offering prey in the smaller size ranges. From day 6/7 the diet could alternate between ongrown larger sized PA and EA. However constant feeding and added probiotic incurs higher costs. This study indicates that the survival, growth and development of *Nephrops* larvae can be improved with response to different feeding regimes and diets. Further experimentation is needed to quantify optimal feed rates, rations and frequencies along with continuing analysis of novel feeds. The highest survival to Z3 observed in this study (36%) is a considerable improvement in larviculture for this species.

Knowledge of *Nephrops* larviculture techniques is in its infancy and still developing. Guidance on husbandry techniques is sparse, but continues to improve with more research efforts investigating key subject areas related to the aquaculture of this species. However even with the limited knowledge available, key findings such as optimising diet and feed frequencies will make it possible to culture *Nephrops* with the aim of larval research or stock enhancement activities in areas of depleted stocks. However, it remains uncertain whether this is commercially economically viable and would be an interesting subject for future research.

5.5. Acknowledgements

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Appendix 5A. Supplementary Figures

Table 5.3. The variance, standard deviation and coefficient of variation of the survival data in experiment 1.

	EA				PA				WP			
DAY	**Mean**	**S**	**SD**	**CV**	**Mean**	**S**	**SD**	**CV**	**Mean**	**S**	**SD**	**CV**
3	96.00	2.67	1.63	1.70	95.99	18.61	4.31	4.49	90.63	5.95	2.44	2.69
6	83.33	54.22	7.36	8.84	87.93	18.40	4.29	4.88	63.93	527.14	22.96	35.91
9	71.33	238.22	15.43	21.64	69.78	3.63	1.91	2.73	55.90	524.97	22.91	40.98
12	62.00	344.00	18.55	29.91	60.34	49.43	7.03	11.65	46.49	369.95	19.23	41.37
15	43.33	856.89	29.27	67.55	48.99	0.67	0.82	1.67	37.07	308.27	17.56	47.36
19	24.67	416.89	20.42	82.78	26.16	18.07	4.25	16.25	11.46	66.55	8.16	71.21
POOLED		890.14	29.84	47.03		567.31	23.82	36.72		890.38	29.84	58.61

S = variance; SD = Standard deviation; CV = Coefficient of variation. EA = Enriched Artemia; PA = Enriched Artemia with probiotic; WP = Wild Zooplankton.

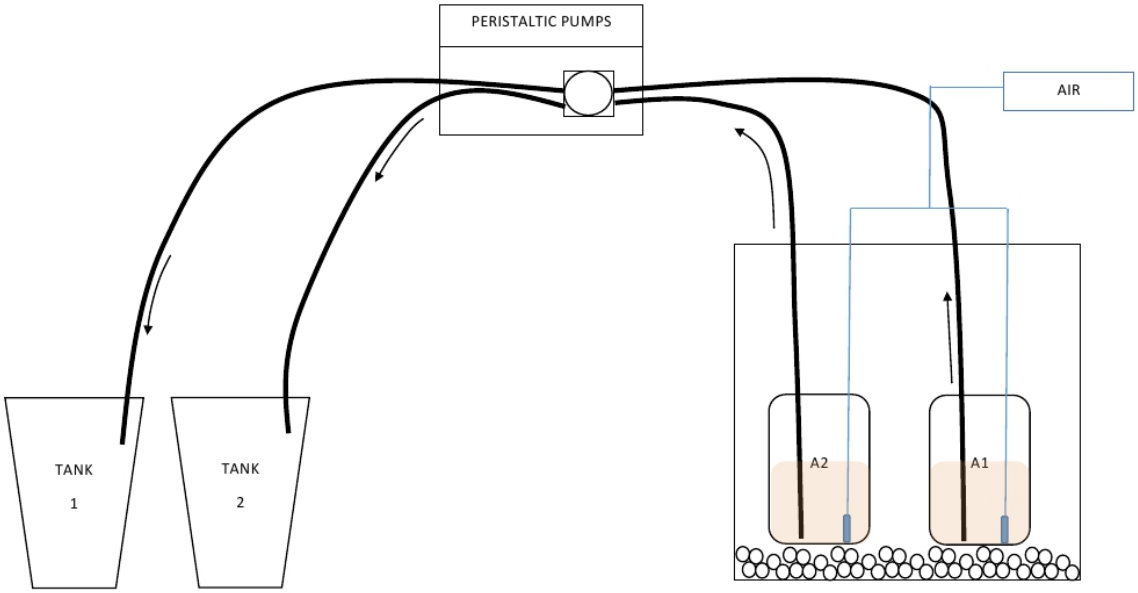


Figure 5.5. Schematic diagram of the equipment used for the continuous feeding treatment of *Nephrops* larvae in experiment 2. Beakers with *Artemia* in aerated sea water (A1 & A2) were kept on ice and the *Artemia* were delivered to the tanks (1 & 2) through a peristaltic pump.

Appendix 5B. Fatty Acid Literature Review

The fatty acid and amino acid profiles gathered from literature investigating the nutritional values of *Artemia* and WP is shown below. The lipid profile for *Artemia* nauplii can vary depending from the origin, strains and parentage. Oleic acid (18:1n-9) and palmitic acids (16:0) comprise a large proportion of the total fatty acids in unenriched *Artemia*.

Several different methods for analysing and reporting amino acid profiles has made reviewing and comparing the data difficult.

Table 5.4. Fatty acid composition (% of total fatty acids) between un-enriched *Artemia*, enriched *Artemia* and wild zooplankton diets.

	Artemia (not enriched)															*Artemia* (enriched)					
14:00	-	-	0.8	-	-	-	-	-	-	-	3.9	1.0	0.9	1.0		0.9	3.2	0.9	-	-	-
16:00	-	-	12.6	**16.3**	3.6	12.6	15.7	18.6	14.4	23.6	21.3	12.8	41.4	37.7	13.5	11	9.9	10.9	-	-	-
17:00	-	-	-	-	-	-	-	-	-	-	-	0.9	-	-		-	0.7	0.8	-	-	-
18:00	-	-	7.4	-	-	-	-	-	-	-	10.8	8.8	14.4	15.3	7.6	4.9	4.3	5.0	-	-	-
∑SFA	-	-	-	-	-	-	-	-	-	-	38.1	24.4	59.1	57.2	24.6	-	16.2	18.1	21.3	-	-
18:1n-9	-	-	22.5	**27.6**	6.1	17.8	23.7	21.2	17.9	40.4	6.5	15.8	**5.0**	**5.4**	20.5	19.7	16.9	18.1	-	-	-
18:1n-7	-	-	10.6	-	-	-	-	-	-	-	-	0.3	-	-	7.3	7.5	7.6	8.6	-	-	-
∑MUFA	-	-	-	-	-	-	-	-	-	-	31.3	16.9	21.7	24.2	35.5	-	33.4	34.8	27.3	-	-
18:2n-6 (LOA)	-	-	6.8	**7.9**	1.8	11.0	12.2	7.4	8.0	6.2	0.0	20.1	**0.9**	**1.2**	5.9	6.8	4.3	4.7	-	-	-
18:3n-6	-	-	-	-	-	-	-	-	-	-	0.0	0.7	0.9	1.1		-	-	-	-	-	-
18:3n-3 (LNA)	-	-	-	**34.3**	8.2	**21.5**	6.8	6.3	16.7	-	11.7	20.9	**0.3**	**0.4**	25.4	-	18.5	18.2	-	39.8	**36.5**
20:3n-3	-	-	-	-	-	-	-	-	-	-	0.0	1.2	0.4	0.3	0.8	-	-	-	-	-	-
18:4n-3	-	-	2.3	-	-	-	-	-	-	-	2.1	7.1	-	-	3.9	2.7	2.0	1.7	-	-	-
20:4n-6 (ARA)	-	-	2.3	-	-	-	-	-	-	-	2.5	2.8	0.1	0.2	0.3	1.7	1.5	1.7	2.1	1.0	**3.5**
20:5n-3 (EPA)	9.3	0.2	3.6	**6.2**	0.6	**4.5**	**9.1**	24.5	0.0	44.7	6.0	3.6	0.1	0.2	1.0	9.8	12.5	10.8	4.9	**14.3**	**12.0**
22:6n-3 (DHA)	0.2	-	0.2	-	-	**0.2**	**0.2**	0.0	0.3	0.2	2.7	0.5	**0.0**	**0.2**	0.1	5	5.2	3.0	6.3	**36.6**	**5.2**
∑PUFA	-	-	-	-	-	-	-	-	-	-	-	59.6	8.4	11.7	39.9	-	46.5	42.1	-	-	-
∑n-6	-	-	9.1	-	-	-	-	-	-	-	2.5	24.0	-	-	6.8	8.5	6.0	6.8	8.1	-	-
∑n-3	-	-	27.1	-	-	-	-	-	-	-	23.5	33.4	-	-	32.8	37.8	40.5	35.5	34.5	-	-
∑n-3/∑n-6	-	-	0.3	-	-	-	-	-	-	-	-	1.4	-	-		0.2	6.7	5.2	-	-	-
DHA/EPA	-	-	0.1	-	-	-	-	-	-	-	-	0.1	-	-	0.1	0.5	0.4	0.3	1.3	-	-
EPA/ARA	-	-	-	-	-	-	-	-	-	-	-	1.3	-	-	2.9	-	8.3	6.4	2.3	-	-
Reference	a	a	b	e *1	e *2	e *3	e *4	e *5	e *6	e *7	f	h	i	i	k	b	c	c	d	e	e

Artemia (enriched) *Cont.*				Wild Zooplankton														
-	-	-	2.5	3.4	4.3	4.2	6.2	4.3	4.6	-	5.8	-	-	4.3	1.8	0.9	-	4.6
-	-	-	16.9	16.9	16.4	15.4	14.3	13.7	13.8	-	13.0	-	-	20.5	64.4	72.1	19.1	11.5
-	-	-	-	-	-	-	1.3	0.9	1.1	-	-	-	-	0.9	-	-	-	-
-	-	-	3.4	3.7	2.6	1.8	2.2	2.5	3.4	-	13.0	-	-	4.7	18.5	22.0	7.2	3.7
-	-	-	24.3	-	-	-	24.9	22.3	22.4	22.5	34.4	-	-	31.2	85.8	95.1	31.3	20.1
-	-	-	9.3	2.9	6.6	11.7	2.0	1.0	3.0	-	8.6	-	-	19.6	1.7	0.1	24.0	0.5
-	-	-	2.8	3.3	1.7	1.1	1.1	2.0	2.5	-	-	-	-	2.0	-	-	5.5	3.0
-	-	-	14.0	-	-	-	6.4	6.2	9.6	10.4	25.6	-	-	22.1	8.2	0.6	38.3	7.0
-	-	3.4	4.2	2.0	2.4	2.8	2.6	1.6	1.6	-	2.1	1.9	0.9	2.5	0.2	0.6	2.0	7.7
-	-	-	0.2	-	-	-	-	-	-	-	0.0	-	-	1.9	0.3	0.1	-	2.5
20.6	**35.3**	15.8	13.3	-	-	-	2.2	1.1	1.7	-	8.3	2.2	0.4	1.7	0.2	0.0	0.3	12.9
-	-	-	0.1	-	-	-	0.3	-	-	-	2.5	-	-	1.4	0.2	0.0	0.3	0.4
-	-	-	2.0	1.5	4.6	7.8	2.3	2.5	3.3	-	2.0	-	-	2.9	-	-	0.0	14.4
8.2	**3.7**	1.5	2.5	0.8	0.6	0.6	0.3	0.6	1.3	0.7	0.0	0.8	1.4	11.0	0.7	0.0	7.1	1.3
17.6	**41.4**	4.2	0.4	21.1	18.1	14.5	9.2	13.2	16.0	18.6	10.0	11.9	14.1	10.9	0.3	0.0	7.4	0.6
10.9	**20.1**	6.2	21.5	32.9	24.8	24.0	39.4	44.4	37.5	28.5	9.4	18.1	34.9	12.5	0.5	0.0	10.0	12.8
-	-	-	-	-	-	-	58.7	67.4	62.7	-	-	-	-	46.7	3.9	4.0	29.7	-
-	-	-	15.4	2.8	3.0	3.4	4.2	3.0	3.2	4.3	4.1	-	-	16.8	-	-	11.1	25.7
-	-	10.9	43.9	58.3	52.4	51.9	54.6	64.1	59.5	55.9	33.9	32.3	50.3	28.4	-	-	18.6	51.3
-	-	-	-	0.0	0.1	0.1	13.0	13.0	18.5	-	-	-	-	1.7	-	-	-	-
-	-	1.5	-	1.6	1.4	1.7	4.3	3.4	2.3	1.6	-	1.5	2.5	1.2	-	-	1.4	-
-	-	-	-	-	-	-	30.7	22.0	12.3	29.0	-	-	-	1.3	-	-	1.0	-
e	e	g	J	b	b	b	c	c	c	d	f	g	g	h	i	i	k	J
											*8	*9	*10		*11	*12		*13

a= Léger et al. (1986); b= Naess et al. (1995); c= McEvoy et al. (1998); d= Hamre et al. (2002); e= Dhont & Van Stappen (2003), fatty acid values displayed as mg/g dry weight; f= Rajkumar & Kumaraguru vasagam (2006); g= Yamamoto et al. (2008); h= Gendron et al. (2013); i= Ladhar et al. (2014); J= Øie et al. (2015); k= Reis et al. (2016); *1= Great Salt Lake, USA *Artemia*; *2= San Francisco Bay, USA *Artemia*; 3*= PR, China *Artemia*; 4*= *A. parthenogentica*; 5*= Madagascar sourced *Artemia*; 6*= *A. persimilis*; 7*= *A. tibetiana*; 8*= *Arcatia clause*; 9*= *Oithona* spp.; 10*= *Acartia* spp.; 11*= *Oithona nana*; 12*= *Oithona similis*; 13*= Cultured *Acartia tonsa*; -= Not stated. Numbers in bold indicate an average from that study.

Table 5.5. Fatty acid composition of *Nephrops* embryo's (µg/mg dry weight).

	Nephrops Embryo Fatty Acid Composition (µg/mg dry weight)							
14:00	5.2	4.7	1.9	1.4	1.8	2.4	1.5	0.5
16:00	49.7	37.7	29.9	14.6	16.3	22.9	10.8	8.9
17:00	1.8	1.7	1.1	0.2	0.5	1.1	0.5	0.7
18:00	9.6	8.2	7.4	3.2	4.2	5.7	3.9	3.7
∑SFA	70.5	56.0	42.2	19.9	23.5	32.8	18.2	15.1
18:1n-9	75.1	41.0	30.3	18.7	18.1	26.1	14.1	16.2
18:1n-7	16.1	13.4	10.6	6.3	5.4	7.4	3.9	4.2
∑MUFA	126.5	85.1	56.0	35.7	34.6	45.5	25.0	25.2
18:2n-6 (LOA)	3.2	2.9	1.9	0.7	0.9	2.5	1.2	1.8
18:3n-6	0.2	0.2	0.3	0.0	0.1	0.2	0.2	0.3
18:3n-3 (LNA)	1.4	1.0	0.6	0.2	0.5	0.8	0.5	0.5
20:3n-3	0.7	0.4	0.3	-	-	-	-	-
18:4n-3	1.0	0.6	0.2	-	-	-	-	-
20:4n-6 (ARA)	5.7	4.8	4.0	2.9	2.2	3.0	3.1	2.4
20:5n-3 (EPA)	21.7	15.3	15.7	8.7	7.2	13.0	11.0	8.0
22:6n-3 (DHA)	42.1	14.2	13.8	20.8	15.7	22.6	19.9	15.8
∑PUFA	85.3	45.9	40.4	1.3	1.8	4.9	2.8	3.5
∑n-6	12.9	10.7	7.6	-	-	-	-	-
∑n-3	72.4	35.1	32.7	-	-	-	-	-
∑n-3/∑n-6	5.6	3.3	4.3	-	-	-	-	-
DHA/EPA	-	-	-	-	-	-	-	-
EPA/ARA	-	-	-	-	-	-	-	-
Reference	l *14	l *15	l *16	m *17	m *17	m *17	m *17	m *17

l= Rosa et al. (2003); m= Pochelon et al. (2011); *14= embryonic stage 1; *15= embryonic stage 2; *16= embryonic stage 3; *17= embryonic stage 2.

Table 5.6. Amino acid analysis between diets.

	Arg	His	Ile	Leu	Lys	Met	Phe	Thr	Tryp	Val	Reference	Analysis
Artemia (not enriched)	9.2	3.2	4.1	6.7	9.0	2.4	6.5	4.6	6.2	4.6	a	g / 100 g protein
	9.7	4.1	4.7	7.5	9.9	1.9	4.3	4.4	8.9	4.5		
	8.3	3.0	4.6	7.1	7.4	2.2	8.8	5.1	6.5	4.7		
	8.2	2.3	5.7	8.4	7.8	3.1	7.2	4.0	5.6	4.4		
	8.3	3.2	5.4	8.5	9.0	3.1	7.2	4.6	4.6	2.6		
Wild Zooplankton	9.9	1.3	2.4	4.5	6.6	4.7	2.1	2.1	0.6	3.8	b	Free amino acids (µmol/g WW)
Artemia (not enriched)	3.6	0.7	1.5	2.5	3.9	2.2	1.5	1.3	0.3	2.1		
Artemia (Enriched)	3.7	0.9	1.4	2.2	4.5	2.0	1.4	1.5	0.2	2.1		
Artemia (Enriched)	18.0	4.1	4.4	12.0	17.0	4.7	7.0	6.2	1.8	8.6	d	Free amino acids (mg/g Protein)
Wild Zooplankton	25.0	4.1	6.5	15.0	17.0	6.3	7.3	7.3	2.1	10.0		
Artemia (not enriched)	11.5	3.0	5.0	7.0	7.7	2.1	4.2	4.8	1.2	5.4	e	g / 100g protein
Artemia (not enriched)	7.2	3.1	5.1	8.8	10.2	2.0	5.3	3.9	-	6.7	f	Amino acid % in protein
Wild Zooplankton	8.6	3.5	2.2	9.9	11.0	2.0	1.1	2.5	-	9.6		
Artemia (Enriched)	6.2	1.5	-	-	4.5	1.2	-	-	-	-	g	Free amino acid composition (mg/g dry matter)
Wild Zooplankton *1	6.9	1.1	-	-	4.2	1.1	-	-	-	-		
Wild Zooplankton *2	22.8	1.2	-	-	6.2	2.0	-	-	-	-		

Arg=Arginine; His=Histidine; Ile=Isoleucine; Leu=Leucine; Lys=Lysine; Met=Methionine; Phe=Phenylalanine; Thr=Threonine; Tryp=Tryptophan; Val=Valine; *1=Oithona spp.; *2=Acartia spp. References; a= Léger et al. (1986); b= Naess et al. (1995); d= Hamre et al. (2002); e= Dhont & Van Stappen (2003); f= Rajkumar & Kumaraguru vasagam (2006); g= Yamamoto et al. (2008); -= Not stated.

Appendix 5C. Cost Factors

Table 5.7. The calculations used to evaluate the cost factors.

Analysis	Formula
a = Cost (£) per day	Cost of Artemia cysts*^{EA,PA,BA,CO}, electricity costs (heating*^{EA,PA,BA,CO}, aerators*^{EA,PA,WP,BA,CO}, lighting*^{EA,PA,BA,CO}, ice machine*^{CO}), enrichments*^{EA,PA,BA,CO}, probiotics*^{PA}, algae paste*^{WP}, fuel*^{WP} used per day
b = Cost (£) per batch	*a* x estimated number of days to reach PL stage
c = Cost (£) / larva	*b* / total number of larvae at day 19

Letters after the asterisks denote which item corresponds to each treatment. EA= Enriched Artemia; PA= Enriched Artemia with probiotic; WP= Wild Zooplankton; BA= Batch feeding frequency; CO= Continuous feeding frequency

Appendix 5D. Comparison of Biovolume in Experiment 1

The WP treatment had a higher total biovolume, wet weight, dry weight and carbon content than the *Artemia* treatments (Table 5.8).

Table 5.8. The total biovolume (*V* mm^3), wet weight (*WW*, g), dry weight (*DW*, g), and carbon content (*CC*, µg carbon)

Feed	Total *V* (x10^{-8} mm3 per tank)	Total *WW* (g)	Total *DW* (g)	Total *CC* (µg C)
Wild Zooplankton (WP)	8.21	268.06	55.78	25.10
***Artemia* (EA and PA)**	5.82	147.49	29.48	13.26

6. Burrow Occupancy Influences *Nephrops norvegicus* Post Larval Release Behaviour

Abstract

There is limited evidence documenting *Nephrops norvegicus* post larval behaviours and the ways in which they are recruited to the benthos. It is speculated that post larvae inhabit the burrows of the adult but there is limited evidence of this within the literature. A repeated experimental design was used to investigate habitat selection and the behaviours of reared post larvae (PL) around adult burrows and alternative habitats. Both treatments used sediment taken from known *Nephrops norvegicus* grounds to form an adult burrow. The behaviours and location of the PL were compared in trials where the burrow was either un-inhabited or occupied by an adult conspecific. The majority of PL were able to find shelter within 15 minutes and spent more time in the burrow when it was empty, but spent more time in alternative habitats when it was occupied. There was a higher percentage of PL that remained in the burrow habitat from first entering when the burrow was empty. However, there were several behaviours that were different when the burrow was occupied, including more vigilant and retreat behaviours from the burrow areas and PL remained further away from the burrow entrance. When PL exhibited burrowing behaviour, the number of burrows excavated, and the number and duration of burrowing behaviour bouts were higher when it was occupied, and the new burrows excavated were at a larger distance away from the artificial burrow. PL were observed entering the burrow by walking on the underside of the burrow wall. The results suggest burrow occupancy influences PL habitat selection and that alternative habitats could be an important habitat in PL recruitment.

Abbreviations:

PL= Post larvae; INB= In the burrow; BE= burrow entrance; BR= burrow ridge; BP= burrow proximity; BPM= burrow proximity mound; INC= in the cobbles

6.1. Introduction

Stock enhancement and restocking efforts have been taking place for clawed lobsters since the early 1900's (Nicosia and Lavalli, 1999). Investigations exploring post larval behaviour to improve survival and recruitment into the wild stock is needed to inform researchers, hatchery managers and governmental agencies in optimising release techniques and protocols (Agnalt et al., 1999; Nicosia and Lavalli, 1999). Hatchery techniques for *Nephrops norvegicus* (referred to as *Nephrops*) are still in their infancy, although recent improvements in larval survival may lead to renewed interest in *Nephrops* stock enhancement, by the release of post larvae (PL) onto known *Nephrops* grounds (Powell and Eriksson, 2013). It is clear from the work undertaken on *Homarus* spp. that many factors influence the success of releasing PL cultivated from the laboratory into an open alien environment (Castro and Cobb, 2005; Van Der Meeren, 2005). One important requirement for improving onward survival is to understand how PL behave when first released (van der Meeren, 2000; 2005).

Within their first stages the PL become semi-benthic and start exhibiting substrate and habitat finding behaviours, eventually settling on their preferred substratum (Farmer, 1975; Smith, 1987; Cobb and Wahle, 1993). The PL are able to excavate burrows, although few independent burrow excavations have been found (Chapman and Rice, 1971; Rice and Chapman, 1971; Chapman, 1980). Therefore, it is assumed that the PL enter the burrow of the adults, and excavate away from the main tunnel, rather than excavate an individual burrow from the benthic surface (Tuck et al., 1994). The PL remain in the burrows for approximately a year, with increasing emergence behaviour proportional to their length / age (Chapman, 1979; 1980; Tuck et al., 1994). However, there are few examples of juvenile and adult burrow complexes or observations of PL on the substrate in the wild. The mechanisms involved in the PL entering an adult burrow, and whether PL can or do inhabit alternative habitats, are not known. Like most decapod crustaceans, *Nephrops* are aggressive towards conspecifics, and therefore entering an adult burrow could be potentially dangerous for the PL (Chapman and Rice, 1971; Farmer, 1975; Smith, 1987; Katoh et al., 2008). There are examples of direct mortality occurring from the adult preying on the PL when trying to enter the burrow (Smith, 1987). To date there is little information on *Nephrops* PL behaviour, the mechanisms used for habitat selection, or of the adult-juvenile burrow complex relationship.

If stock enhancement activities develop for this species, it is important to have sufficient knowledge of how *Nephrops* PL behave following their release onto *Nephrops* grounds.

The aims of this study were to understand *Nephrops* post larval habitat selectivity and the behaviours of naive PL around adult burrows. The behaviours of PL were observed in relation to different habitats including that of adult burrows. The results of this research will inform any plans related to the release of PLs for stock enhancement programmes.

6.2. Methodology

6.2.1. Animals

Ovigerous females were creel-caught between May and June 2014 in the Skagerrak, Sweden at an approximate depth of 40 m and brought to the culture facility (Kristineberg Marine Research Station) where they were housed in separate compartments. First stage larvae were collected daily and transferred to 80L cylindrico-conical tanks (4 larvae/L) maintained by heated (15^{o}C) flow-through upwelling water and fed daily with live enriched *Artemia* (3-5 *Artemia*/ml) (similar to methods used in 5.2.3.1). Once the larvae were at stage 3 they were removed and placed into individually into separate cylindrical beakers (350 ml) and maintained at a temperature of 15^{o}C and continued to be fed live *Artemia*. After the larvae had metamorphosed into post larvae (PL), the beakers were transferred to a dark room prior to experimentation for a minimum of 30 minutes. Experiments took place in July and August 2014.

6.2.2. Experimental Design

Each PL (mean carapace length 4.17 ± 0.38 mm and total length 9.98 ± 0.69 mm, n=22) was checked for deformities, good health and vitality. Deformed and low vitality individuals were not used in the experiment. Each individual was used twice for each treatment in a repeated measures design. Treatment 1 (Control) had two variables being an uninhabited artificial burrow (empty) and cobbles as habitat choices (n=22). Treatment 2 was the same but had an inhabited artificial burrow (occupied; n=22).

The beaker was placed into the first treatment (empty burrow) and the PL was gently released from the beaker in the centre of the tank and allowed to swim towards the substrate. Once the PL had made contact with the substrate the timer was started and the behaviour and

location within the tank noted every 5 seconds. After 40 minutes of continuous observation, if the PL had chosen a habitat (either burrow or cobbles) and had remained in the same location for over 5 minutes and further exploration had ceased, then the observation and location would be noted every 5 minutes for a further 20 minutes. If the PL had not chosen a habitat, then the location and behaviour were further noted every minute for a further 20 minutes. An ethogram was developed through preliminary investigations (Appendix 6A: Table 6.2).

If the PL chose a location other than the burrow or cobble habitat, the location and its defining features were noted. If the PL started burrowing behaviour, the exact location was also noted. After a total of 60 minutes of observation the PL was removed and placed in a beaker and left to rest for over 30 minutes. After this time the PL was placed in treatment 2 (occupied), containing the occupied burrow and the same procedure was repeated as above. After each experiment the sediment was disturbed, any constructed burrows filled in and the tank was left for ~3 hours to remove any chemical traces of the previous experiment. The water was exchanged every 1:41 hours.

Repeated experiments were designed to determine if PL were attracted towards the burrow itself and if so, if it would enter the burrow. The second treatment therefore would test if this was still the case when an adult is occupying the burrow, or whether its behaviour would be altered the behaviour, and perhaps favour an alternative habitat such as cobbles. All the experiments were performed in the same sequence (empty burrow followed by occupied burrow) and under red light conditions to reduce light effecting behaviour.

The burrow inhabitants were both randomly chosen males (CL=30.75 ± 1.63 mm) and checked for health every other day. The inhabitants were changed after treatment number 6. This is a repeated measured design and therefore in treatment 2 the PL were in contact with the substrate for the second time, albeit only 30 minutes later. Preliminary trials indicate that the treatment order had effect in habitat preference results in this study.

6.2.3. Tank and Burrow Design

Each tank had a total capacity of 15.7 litres with a benthic surface area of 510.71 cm^2. The tanks had flow through water maintained at a temperature of 14.8 – 14.9^oC and flow rate of 0.55 ml/s.

The burrow was constructed using a mesh cylinder with a mesh size of 2mm which allowed the sediment to pass through, yet keeping the integrity of the burrow structure. The burrow opening was 7 cm in length (35 mm radius) and the burrow had a total length of 20 cm with a mesh posterior end. The burrow was placed at a slope of between 20-30^{o} replicating natural burrow openings (Rice and Chapman, 1971). Natural burrows are typically U shaped to allow water flow through the burrow and oxygen levels to be maintained. Therefore a smaller hose (10 mm) was attached to the posterior end of the burrow with a flow rate of 0.44 ml/s. Subsequently the water flow was facing towards the opening of the burrow. The burrow door which kept the adult within the burrow was attached 3 cm from the anterior end of the burrow, which allowed an unambiguous determination that the PL entered the burrow but did not allow any contact with the adult and possible mortality due to predation. A further 3 cm cap was attached on the top anterior to elongate the within burrow length to 6 cm. The burrow design was identical for each treatment. To ensure that there was an equal chance of the PL entering either the burrow and cobble habitat, the areas were the same size (80cm^{2}).

The sediment was collected with a Van Veen Grab from known *Nephrops* grounds at the mouth of the Gullmarsfjord. The sediment was then sieved through a 1cm sieve then a finer 1 mm sieve to remove any macro fauna. The sediment was held in a flow-through system and placed into the tanks around the burrow and allowed to settle over three days with continuous flow through. The burrow area was manipulated to replicate that of naturally occurring burrows: namely with a burrow mound (BPM) rising around and into the entrance, a burrow ridge around the borrow entrance, replicating material excavated from the burrow tunnel. This made is possible to clearly classify the various areas close to the burrow (Figure 6.1). The sediment was approximately 13cm and the water approximately 10.5 cm deep. Each cobble was approximately 3 x 2 cm in size.

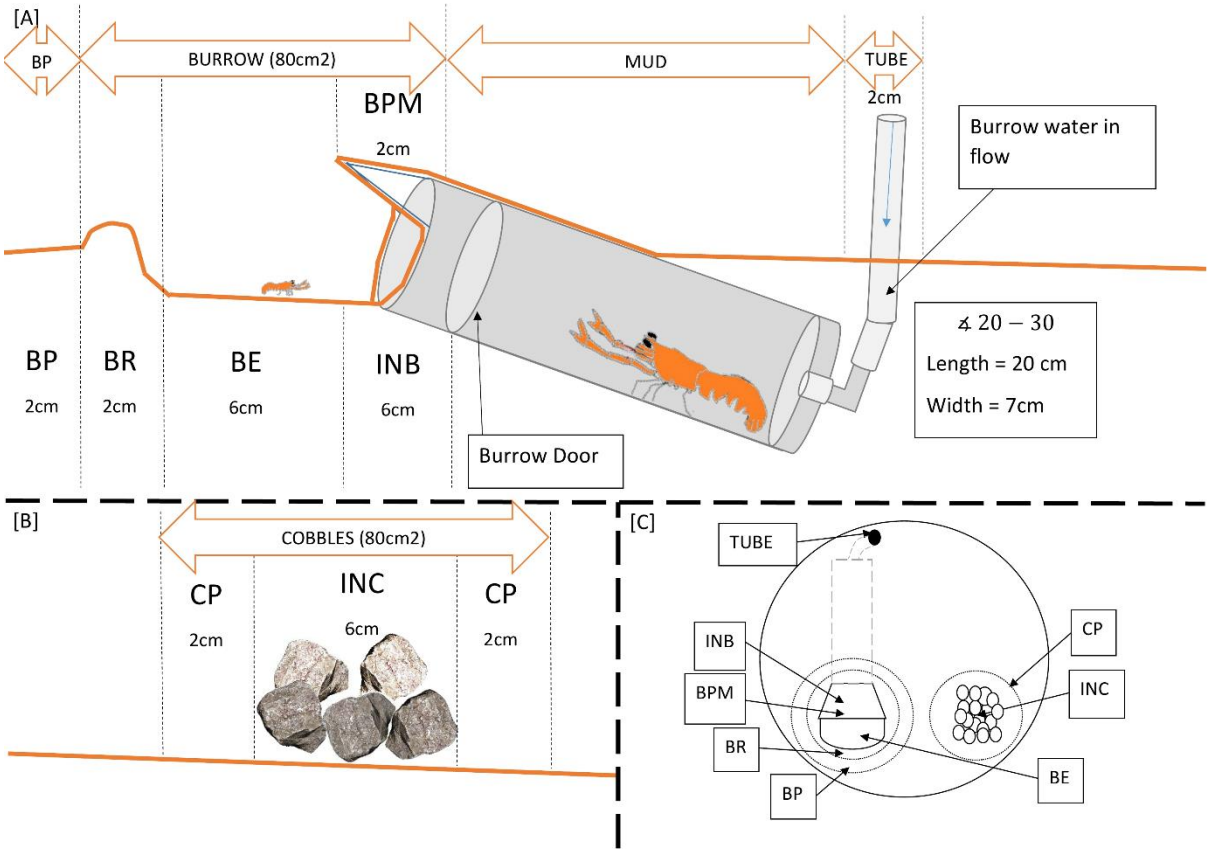


Figure 6.1. Diagram illustrating the perimeters and dimensions of (A) the burrow and proximity areas; (B) Side view of the Cobble perimeters and dimensions; (C) A bird's eye view of the tank setup with habitat locations. The brown line indicates the level of substrate including around the burrow entrance and manipulated mound. BP = Burrow Proximity; BR = Burrow Ridge; BE = Burrow Entrance; INB = In the Burrow; BPM = Burrow Proximity Mound; TUBE = Inflow Burrow Pipe; CP = Cobble Proximity; INC = In the Cobbles. N.B. not drawn to scale.

6.2.4. Statistics

All data were tested for conformity to normal distribution (Kolmogorov Smirnov test) and equal variances (Levene's test). If the data did not conform it was either transformed (logit and arcsine square root) or a non-parametric test was used such as the Wilcox signed rank test. If the assumptions of the Wilcox sign rank test could not be met the sign test was used. Habitat preference and burrow behaviour (time spent) data were tested using a paired t-test and Anova was used to analyse the proportion of time spent in different locations within each treatment. Habitat area data was normalised. Frequency data and the number of burrowing behaviour bouts were analysed using a sign test. The proportion of time in each location and frequency data was then ranked and analysed using a spearman rank correlation coefficient. The number of PL burrowing, burrow distance data and burrowing behaviour data were analysed using the Wilcoxon signed rank test. Categorical frequency data (number of

burrows, behavioural sequences, location sequences, time to reach and shelter preference data) was analysed using a chi squared test.

For analysis into how the PL used several habitats, the first habitat (the area which the PL entered first, excluding mud) and chosen habitat (where the PL spent ≥ 20% of their time within the same location, excluding mud) were combined and the percentage of PL going from habitat A to B was calculated. If the PL did not spend above 20% in the same location, the PL was categorised as undecided.

The time to reach sheltering habitat and habitat of choice were analysed by categorising the total time (40-60 minutes) into five-time categories (0-15; 15-30; 30-45; 45-60 and 60+ minutes) and identifying the time at which the PL first entered into sheltering habitats and the chosen habitat (remaining within the chosen habitat for over 35 seconds). The sheltering habitats were the “burrow”, “cobbles” and “tube” habitats.

Distance from the burrow was calculated using ImageJ software (Schneider et al., 2012) with burrow locations taken during data collection. The approximate positions were overlaid on an image of the tank and calculated from the mid-point of the burrow entrance.

6.3. Results

The behaviour of 22 PL were investigated for habitat preference with and without the presence of an adult in the burrow. In addition, the time to reach the habitats and how the PL subsequently moved between each habitat was investigated.

6.3.1. Habitat preference

There was a significant positive correlation between the frequency of entering a habitat and the proportion of time spent (s) within that location (Spearman Rank Correlation Coefficient, $r = 0.91$, $df = 200$, $P<0.001$; see Appendix. 6A).

There was a significant difference in the proportion of time spent (s) in all the locations between treatment 1 (empty) and treatment 2 (occupied) (Paired t-test, $t=13.25$, $df=39$, $p< 0.001$; Figure 6.2).

In both treatments (empty and occupied) the PL spent over 40% of the time in the mud habitat without any disguising features.

Comparing between the two treatments and excluding the mud area, PL in treatment 1 (empty) spent the majority of the time within the burrow habitat (33.70% ± 6.58 s) which was significantly higher than in treatment 2 (occupied, 7.50% ± 2.94 s). However, in treatment 2 (occupied) the PL spent the majority of the time (22.06% ± 5.76 s) within the cobbles habitat which was significantly higher than in treatment 1 (empty) (12.80% ± 3.68 s), and other areas such as the burrow proximity (BP; 18.21% ± 4.79 s) and tube habitats (9.46% ± 3.40 s; Treatment 1; 11.22% ± 1.94 s; 1.94% ± 0.55 s respectively).

The frequency of PL entering the burrow and tube habitats was significantly different between the two treatments (Sign test, P<0.01). However, there was no significant difference in the frequency of PL entering the mud, BP and cobble habitats between the two treatments (Sign test, P>0.05).

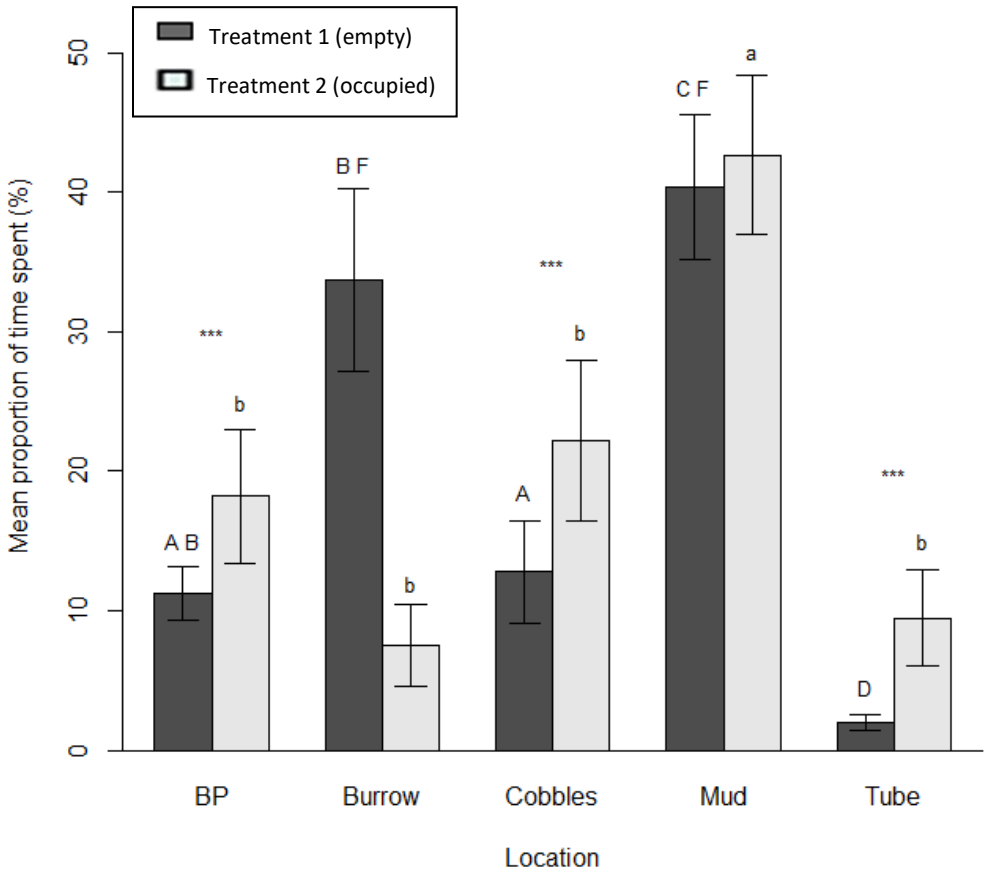


Figure 6.2. Proportion of time spent (s) in each tank location (mean ± SE), N=20. Data was transformed and normalised for analysis, but the original data is shown. Asterisks denote where significant differences lie between the two treatments for each location (Paired t-test, *** P<0.001). Means with different letters are significantly different to other locations within the same treatment (Tukey's HSD, p<0.05). BP= Burrow Proximity; Burrow= In the burrow areas including in the burrow, burrow entrance and burrow ridge.

Time budget analysis comparing time spent in each location within each treatment indicates that there was a significant difference in the time the PL spent in each location for both treatments (ANOVA, P<0.001). In both treatments, the PL spent significantly more time in the mud habitat than in every other habitat (ANOVA, P<0.05), except for within the burrow in treatment 1 (empty) (ANVOA, P= 0.798). The PL also spent significantly more time within the burrow than the cobbles and tube habitats in treatment 1 (empty) (ANOVA, P<0.05). However, in treatment 2 (occupied) there is no significant difference in the time spent in the cobble or borrow areas compared to other locations (ANOVA, P>0.05).

6.3.2. First Shelter Habitat

Overall 55% and 30% of the PL entered the "burrow" and "cobbles" as the first sheltered habitats respectively (Figure 6.3). There was a significant difference in the number of PL that entered the "burrow" and "Tube" as first sheltered habitats between the two treatments (Chi Squared test, χ^2 = 4.55, 5.0, df=1, p<0.05).

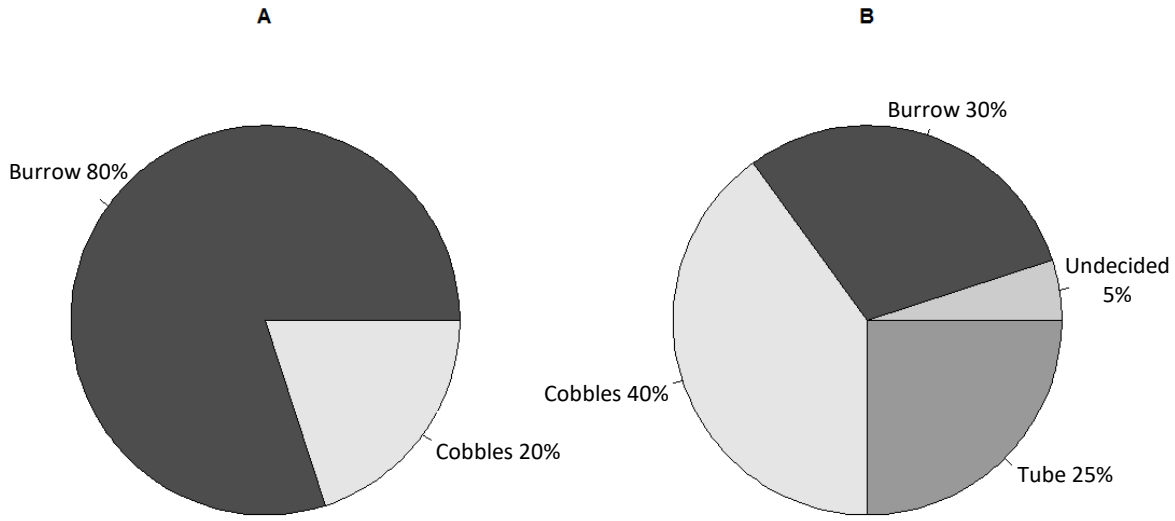


Figure 6.3. First shelter habitat preference of PL for (A) treatment 1 (empty) (B) treatment 2 (occupied). N = 20.

6.3.3. Time to Reach Shelter

PL in treatment 1 (empty) required a greater duration to find sheltered habitat (1046.75 s ± 222.91) but required less time (1420.00 s ± 244.93) to choose the preferred habitat than in treatment 2 (occupied) (817.36 s ± 185.97 and 1786.79 s ± 242.44 respectively), although these were not significantly different (t-test, P>0.05).

Overall 60% of the PL encountered suitable sheltering habitat within the first 10-15 minutes (Table 6.1). In treatment 1 (empty) 25% of the PL took significantly longer (>30 minutes) to reach suitable sheltering habitat, when compared to treatment 2 (occupied; Chi Squared test, χ^2= 5.0, df =1, p<0.05). In comparison 95% of the PL in the occupied treatment found sheltering habitat within 30 minutes. Overall 2.5% of PL did not enter any sheltering habitat within 60 minutes and all were within treatment 2 (occupied).

There was no significant difference found between the two treatments in the time for the PL to choose their preferred habitat (Chi Squared test, χ^2= 0.12, df =1, p>0.05), although 40% and 15% of PL chose within 15 minutes in empty and occupied treatments respectively (Table 6.1). Overall significantly more PL (82.5%) chose their preferred habitat in less than 45 minutes, than taking longer (>45 minutes).

Table 6.1. Time of the PL to find shelter and final preferred habitats between treatments. N=20.

	PL reaching sheltered habitat (%)		PL choosing preferred habitat (%)	
Time (min)	Treatment 1 (empty)	Treatment 2 (occupied)	Treatment 1 (empty)	Treatment 2 (occupied)
0 -15	55	65	40	15
15 - 30	20	30	25	50
30 - 45	20	0	20	15
45 - 60	5	0	5	0
60+	0	5	10	20

6.3.4. Habitat Changing

Figure 6.4 illustrates that in treatment 1 (empty) when the majority of the PL chose the "burrow" as the first sheltering habitat, they remained there as their choice habitat (45%) which was significantly higher than in the occupied treatment (Chi Squared test, χ^2 = 6.40, df=1, p<0.05). In treatment 2 (occupied) the PL had a larger range of shelter habitats and migration to subsequent choice habitats.

Overall net migration from the “burrow” habitat to alternative habitats was >20% in both treatments. More than 15% net migration to the “BP” habitat was observed within both treatments.

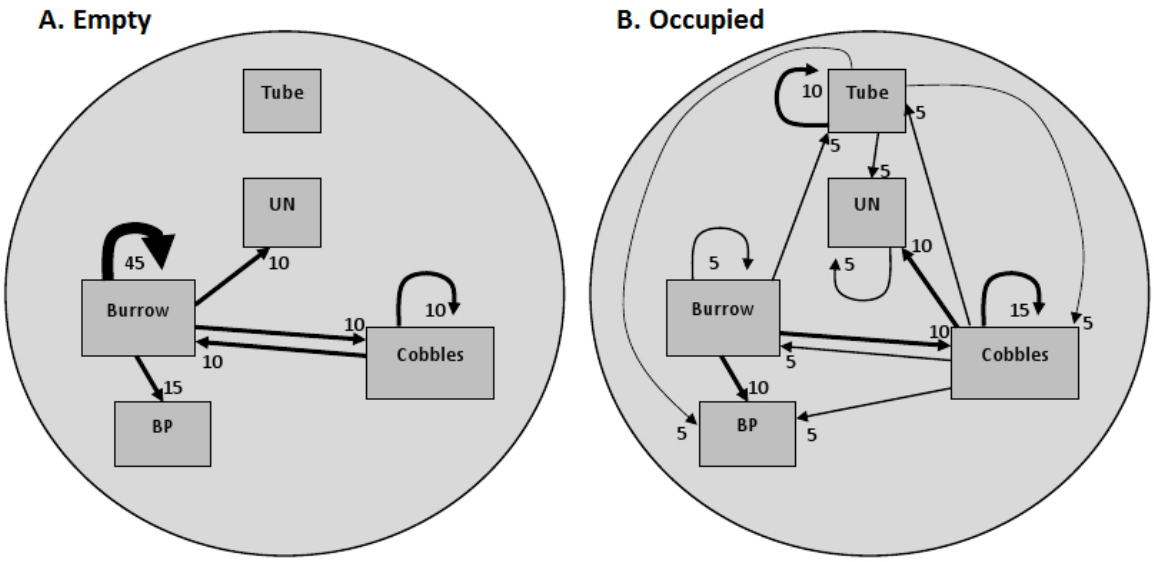


Figure 6.4. A= Treatment 1 (empty) B= treatment 2 (occupied) N=20. Kinematic graph of the PL habitat selection from the first sheltering habitat to the overall choice habitat. Numbers represent percentage of that movement for each treatment. BP= burrow proximity; UN= undecided.

6.3.5. Habitat Behaviours

The majority of all behaviours were followed by “exploratory” (33 – 80%) and/or “stationary” (6 – 50%) behaviours (Figure 6.6). “Retreat” behaviours usually followed “exploratory” behaviour (8%) and “entering the burrow” (5%). The total duration of retreat behaviours exhibited in treatment 1 (empty; 1.25s ± 3.58) was significantly lower than in the occupied treatment (5.5s ± 9.16; Wilcox Signed Rank Test, $P<0.05$). Figure 6.5 illustrates the duration of PL behaviours in each location between the two treatments.

Within the cobble habitat the PL exhibited a range of behaviours that were not significantly different in duration between the two treatments (Wilcox signed rank test, $P>0.05$) except for “retreat” behaviours (treatment 1; empty = 7.00s ± 1.60, treatment 2; occupied= 3.50s ± 1.21; Wilcox signed rank, $P<0.05$). Overall there was a significantly higher percentage of “burrowing” behaviour following behaviours within the cobble habitat (35%), than following within the burrow habitat (5%; Chi squared text, $\chi^2 = 22.5$, df=1, $p<0.001$) (Figure 6.6).

The PL approached the cobble area by “walking” in the cobble proximity (W) and “walking around the cobbles” (WS). These behaviours were followed by “walking on top” (WON; 15.5%) and “under the cobbles” (i.e. walking through and between cavities within the cobbles) (WUN; 13%). There was a significant association of “burrowing” behaviour following from preceding behaviours such as “walking within the cobbles” (INC) and “walking under the cobbles” (WUN; Chi squared text, $\chi^2 = 374.25$, df=10, $p<0.001$; Figure 6.7). There was a high percentage of PL “walking under cobbles” followed by “walking within the cobbles” (INC) behaviours (47%). However, there was no significant difference in the duration of “walking within the cobbles” (INC) behaviours exhibited between treatments (Wilcox signed rank test, $P>0.05$).

PL entered the burrow habitat following “exploratory” behaviours (9%; Figure 6.6). The PL would enter the burrow proximity (BP) area or the BP mound area (BPM) first to enter subsequent burrow areas. In the BP area the PL remained “walking” (44%), with some “burrowing” and “stationary” behaviours. In treatment 1 (empty) PL exhibited longer durations of “stationary” behaviour in the BP mound (11.25s ± 7.31) and burrow entrance areas (17.5s ± 5.61) when compared to treatment 2 (occupied) (2.5s ± 1.38 & 11.0s ± 5.54 respectively; Wilcox signed rank test, $P<0.05$). However, in treatment 2 (occupied) PL remained “stationary” for longer on the burrow ridge (10.75s ± 9.12) and proximity areas (21.5s ± 8.38) when compared to treatment 1 (empty) (1.88s ± 1.0 & 8.0s ± 3.74; Wilcox signed rank test, $P<0.05$).

From the BPM area the PL could enter the burrow entrance area and resume “walking” (14%) or directly enter inside the burrow (34%). This was achieved by walking upside-down on the underside of the burrow ceiling (indicated as TOP; Figure 6.8 & 6.9) and was significantly longer in total duration in treatment 1 (empty; 7.25s ± 2.0) than in treatment 2 (occupied; 2.0s ± 0.84; Wilcox signed rank test, $P<0.05$).

There was a significantly higher total duration of PL exhibiting “within burrow” behaviours (INB) in treatment 1 (empty; 416.25s ± 126.49) than in treatment 2 (occupied; 37.25s ± 18.13; Wilcox signed rank test, $P<0.05$). The PL were observed “burrowing” and “sediment picking”, although the majority of behaviours could not be observed (INB). The PL did exhibit “retreat” behaviours from within the burrow to the burrow entrance and burrow ridge areas, and were

followed by “stationary” behaviours (25 & 28% respectively). The retreat behaviours from all burrow areas were significantly longer in total duration in treatment 2 (occupied; 1.34s ± 0.71) than in treatment 1 (empty; 0.31s ± 0.41; Wilcox signed rank test, $P<0.05$). “Burrowing” behaviour was observed in both the burrow and cobble habitats and was significantly higher in total duration in treatment 1 (empty) for the BP mound (1.38s ± 0.98) and burrow entrance areas (7.25s ± 3.73) when compared to treatment 2 (occupied; 0.0s ± 0.0 & 0.38s ± 0.39; Wilcox signed rank test, $P<0.05$).

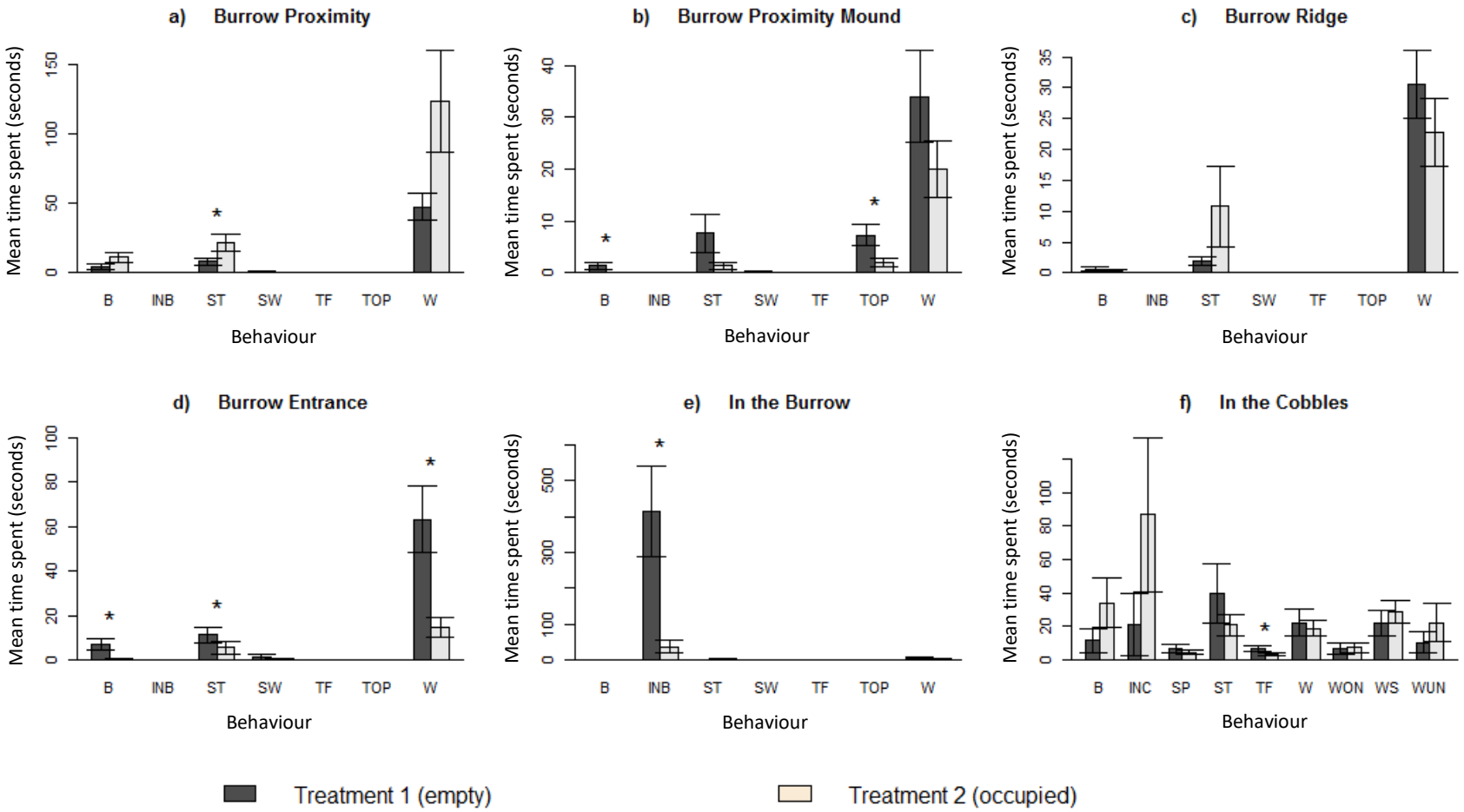


Figure 6.5. Average duration in seconds (Mean ± SEM) of each behaviour exhibited in total for different habitat areas between treatment 1 and 2. N=20. Asterisks above the error bars denote where significant differences lie between treatments for the discreet behaviour and area. B = burrowing; INB = In burrow behaviour; ST = Stationary; SW = Swimming; TF = Escape / Retreat; TOP= Walking on the underside of the burrow ceiling; W = Walking.

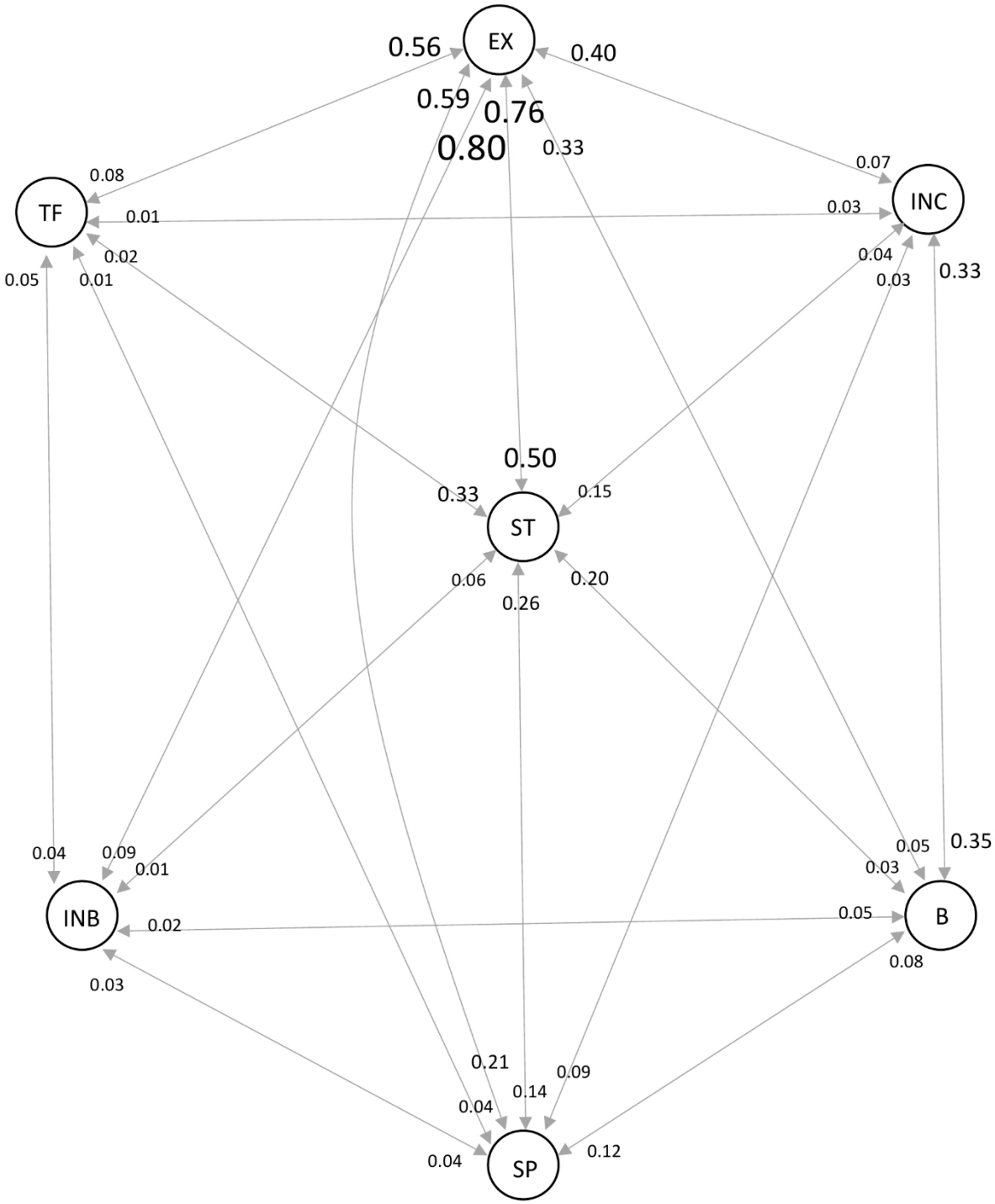


Figure 6.6. The behavioural sequences from both treatments combined. Numbers denote condition probability of one behaviour following the other. N=20. EX= Exploratory; TF= Escape/ retreat; INC=Behaviours associated within the cobble habitat; ST= Stationary; B= Burrowing; SP= Sediment probing; INB= Behaviours associated with the artificial burrow habitat.

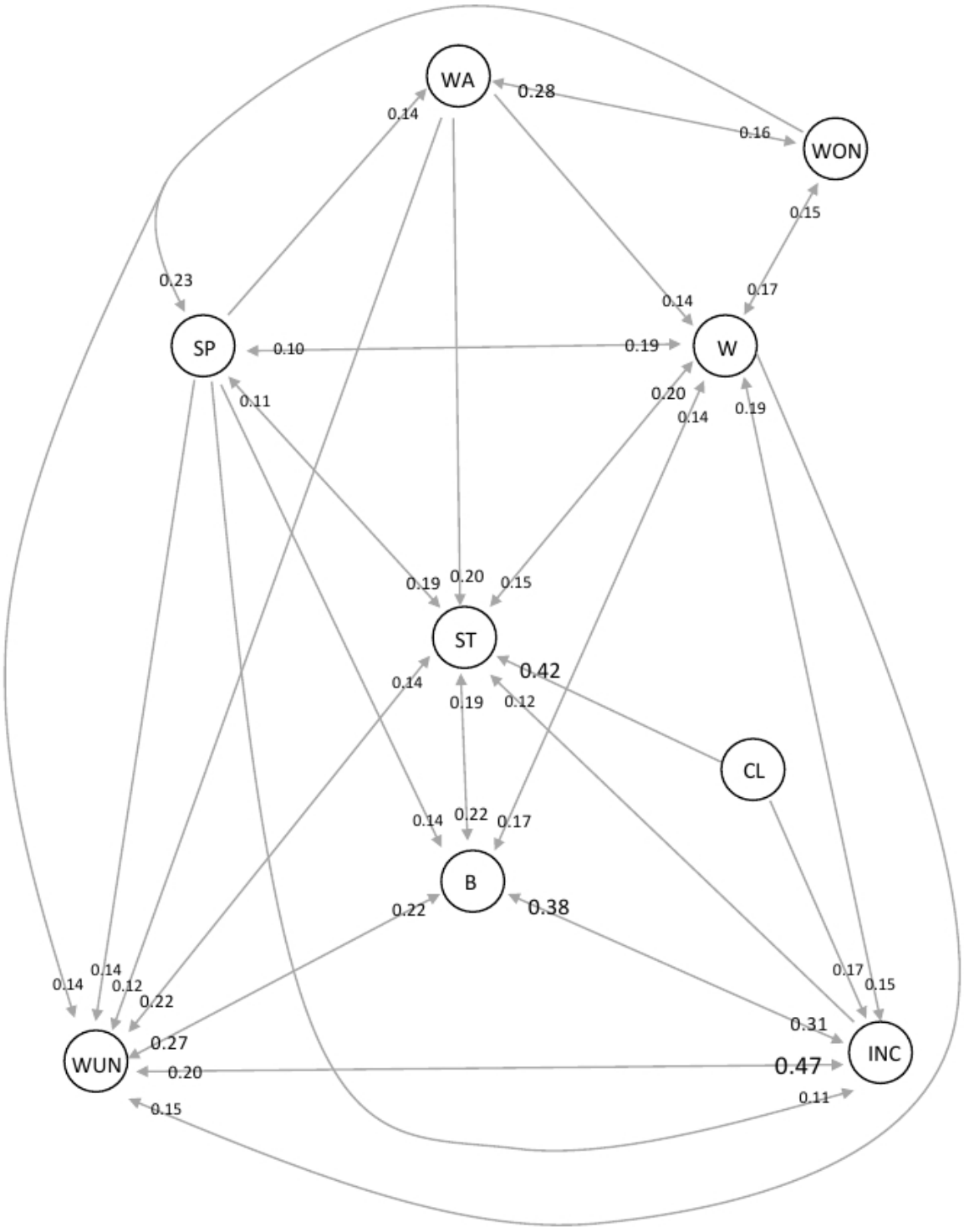


Figure 6.7. Key cobble habitat sequence behaviours. Numbers denote condition probability of one behaviour following the other. WA= Walking around the cobbles; W=Walking in cobble proximity; WON= Walking on top of the cobbles; SP= Sediment picking/eating; ST= Stationary; CL= Cleaning; B= Burrowing; WUN= Walking under the cobbles; INC= Unclear behaviour within the cobbles. Note that only behaviours sequences above 10% are shown.

There were several differences in the frequency of the behavioural sequences in the burrow habitats (Figure 6.8). For example, PL within treatment 1 (empty) had a behavioural sequence of "walking" from the burrow proximity (BP) area to the burrow ridge (BR) area more frequently than treatment 2 (occupied) (Chi squared test, χ^2= 5.12, df =1, p<0.05). This is also illustrated in Figure 6.9 indicating movement probability between areas. Post larvae in treatment 1 (empty) had 13% probability of moving into the BR area which was significantly more than treatment 2 (occupied) (Chi Squared test, χ^2= 3.93, df =1, p<0.05). The PL also showed a higher frequency of "walking" from the burrow entrance (BE) into the burrow (INB; Chi Squared test, χ^2= 33.38, df =1, p<0.05) than in treatment 2 (occupied).

The PL also displayed more exploratory behaviours such as continuous "walking" in the areas closer to the burrow entrance in treatment 1 (empty) compared to treatment 2 (occupied) (Chi Squared test, χ^2= 67.33, df =1, p<0.05). The PL also showed more of the following burrowing related sequences in treatment 1 (empty) such as "walking" followed by "sediment picking" (Chi Squared test, χ^2= 19.0, df =1, p<0.05), continual "sediment picking" (Chi Squared test, χ^2= 14.0, df =1, p<0.05), and "sediment picking" followed by "stationary" behaviours (Chi Squared test, χ^2= 10, df =1, p<0.05) in the BE area when compared to treatment 2 (occupied). PL had a 65% probability of remaining in the BE area in treatment 1 (empty) which was significantly higher than treatment 2 (occupied; 58%; Chi Squared test, χ^2= 107.92, df =1, p<0.05).

The method used by the PL to enter the burrow was also different between treatments. A higher frequency of PL entering from the "TOP" following "walking" in the BPM area was higher in treatment 1 (empty) than treatment 2 (occupied) (Chi Squared test, χ^2= 11.00, df =1, p<0.05), and "walking" from the BE into the burrow (INB) was higher in treatment 1 (empty) than the treatment 2 (occupied) (Chi Squared test, χ^2=20.45, df =1, p<0.05). Overall there was a significantly higher probability of PL going from the BPM and BE areas into the burrow (INB) in treatment 1 (empty) than treatment 2 (occupied) (Chi Squared test, χ^2= 12.45; 48.0, df =1, p<0.05).

If the PL were in the burrow, the frequency of the next sequential behaviour being exhibited and located "within the burrow" was higher in treatment 1 (empty) than treatment 2 (occupied) (Chi Squared test, χ^2= 1212.36, df =1, p<0.05).

However, in treatment 2 (occupied) the PL showed a significantly higher frequency of "walking" from the BE to BR area than in treatment 1 (empty; Chi Squared test, χ^2= 6.15, df =1, p<0.05) and going from INB to the BE area (Chi Squared test, χ^2= 43.63, df =1, p<0.05). More frequent vigilant behavioural sequences were observed in treatment 2 (occupied) than treatment 1 (empty), such as "stationary" behaviours following "stationary" behaviours in areas just out of the burrow, in the BR and BP areas (Chi Squared test, χ^2= 51.95; 30.55, df =1, p<0.05).

PL in treatment 2 (occupied) also showed a higher frequency of the following behavioural sequences in the BP area: "walking" (Chi Squared test, χ^2= 102.57, df =1, p<0.05), "walking" to "stationary" (Chi Squared test, χ^2= 10.67, df =1, p<0.05), and "stationary" to "walking" (Chi Squared test, χ^2= 10.26, df =1, p<0.05) when compared to treatment 1 (empty).

In addition, PL showed higher frequencies of exhibiting behavioural sequences such as "burrowing" following from "walking" (Chi Squared test, χ^2= 6.04, df =1, p<0.05) and *vice versa* (Chi Squared test, χ^2= 6.04, df =1, p<0.05) than in treatment 1 (empty).

Figure 6.9 illustrates the overall movement of the PL from one habitat area to another within the burrow area. The figure illustrates that there is an overall trend that the sequence probability of PL moving towards the INB area is higher in treatment 1 (empty) from where ever the PL first entered an area, compared to treatment 2 (occupied). However, in treatment 2 (occupied) there is an opposite trend that the PL had a higher probability of moving away from the INB area, for example a higher sequence probability of PL moving from the INB to the BE and the BPM to the BP areas. PL also had a higher probability of remaining in the BP and BR areas in treatment 2 (occupied), but had a higher probability of staying in the BPM, BE and INB areas in treatment 1 (empty). The frequency of PL moving into the INB area was significantly higher in treatment 1 (empty) when PL came from the BPM and BE areas.

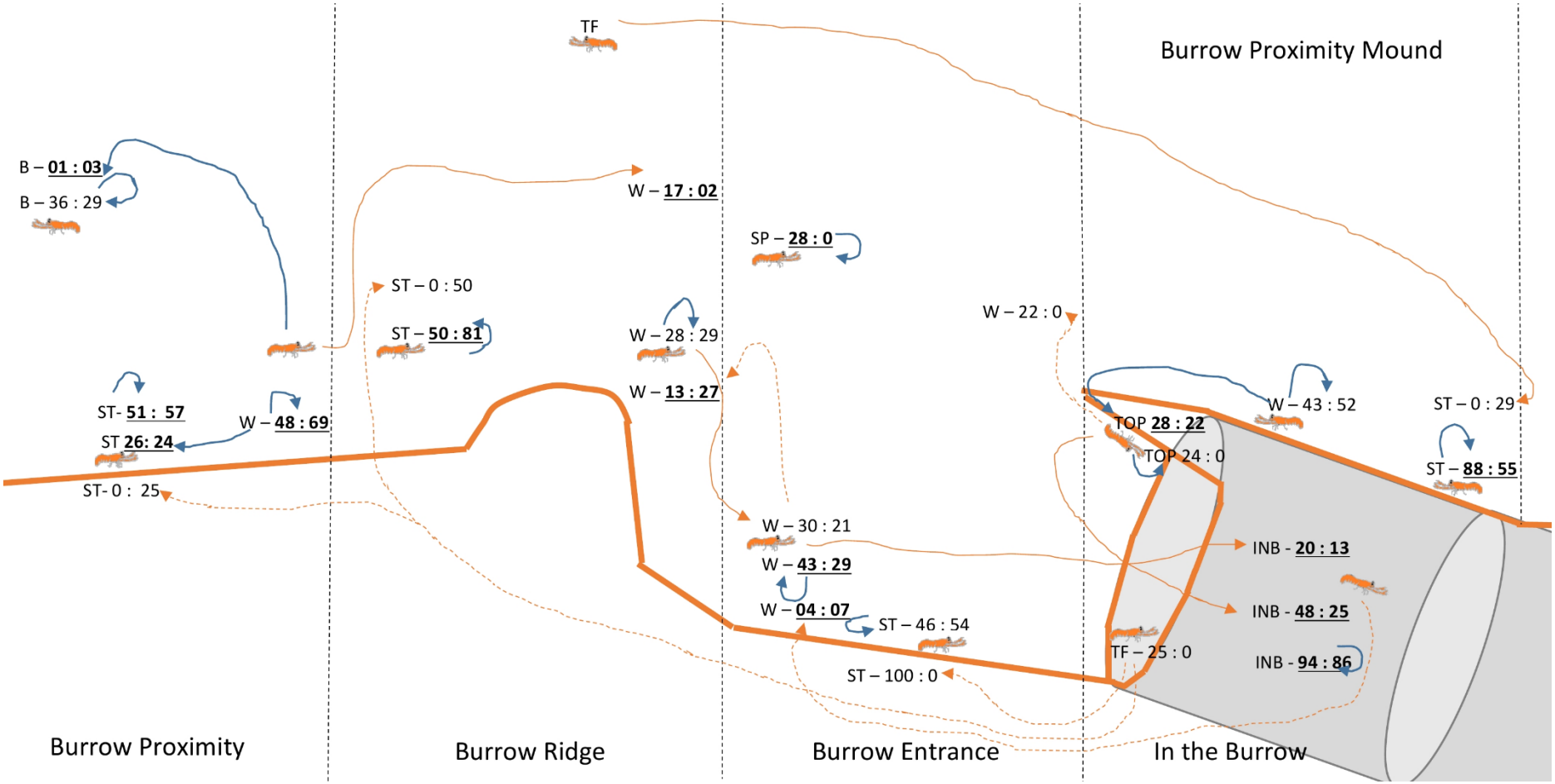


Figure 6.8. Major sequence behavioural probabilities in the burrow areas between treatment 1 (empty) and treatment 2 (occupied) displayed as (empty : occupied). Probabilities (%) that are underlined indicate a significant difference in the observed frequencies of behavioural sequences between the treatments, rather than the probabilities (Chi Squared, P<0.05). Arrows indicate the direction of sequence, blue arrows indicate sequences within the same burrow area, orange arrows indicate sequences going into another burrow area. Dashed arrows denote behaviours moving away from within the burrow. B= burrowing; INB= in the burrow; SP= sediment picking; ST= Stationary; SW= Swimming; TF= retreat; TOP= entering into the burrow on the underside of the burrow; W= walking.

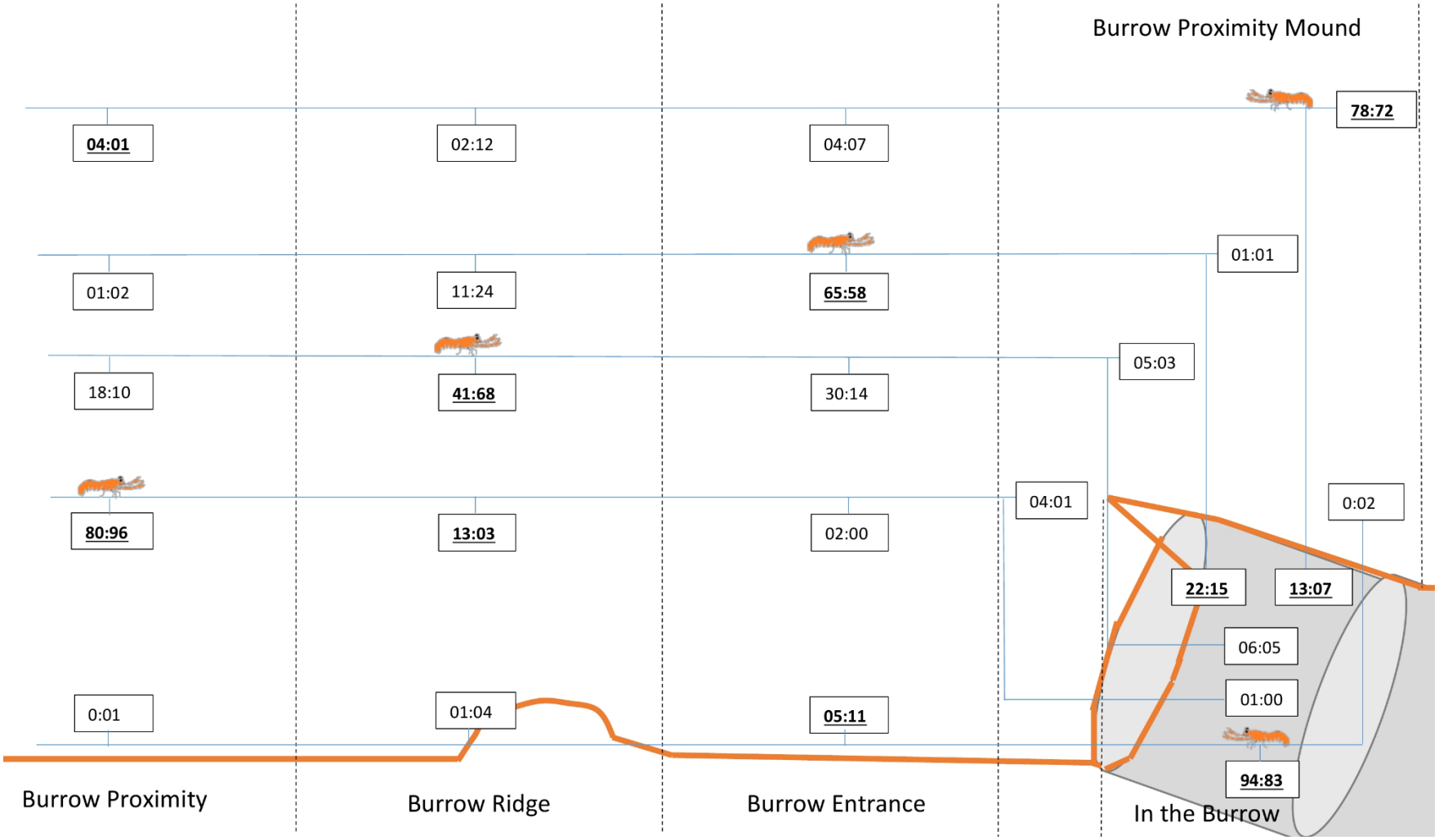


Figure 6.9. The sequence probability of the PL moving from one burrow habitat to another burrow habitat between treatment 1 (empty) and treatment 2 (occupied) (indicated by empty : occupied). The illustrated PL indicates the starting burrow habitat area with each line depicting the movement to each burrow area. Numbers in bold and underlined denote where significant differences lie in the location frequency sequence between treatments (Chi Squared test, P<0.05).

6.3.6. Burrowing Behaviour

The number of PL that exhibited burrowing behaviour was significantly higher in treatment 2 (occupied; n=16) with significantly more burrows excavated (n=27) than in treatment 1 (empty; n=6 & 12 respectively; Chi Squared test, χ^2= 4.55; 5.77, df =1, p<0.05). The total number of burrowing behaviour bouts (defined as a set of behaviours observed in continuous sequence, *i.e.* over five seconds) was larger during treatment 2 (occupied; n=118) than in treatment 1 (empty; n=37; Sign test, p<0.001), with each bout having an average duration of 8.48s ± 0.52.

The total duration of burrowing behaviour exhibited (Figure 6.10) was also higher in treatment 2 (occupied; 60.94s ± 12.71) than treatment 1 (empty; 18.75s ± 11.29) (Paired t-test, t=2.36, df=15, p<0.05).

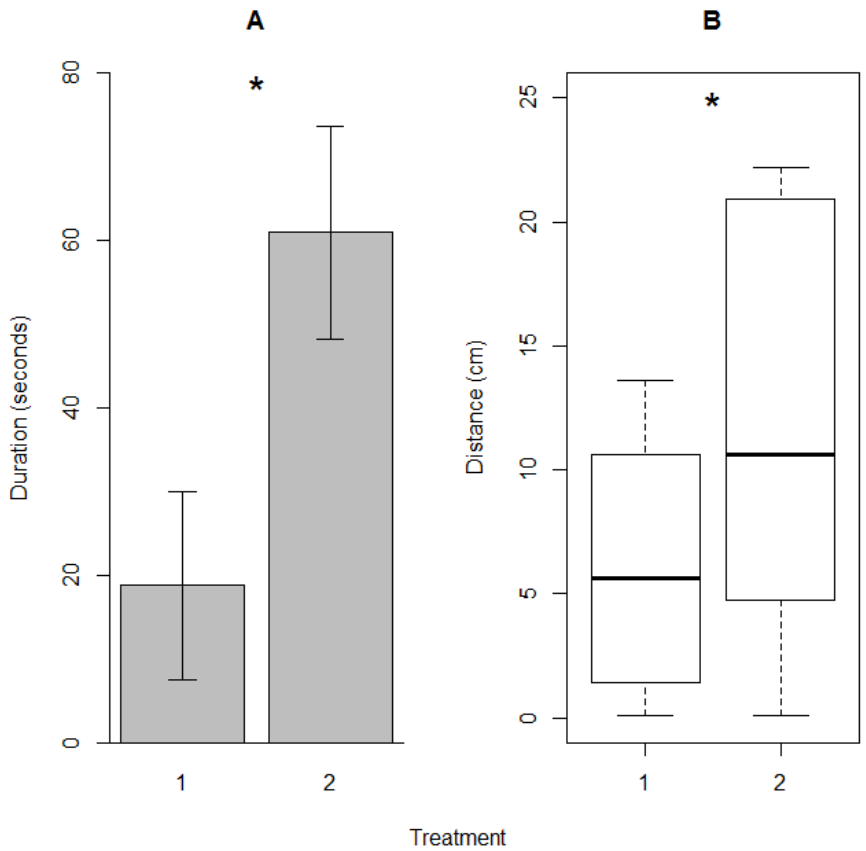


Figure 6.10. (A) The duration of burrowing behaviour (mean ± SEM, n=20) and (B) the average distance of constructed burrows from the artificial burrow entrance between treatments (mean ± SEM, n=20). Treatment 1 = empty; Treatment 2 = occupied. Asterisk denotes where significant differences lie between the treatements (P<0.05) using (A) t-test and (B) Wilcoxon signed rank test.

The number of burrows excavated was significantly higher only in the tube habitat when compared between the treatments (Chi Squared test, $\chi 2= 5.0$, df =1, $p<0.05$), although there was no difference in the number of burrows (Chi Squared test, $p>0.05$) or the duration of burrowing behaviour between habitats (Wilcoxon signed rank test, $P>0.05$).

The average distance of the burrows excavated from the burrow entrance within treatment 2 (occupied; 11.58 cm ± 1.40) was significantly larger (almost double) when compared to the treatment 1 (empty; 6.02 cm ± 1.41; Wilcoxon signed rank test, $p<0.05$).

Burrowing behaviour consisted of the following behaviours: The sediment was probed and manipulated firstly with the third maxillipeds and chelae. The excavated sediment was transported by use of the chelae, maxillipeds and pereopods to bulldoze the sediment away and form a groove in the sediment. The PL worked in opposite directions to create a 'groove' within the sediment and used pleopod fanning to flush loose sediment away. The PL also performed backward digging by pulling the substrate backwards.

6.4. Discussion

Previous attempts to create artificial burrow in sediment have proved unsuccessful with little interest shown by the PL (Smith, 1987). However, this study highlights that PL were interested in an artificial burrow that had not been excavated by an adult, but rather mimicked the characteristics of one (albeit the adult cannot leave).

The PL spent more time in the burrow habitat when it was empty compared to when it was occupied. The high frequency of PL entering the burrow and the time remaining within the burrow in the empty treatment (treatment 1) indicates the attractiveness of the burrow and the minimal interest in alternative habitats, whereas this was the opposite within the occupied treatment (treatment 2). An interesting observation was made of how the PL entered the burrow. The PL entered by walking from the burrow mound and into the burrow opening on the underside of the burrow ceiling. It is unclear as to why the PL exhibited this behaviour, which occurred significantly more when the burrow was empty than when occupied. One could assume that this offers a strategical advantage by reducing the likelihood or efficiency of an attack from an adult (Arnott et al., 1999) or is related to the body orientation and potential escape trajectory (Guerin and Neil, 2015), although this has not been tested within *Nephrops*. Another explanation may be due to the PL initiating its own

burrow complex within the adult burrow. The study from Tuck et al. (1994) indicates that 17% of the adult burrows sampled incorporated juvenile burrows, although this percentage may be an overestimation due to bias, no independent juvenile burrows were recorded. Visual inspection of the casts of adult-juvenile burrow structures indicates that that the juveniles excavate narrow tunnels connected from the adult opening and main tunnel channels that rise steeply to the surface providing independent access or ventilation (Chapman, 1980; Smith, 1987; Tuck et al., 1994). Other narrow tunnels are also excavated along the upper and side surface of the adult tunnel. The number of PL that were observed in this study exhibiting burrowing behaviour within the artificial burrow may be under-represented in our results (12.5%), as it was difficult to observe any behaviour more than 1-2cm into the burrow tunnel.

It is interesting that although the frequency of entering the burrow proximity and cobble locations were not significantly different between the two treatments, there was however a significant difference in the proportion of time spent in those locations. The PL in the occupied treatment remained in these areas longer than in the empty treatment. The significant difference between the two treatments may indicate that the PL could sense the adult occupying the burrow in treatment 2 and thus may indicate evidence of PL displaying vigilant behaviour. *Nephrops* use a range of receptors to detect and perceive the environment. The olfactory sensilla are used to detect chemical signals and odour detection in social interactions, whilst mechanoreceptors detect movement (Katoh et al., 2013). This may illustrate the trade-off between being near the burrow entrance and waiting for an opportunity to quickly enter the burrow at a time when it is less guarded, and so less likely for an attack from a predator or the guarding *Nephrops* adult. Stationary behaviours within the burrow proximity and ridge may indicate that these locations are a reasonable distance away from initiating an encounter with the residing adult. Chapman and Rice (1971) describe that fighting between two adults was initiated when an intruder entered the burrow entrance area to the burrow, but not in the burrow proximity.

The detection of the adult would also explain more retreat behaviours exhibited from the burrow habitat when it was occupied, which is similar to the findings of Smith (1987), where PL were chased from the burrow opening by the occupying adults. The PL in this study retreated by tail flipping, swimming and rapid walking to areas such as the burrow entrance and ridge. The PL subsequently re-located and explored a range of alternative habitats. The

lower rate of PL entering the burrow could have significant influences upon recruitment to a sub-population. It is not known if larger conspecifics are an important factor in regulating *Nephrops* recruitment in the wild.

When the burrow was occupied the PL spent a large majority of time within the cobble habitat. The cobbles and tube habitat provide a thigmotactic stimulus and offer relative shelter from predators. Several times the PL were observed to press themselves alongside the tube or tank side and to start burrow excavations against key features within the cobble and tube habitats. Previous research indicates that stage 1 PL show little interest in burrowing and are still in the stage of changing from planktonic larvae to benthic PL (Smith, 1987). However, PL within this study were intent in burrowing behaviour and burrow area investigation. Within the cobble area the PL would walk under and within the cobbles before burrowing. If there was an opening between the substrate floor and an overhang of part of the cobble the PL would start burrowing behaviour under the cobble overhang. Similarly other juvenile crustaceans burrow under features such as stones, cobbles and tiles to provide a burrow shelter, and are potentially a key habitat for benthic recruitment (Berrill, 1974; Wahle and Steneck, 1991; Wickins and Barry, 1996). Burrowing behaviour in this study was similar to that previously described for *Nephrops* (Rice and Chapman, 1971; Farmer, 1974a; Smith, 1987; Wickins et al., 1996), although the PL also performed backward digging by pulling the substrate backwards, which has not previously been described.

The potential of using substrata that provide shelter for *Nephrops* PL could be a promising development in knowledge and could be used to enhance recruitment, especially on high density *Nephrops* grounds, where survival is lowered due to the apparent unwillingness of PL to enter an occupied burrow. It may be possible that cobble habitats could provide settlement areas near to *Nephrops* grounds, with a later ontogenetic shift to a preference for mud habitat as the juvenile grows and starts to construct its own burrow complex (Chapman, 1980). In contrast to Smith (1987) the PL in this study did not remain stationary in the excavated substrate, rather the PL would either continue to burrow when in the cobble habitat, or further explore the tank when not in the cobble habitat.

More than half of the PL came across suitable shelter habitat (the burrow or cobbles) within 15 mins of entering the tank. This indicates that the PL were actively exploring the tank when

released onto the substrate during this time. PL within the empty treatment took longer to come across shelter habitats when compared with the occupied treatment. The presence of conspecifics has been shown to reduce settlement time due to attraction to the suitable habitat when in the planktonic stages and by further reducing the time to find suitable shelter habitat (Fletcher, 2006; Burdett-Coutts et al., 2014). It is hypothesised that added intraspecific competition or a potential predator as in this study, has caused increased burrowing behaviour (frequency, duration and number of burrows) and has reduced the time to reach shelter habitats. This has been shown in other lobster behavioural release studies where preference for shelter-providing habitats has reduced mortality from potential predators (Chapman, 1980; Wahle and Steneck, 1992; Meager et al., 2005; Van Der Meeren, 2005; Burdett-Coutts et al., 2014). However, some studies contradict this and indicate that naïve hatchery-bred individuals 'freeze' in the presence of predators and/or increase the time to find shelter due to increased fighting over resources (Johns and Mann, 1987; Van Der Meeren, 2005).

The PL in this study excavated burrows that were at a greater distance away from the artificial burrow entrance when there was an adult residing within. The burrows in the wild are described as occurring in discrete groups, not evenly spaced or distributed at random, with a maximum density (no/m^2) of 2-2.5 (dependent upon favourable sediment composition) (Chapman and Rice, 1971; Rice and Chapman, 1971; Johnson et al., 2013). It is clear that the survival of released lobsters is dependent upon a range of factors (Van Der Meeren, 2005). Finding suitable habitat and shelter is one key factor in reducing predation especially in open sandy habitats such as *Nephrops* grounds. However, there is little evidence in the field of independent juvenile burrows in close proximity to the adults. Therefore one may assume if released *Nephrops* cannot gain entry to adult burrows, or find alternative shelter-providing habitats, then survival could be significantly reduced. Further to this, experiments indicate that released lobsters could be attacked by predators within 15 minutes and are most vulnerable within the first 24 hours (Wahle and Steneck, 1992; van der Meeren, 2000; Van Der Meeren, 2005). Overall the PL in this study found the preferred habitat and remained in that location within 45 minutes of release. If shelter-providing habitats are provided in close proximity to the release site, the time in which PL explore and enter sheltering habitats can be reduced, potentially reducing predator related mortality. The results also suggest the

importance of specific substrates and structures for holding animals, allowing them to perform natural behaviours, thus improving welfare and potentially survival.

In summary there was found to be a difference in PL behaviour and habitat selection dependent upon burrow occupancy. Alternative habitats such as cobbles were used and may be an important settlement habitat similar to the case in *Homarus* sp. The mechanisms of entering the adult burrows are complex, with the PL quickly observing if the burrow is occupied or not.

Appendix. 6A Supplementary Data

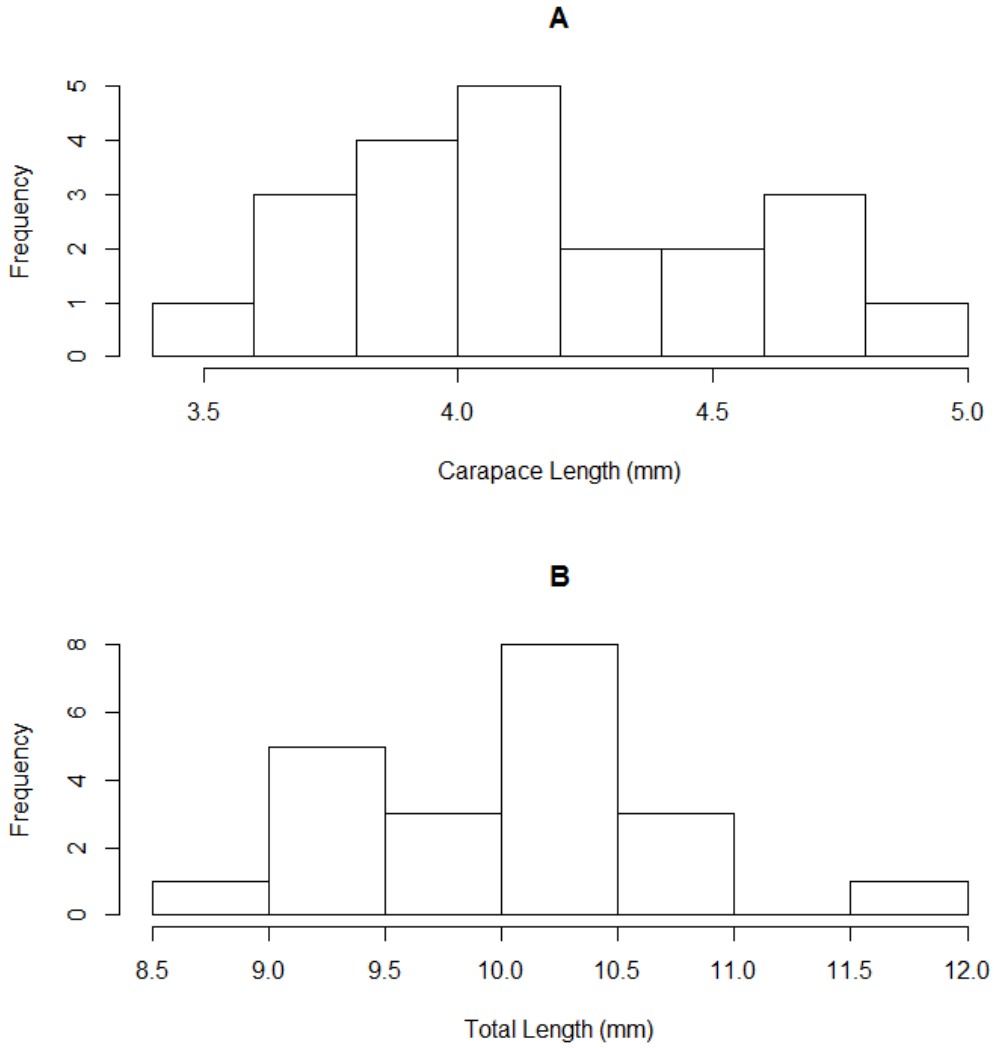


Figure 6.11. Histogram plot of A) carapace length (mm) and B) total length (mm) of PL.

Table 6.2. Ethogram of behaviours used for the PL release behavioural trials. Modified from Wickins et al. (1996).

Code	Analysis grouping	Description
W	W	Walking (not with thigmotactic influence)
WS		Walking along the side of a solid object. For example tank walls, cobbles, IN pipe.
WPL / WSPL		W or WS with pleopod fanning
Sw	SW	Swimming
S	ST	Stationary
Pl		Pleopod Fanning
SPL		Stationary pleopod fanning
Wall	WALL	Climbing on the walls of the tank or IN pipe
WON	WON	Walking on top of the cobbles
UN	WUN	Walking under the cobbles (where gaps between two or more cobbles create an enclosed area / tunnel)
CL	CL	Cleaning – this could be either the eyes, telson or pleopods.
SP	SP	Substrate probing or picking – using the cheliped to manipulate the substrate
SE		Sediment Eating – similar to SP but then using the mandibles to further test the substrate
B	B	Bulldozering or burrowing behaviour
BD		Backward Digging – similar to B but using the cheliped to pull the sediment rather than push the sediment
BE	W	Burrow Entry – walking into the burrow entrance
TF	TF	Tail flick / escape response
R		Retreat – rapid movement away or withdrawing
T		Touch and then stop moving or Retreat
UP	ST	Upside down in dorsal recumbency
INB	INB	Inside the burrow and unable to observe the behaviour
INC	INC	Inside the cobble area and unable to observe the behaviour
TOP	TOP	Walking into the burrow upside down along the ceiling of the burrow
TUBE	TUBE	On the burrow inflow pipe
OTHER	OTHER	Curling or squeezing all the pereopods forwards

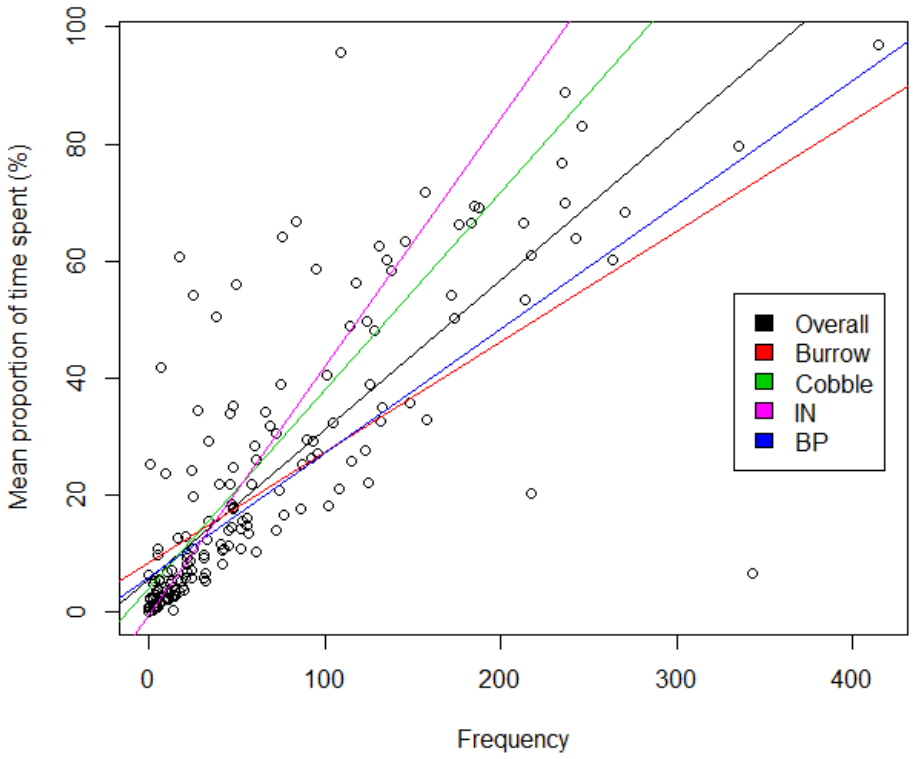


Figure 6.12. Scatterplot of frequency of the PL entering habitats and the proportion of time spent in the habitats.

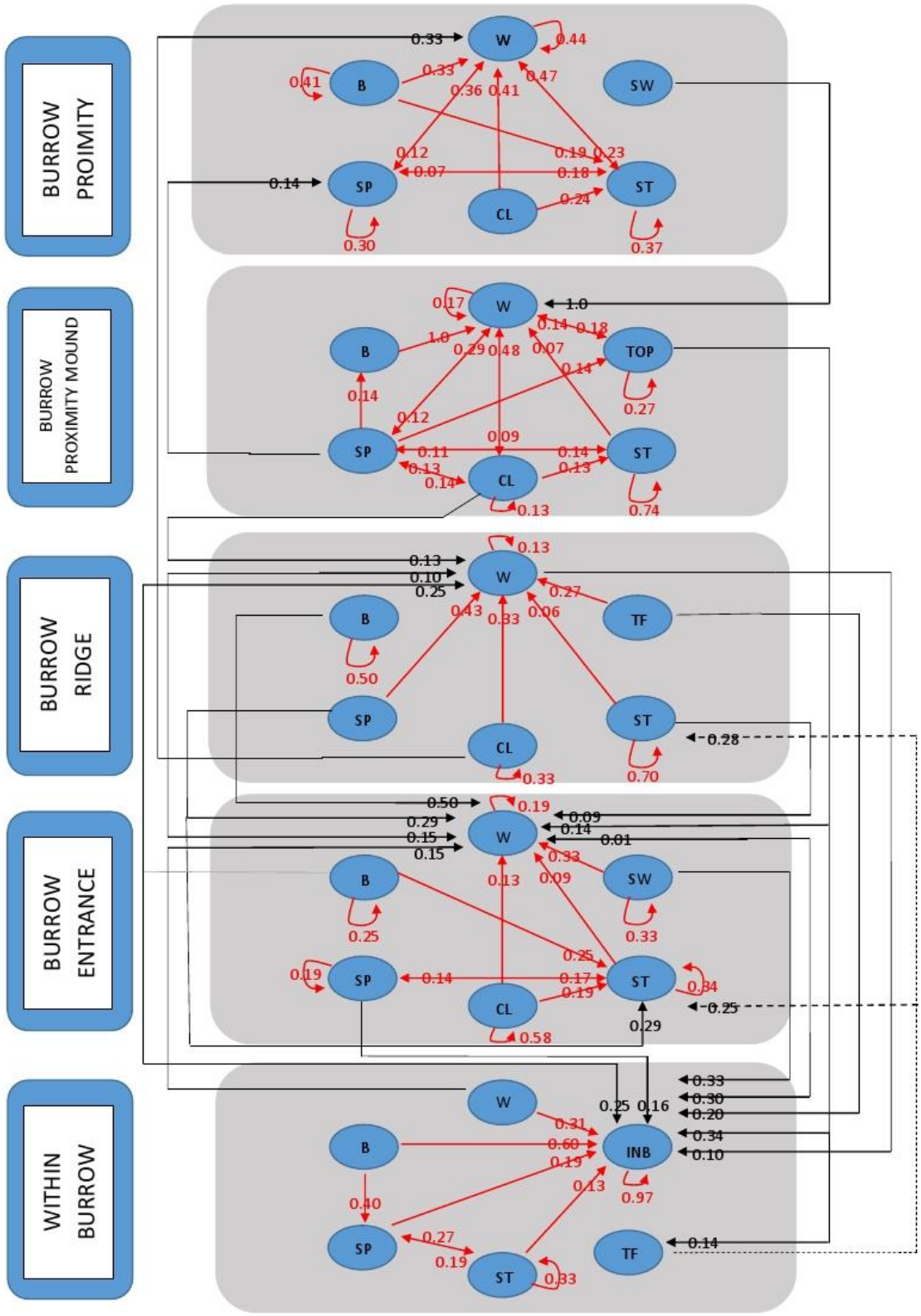


Figure 6.13. Key sequence behaviours and movement between burrow habitats. Numbers denote condition probability of one behaviour following the other. N.B. the dashed line indicates retreat behaviours. W= Walking; ST= Stationary; SP= Sediment Picking; SW= Swimming; CL= Cleaning; B= Burrowing; TF= Retreat; INB= within burrow; TOP= Underside entrance walking.

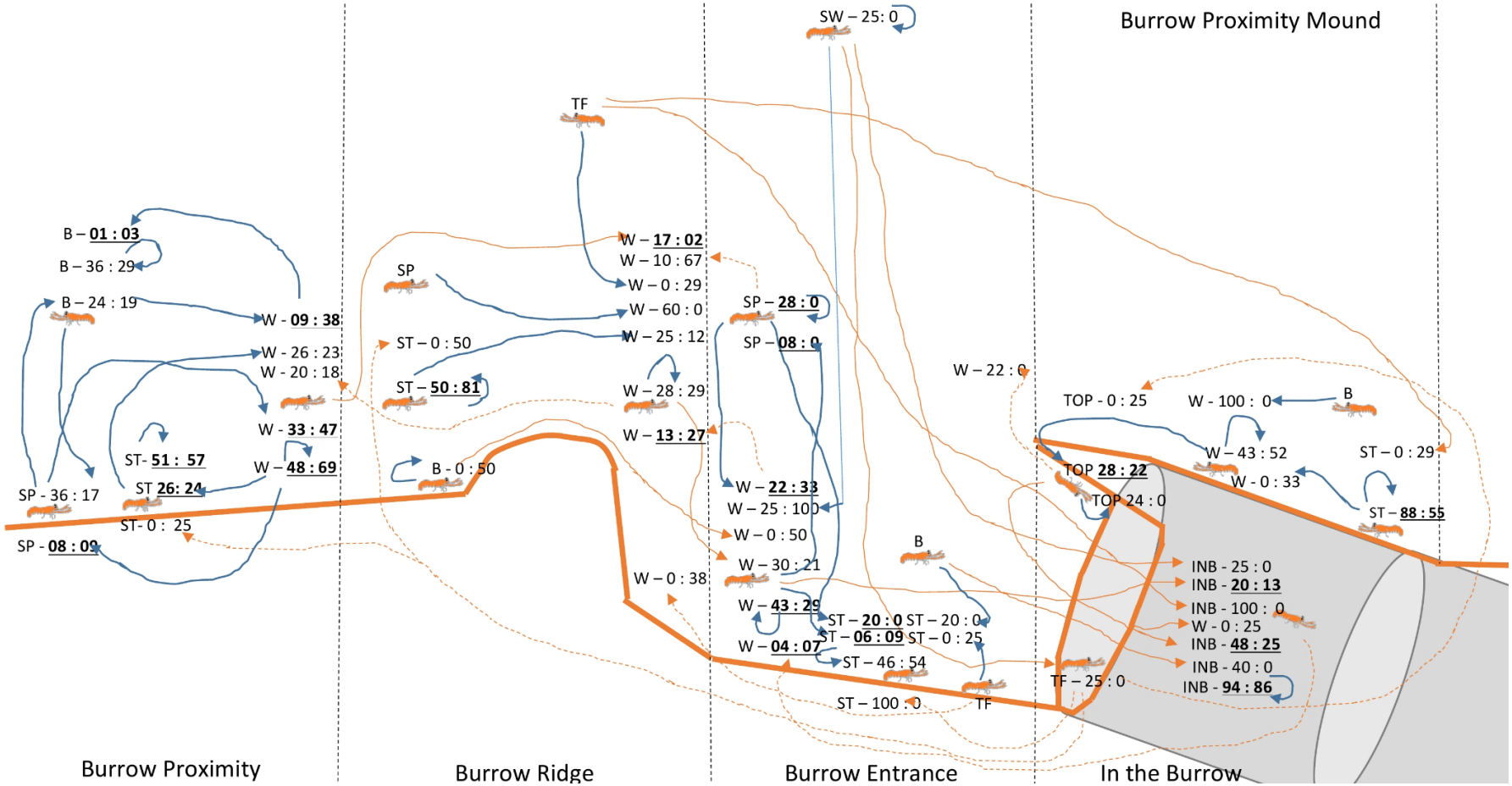


Figure 6.14. A more comprehensive figure (complementary to figure 6.8) on the sequence behavioural probabilities in the burrow areas between treatment 1 (empty) and treatment 2 (occupied) displayed as (empty : occupied). Probabilities (%) that are underlined indicate a significant difference in the observed frequencies of behavioural sequences between the treatments, rather than the probabilities (Chi Squared, P<0.05). Arrows indicate the direction of sequence, blue arrows indicate sequences within the same burrow area, orange arrows indicate sequences going into another burrow area. Dashed arrows denote behaviours moving away from within the burrow. B= burrowing; INB= in the burrow; SP= sediment picking; ST= Stationary; SW= Swimming; TF= retreat; TOP= entering into the burrow on the underside of the burrow; W= walking

Table 6.3. % of PL first entering a habitat and then relocating to the preferred habitat.

Habitat A → Habitat B		Treatment 1 (Empty) %	Treatment 2 (Occupied) %	Overall %
Burrow	Burrow	45	5	25
	BP	15	10	12.5
	TUBE	0	5	2.5
	Cobbles	10	10	10
	Undecided	10	0	5
Cobbles	Cobbles	10	15	12.5
	BP	0	5	2.5
	TUBE	0	5	2.5
	Burrow	10	5	7.5
	Undecided	0	10	5
TUBE	TUBE	0	10	5
	Cobbles	0	5	2.5
	BP	0	5	2.5
	Undecided	0	5	2.5
Undecided	Undecided	0	5	2.5

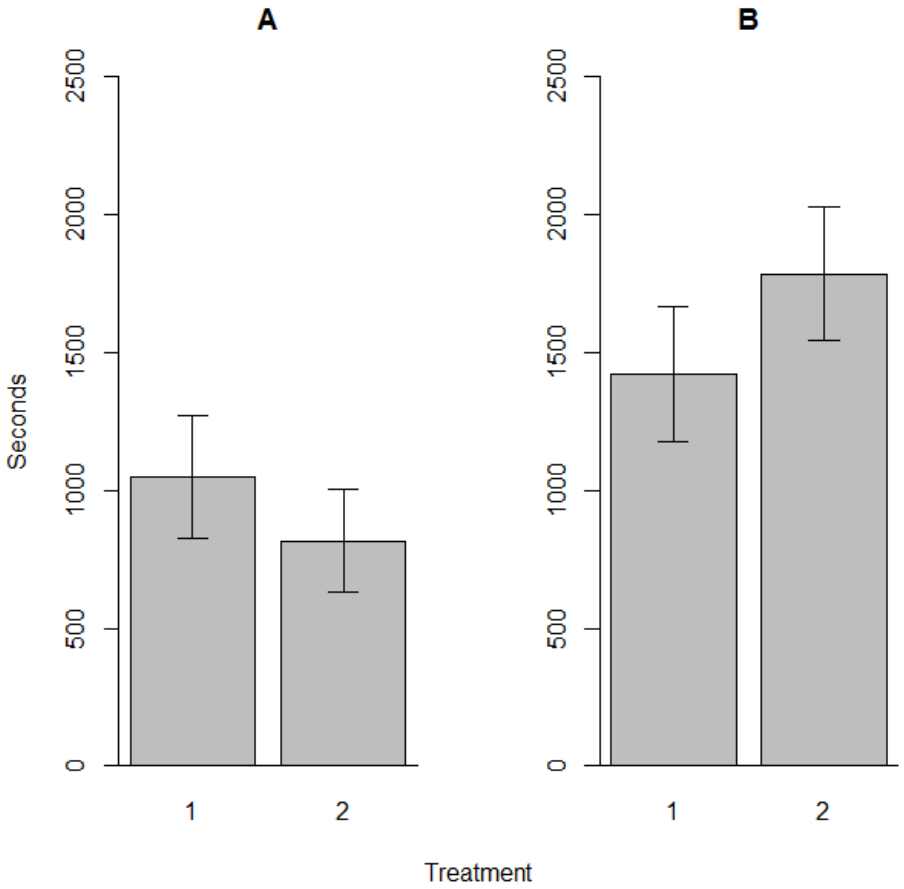


Figure 6.15. Average time (seconds) to (A) reach sheltered habitat and (B) to choose the preferred habitat. N=20. 1= Treatment 1; 2= Treatment 2.

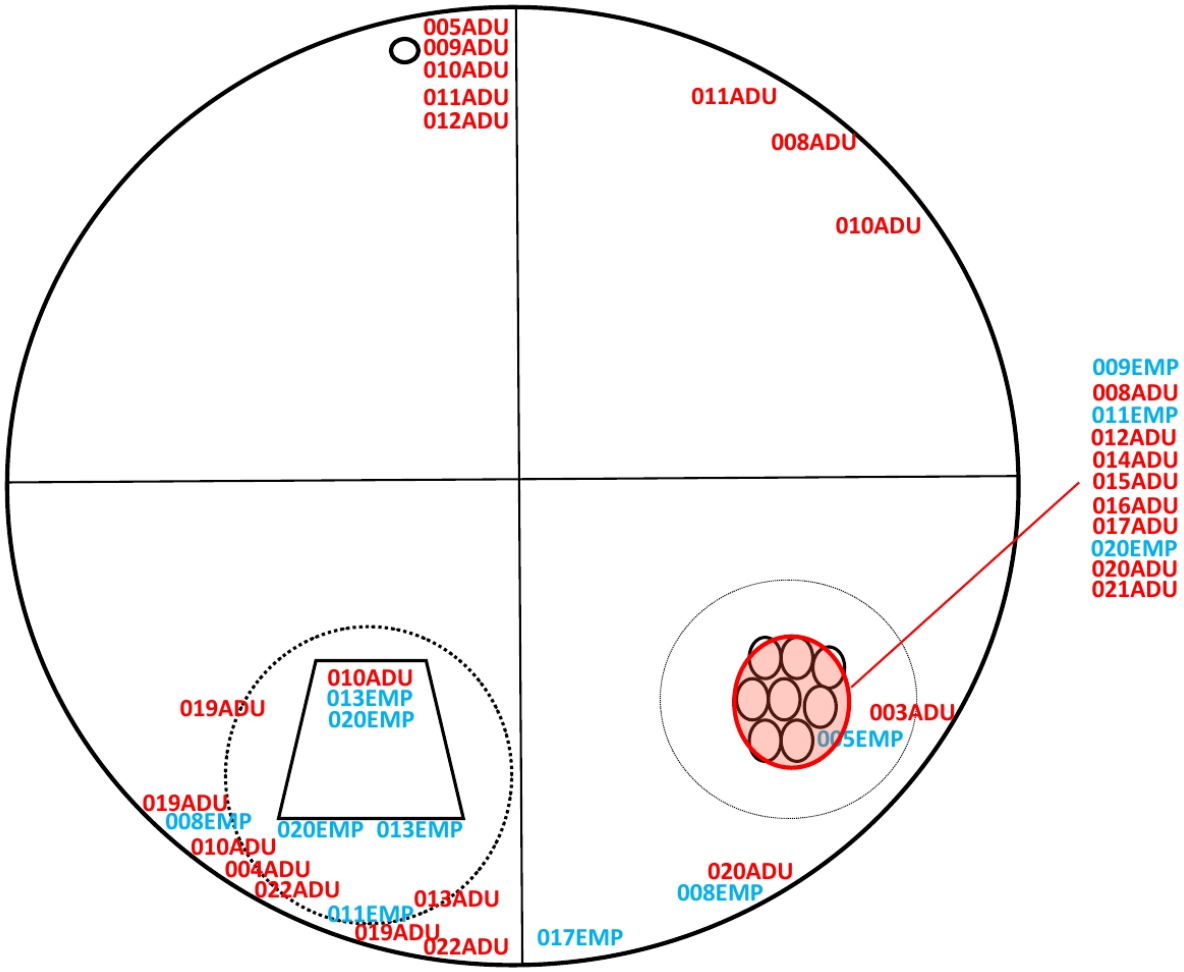


Figure 6.16. Location of PL burrows. EMP= Treatment 1; ADU = Treatment 2.

7. Conclusion

The aims of this thesis were to advance *Nephrops norvegicus* aquaculture techniques, from broodstock procurement through to PL release, and to gain insight into their biology, physiology and behaviour. Therefore, the aims of this thesis were to:

- Identify *Nephrops* stress parameters, the principles that govern social interaction and the implications this has for hatchery engineering and husbandry as well as fisheries practices and their ecological impacts;
- Investigate and develop a methodology to reduce stress levels in broodstock;
- Improve the survival and shorten the development time of larvae;
- Gather behavioural data that would benefit the release activities of PL.

The information provided in this thesis could be used to evaluate the viability and potential of developing hatchery activities for the potential purpose of stock enhancement, restocking, and the aquaculture of *Nephrops*. Information thus far on larval rearing and broodstock has been limited to small-scale experiments and ecological studies, without specific focus on commercial applications. This thesis has provided an overview of key issues encountered throughout the hatchery process such as;

- High mortality of broodstock and egg loss during capture, transport and holding;
- Low survival of larvae and bottlenecks during the development stages;
- The lack of knowledge on PL behaviour to adapt and develop releases strategies.

7.1. Review of Findings

The findings of each chapter are discussed below in relation to the key issues described above, highlighting the study limitations, and potential direction for future research.

Behavioural and physiological studies have been used to further understand practical issues such as stocking densities, heightened aggression, lower growth and development in aquaculture for commercially important species (Ellis et al., 2002; Sloman and Armstrong, 2002; Gilmour et al., 2005). This thesis has widened our knowledge on the physiological consequences of social stress between conspecifics and the successful identification of several haemolymph-based stress parameters within *Nephrops* that identify change in their baseline levels due to social interaction. Some parameters that have been used elsewhere to

indicate fishing related stress did not significantly change in conjunction with social related stress or were masked by individual state-specific differences. It was found that there are significant differences in exhibited levels of aggression between different sexes and sizes. The levels of aggression were reduced within females and between individuals of differing sizes. However, this study did not investigate mixed sex with mixed size, for example, small male vs large female. The implications from chapter 1 highlights the complexity of linking behaviour with physiology and the mechanisms that are involved in *Nephrops* social interactions and the establishment of hierarchies. This is important as individuals are frequently interacting with conspecifics for mating, burrow habitat, food and throughout the capture process. It also implies that broodstock should either be held individually or with individuals that would cause the least amount of stress (i.e. large and small females). It also highlights the potential of fishery related pressures due to selecting certain sized or sexed individuals from populations. It would be interesting to assess the physiological cost of having telescoped populations where fishing has selected and removed larger individuals from the population, meaning that the size distribution is smaller and similar sized individuals will have increased interactions (Steneck, 2006). Spiny lobsters demonstrate how smaller males have reduced fertilisation success when compared the larger males. Therefore if current fishing practices focus on the capture of only the larger individuals from a population, it could reduce the ability for the population to maintain its size or it could reduce the genetic diversity within a population (Butler et al., 2015).

The complexity of the social interactions and behavioural patterns in *Nephrops* are an interesting aspect and an ideal species for such research. One could investigate different behavioural models further, such as, the sequential assessment model, identifying and quantifying the ways in which similar sized individuals establish a winner or a loser and comparing this between different sized opponents.

Chapter 3 has also given information on baseline physiological "stress" levels, out with fishing related stressors which will prove useful for further studies investigating metabolites in *Nephrops*. These baseline levels could be measured against stress levels caused by the creel capture methods and also give an insight into the behaviours that occur between random individuals in a confined space, such as found within a creel.

Further knowledge in this field will benefit both the development of the fisheries and aquaculture sector for *Nephrops* and potentially other crustaceans in attempting to reduce stress caused by conspecific interaction. The study has indicated the complexity of how the metabolites relate to one another, and would be interesting as further research in elucidating the causes and consequences from a range of stressors. **Chapter 3** suggests dual roles for the metabolites glucose and ammonia, increasing and decreasing in relation to the stressor and environmental situation. Briffa & Elwood (2001) also found that, for example glucose is a main determinant of the aggression levels shown in hermit crabs. Further investigation as to the identification of whether the changes in these parameters are a causal agent or a consequence from stress would also give an insight into the behavioural and physiological interface. In addition, the assessment of whether social stress, including competition, can cause a variation in the reproductive success and embryo development having implications on future hatchery development. Literature already suggests that *Nephrops* on high density grounds have reduced growth (Maynou and Sardà, 1997). The findings also highlight important behavioural parameters associated with the internal physiology of the individual, such as the association of total haemocyte count (THC) with aggression and submission, and the association of lactate and glucose with submissive behaviours (such as retreat behaviours).

The amount of stress incurred during transport, holding and handling can be severe. Powell et al. (2017) demonstrate that simple but effective techniques can utilised to lower post-transport mortality in *Nephrops*. A practical method with the aim of further reducing stress and immobilizing groups of individuals, was investigated in **chapter 4.**, A suitable sedative solution (eugenol) and the administration techniques and baseline information that could be used to sedate and anaesthetise groups of *Nephrops* was identified. Using sedatives could prove useful in increasing the survival in broodstock post-transportation and within the holding facilities, as shown in other species (Akbari et al., 2010; Pramod et al., 2010). However, transport experiments were not carried out and should be trialled before commercial use. The impact that eugenol has on the mortality/survival of both broodstock and PL during live transport over an extended period would need to be validated.

Commercial hatcheries rely upon the supply of healthy stock. It is imperative that *Nephrops* hatcheries are supplied with healthy broodstock and that husbandry practices maintain high

survival. Stressors may influence the broodstock fecundity by direct embryo mortality, loss, and under development. It is clear that physical disturbance and temperature influences the reproductive performance and larval competency within crustaceans (Smith et al., 2002; Smith and Ritar, 2010). Therefore, the use of eugenol as described in **chapter 4** could prove beneficial in prolonged transportation activities of *Nephrops*, i.e. above 1 hour. Transportation of *Nephrops* in shorter time frames may benefit from alternative methods, such as damp, or wet transportation.

However, the thesis did not investigate if eugenol has an impact upon stress metabolites, such as the parameters discussed in **chapter 3**, and therefore this would be an obvious direction for future research. It is also unclear if eugenol influences embryo development and later larval viability, although as discussed in **chapter 4** this seems unlikely at the concentrations being used (Usta et al., 2002; Wagner et al., 2002; Hoskonen et al., 2013).

Historical larviculture of *Nephrops* has had limited success, with only relatively recent advancements made to produce PL (Powell and Eriksson, 2013). The reasons for failure has been attributed to stress in broodstock, unsuitable diets for larviculture and non-optimal hydrodynamics and tank configurations (Pochelon, 2011). As aquaculture success is defined by overall productivity and cost effectiveness (Goddard, 1996), the objective of **chapter 5** was to improve the practices for larval development and survival. **Chapter 5** therefore investigated larval diets and feeding regimes that optimise growth and survival, and that are practical for use within a commercial hatchery.

Chapter 5 has given valuable insight into the choice of diets commonly used in commercial lobster and shrimp hatcheries. The preliminary experiments (**Chapter 2**) also indicate that the hydrodynamics that improve *Nephrops* larval survival are not adequate to support the use of inert diets. The study noted that using wild zooplankton did not have the same outcome it had with experiments associated with *Homarus* spp (Gendron et al., 2013). Although there was no significant difference in survival to Z3 between the diets, feeding enriched *Artemia* or enriched *Artemia* with probiotics would be (to date) a satisfactory choice of diet due to the relative ease of use at a commercial scale compared to wild captured zooplankton. The diets and techniques used in this study enhanced larval survival (*i.e.* above 4%) and reduced development time compared to that reported in published literature and preliminary

experiments. Feed management must consider not only the requirements of the larvae but also the associated costs. A continuous feeding regime could reduce labour, in return for shorter development time and increased survival, but whether this is economically viable remains questionable due to the price of *Artemia* cysts, hatching set up and running costs. Multidisciplinary nutritional research needs to continue, developing diets and assessing these against key performance measures of the stock, whilst also minimising labour and costs, which is of paramount importance for hatchery success (Bengtson, 2003).

The development time, survival, and growth data have been used as the key performance measures within the shrimp industry, but a thorough investigation in growth and development rates between diets and feeding regimes, with repeated sampling over the sampling period is highly recommended for comparison, rather than for example using only absolute growth, as in **chapter 5**.

The nutritional quality of the diets, i.e. lipid and protein levels were evaluated from available literature. Biochemical analysis of the feeds could indicate key performance attributes that can influence the development of crustacean larvae. For example, good amino acid and fatty acids profiles can influence the digestibility and enhance development through key moult stages (Wickins and Lee, 2002). In addition, the results from **chapter 5** indicate that larval behavioural investigations in relation to different prey is of high importance and can provide a useful guide for optimising larval diets.

The larviculture techniques developed and described in this study may also benefit laboratory experiments in other research programmes investigating issues such as climate change, micro-plastics and noise pollution.

The limitation with the majority of larviculture studies, including **chapter 5**, is that the survival is measured only up to stage 3 and not to the PL stage (de Figueiredo and Vilela, 1972; Thompson and Ayers, 1989; Dickey Collas et al., 2000; Rotlland et al., 2001; Pochelon et al., 2009). If the larvae were allowed to continue to develop to PL, differences in their viability could be assessed, and estimates of recruitment per batch could be made. However, PL were successfully raised using the larviculture techniques from **chapter 5** for **chapter 6** and thus indicates that the methods could be further developed for this species with an aim for stock enhancement activities. The NEPHROPS manual (EU funded FP7 NEPHROPS project, the IP for

which is currently under confidentiality agreements) and this thesis could give guidance on technical methods, recent developments and areas that need further improvement.

The results from **chapter 6** indicate important behavioural considerations for release activities. It seems that PL do use adult burrows but have a preference to enter a burrow when it is un-occupied by a larger individual. However, recruitment to the fishery would be hindered if released PL were not able to gain sheltered habitat, due to the burrows being occupied. Alternatively, **chapter 6** indicates that PL do explore and remain in alternative habitats such as cobbles. This implies that PL could be released on either a suitable muddy substrate, or on one comprising of cobbles with soft sediments. Release programmes for *Homarus* spp. have used habitat alteration and enhancement, such as artificial reefs, to increase the carrying capacity and survival of released PL (Nicosia and Lavalli, 1999); similar strategies could be adopted for *Nephrops* PL release. These results have important implications for the fisheries, in that PL recruitment to the fishery may be hindered due to habitat restrictions such as unsuitable substrate and a high number of occupied burrows in high density populations (Wahle and Steneck, 1992). The post release survival and performance are dependent upon optimising the release strategies for responsible stock enhancement programmes (Lorenzen et al., 2010). Both temporal and spatial considerations need to be made such as; the substrate type, time of release and abiotic factors that can optimise the survival of released PL. Future work should identify "proof of concept" with collaborative restocking experiments in several locations to identify if released *Nephrops* PL survive to contribute the fishery. Results from before-after-control-impact (BACI) methods have been utilised to compare lobster population abundance in released sites to that of comparable non-release sites (Ellis et al., 2015), and could be used for future *Nephrops* release programmes. In comparison, there are several methods to definitively identify hatchery reared individuals, the most common is the use of physical tags. Several tags have been tested with decapod crustaceans such as the coded wire tags (CWT, Bannister and Addison, 1998; Latrouite and Lorec, 1991; Agnalt et al., 2004) and visible implant elastomers (VIEs, Schmalenbach et al., 2011), but these have been used on juveniles considerably larger than a PL1 *Nephrops* larvae, therefore the feasibility of using such tags would need to be investigated. Genetic markers are currently used in several culture fish species such as *Salmo salar* which allows for the identification of individuals with a hatchery origin and their

parentage (Ellis et al., 2015). The advantage of using genetic tagging methods is that it has no requirement for minimum juvenile size, no tag loss, researchers can trace multiple generations and they can test the genetic pool of released populations against that of native populations. In the longer term, it would be interesting to identify PL movement from release on several substrate types and the potential for re-introduction (re-stocking locally extinct populations). Biotelemetry offers a technique to monitor short term movement, activity patterns and habitat utilisation and has been utilised in several crustacean decapods (Guerra-Castro et al., 2011), however the equipment would need to be developed for use with *Nephrops* PL. Although there is a need to assess the effectiveness of stock enhancement programmes, most hatcheries find it difficult to finance long term survival and/or movement monitoring projects (Ellis et al., 2015), and so assessing the true effectiveness of release activities could be challenging.

7.2. Wider Implications

7.2.1. Behaviour

The benefits of using *Nephrops* as a species to study behaviour are shown in Table 7.1 which illustrates some of the major factors to consider when selecting a species for behavioural studies. *Nephrops* are an ideal candidate species as they are readily available, do not require a home office licence (as for vertebrates), can be transported live, have a lower market value compared to other crustacea such as *Homarus* spp., and have been shown to display extensive social interactions.

Table 7.1. Factors to be considered for choosing subject species in behavioural studies. Adapted from Lehner (1996) in relation to *Nephrops norvegicus*.

	Factor	Potential answer regarding *Nephrops*
Suitability	Is the species suitable for the concept being studied	Yes, this includes social behaviour, husbandry, stress, release strategies, naiveté in reared individuals.
	Can you identify individuals	Yes, by the use of markings on the carapace, internal and external tags.
	Does it engage in interesting behaviour which you can observe repeatedly	Yes, *Nephrops* show interesting hierarchal relationships and behaviours in response to stress, and environmental variables.
	Can you make the necessary manipulations on this species	Yes, this has usually been by environmental manipulation (light levels, flow through *etc*.) but also includes the presence of disease(s), chemicals and conspecifics.
Availability	Is the species found locally or will you have to travel to study it in the field	Broodstock can be sourced throughout the UK either direct from fishers, or through merchants and transport services. The technology is available for *in-situ* behavioural observations; however, this could be costly if in deep water.
	Is it found in a foreign country	*Nephrops* have a wide distribution throughout Europe and are a highly valued commodity, therefore research can feed directly into international fishery practices.
	Can observations be made without altering its behaviour	The use of cameras using light filters could enable behaviours to be captured remotely. The species emergence patterns are related to depth, light levels and season. These also play a part in how easy it is to capture *Nephrops* in the wild.
Adaptability	How will it adapt to life in captivity	This is thesis may potentially indicate that *Nephrops* vitality and wellbeing may suffer from prolonged captivity.
	Can you simulate its natural environment	Yes, using aquaria with the option of using mud taken from known grounds to stimulate burrowing behaviour. However, mimicking environmental factors such as depth and prey availability may be difficult.
	Are its habitat compatible with what is available	Yes, larval tanks seem sufficient in larval rearing. Aquaria and perforated cells set in tanks seem adequate to hold juvenile and adult stages. However, improvements are required to simulate the natural environment.
Background information	Is there information and data already on the species	There is a multitude of data on fishing related population studies and trawl equipment development. However, 20 articles have been published between 2000 and 2017 on behavioural related studies specifically focussed on *Nephrops.*

Assessing behaviour has practical applications, for example to assess the health of an individual and the assessment of the treatment being given. Using behavioural analysis during sedation is one practical example that is illustrated in this thesis. Stoner (2012) describes different methods to assess stress and anticipated mortality in crustaceans, and of note is the usefulness in using behavioural assessments and scores to quantify the vitality of an individual, which in turn can be used, with a high degree of success, to predict mortality. Using behavioural assessments has the advantages of being non-invasive but can also be non-intrusive. Identifying behaviours that indicate certain preferences in given situations or environments could be crucial in developing aquaculture and fisheries practices. Gathering information on "normal" behaviours and comparing them to given situations and environments that cause stress (for example capture, handling, transportation and captivity) could give an indication on how to improve techniques, husbandry and their environment. Since 2010 the main focus on *Nephrops* related behavioural research has been on exploring emergence patterns, however, fishery and aquaculture related behavioural research has also started to be published (Figure 7.1).

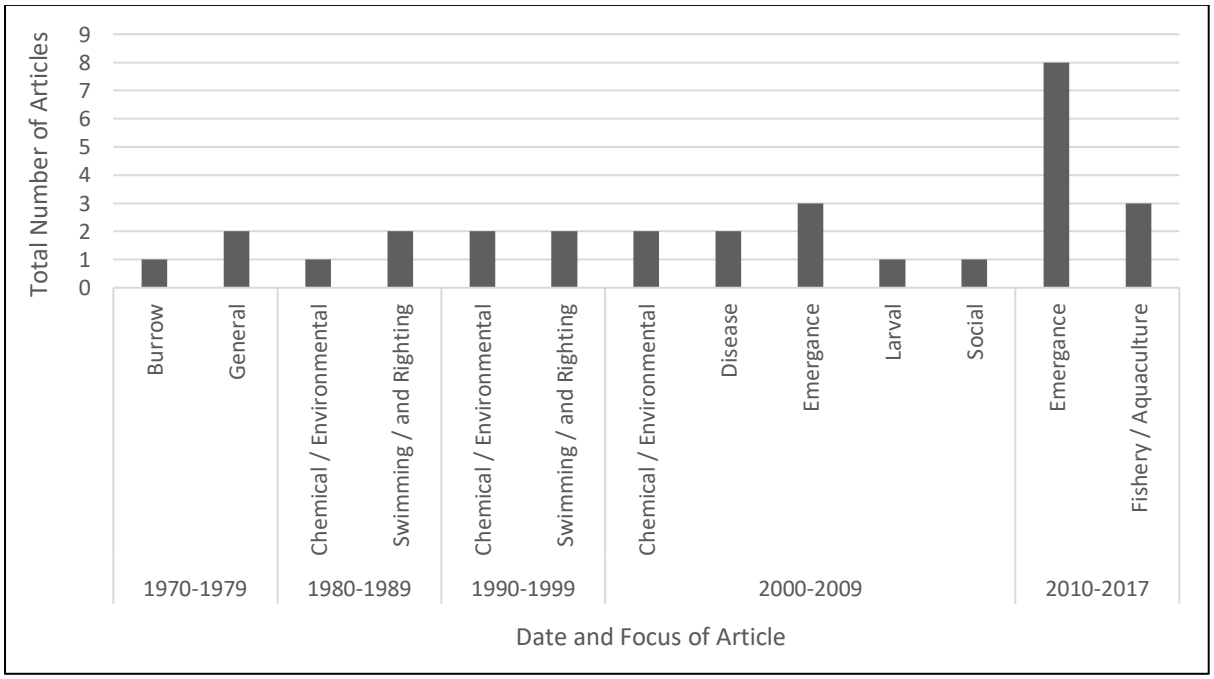


Figure 7.1. Number of articles directly investigating the behaviour of *Nephrops norvegicus* between 1970 and 2017. A search criterion was used on Scopus ("*Nephrops*" AND "behaviour") to estimate the number of articles published on *Nephrops* behaviour.

The information gathered in this thesis adds to the growing research using behavioural data in the wider field of crustacean welfare (Dawkins, 2004). The rationale to assess the welfare standards in crustaceans has been supplemented by the knowledge gained about nociception and the central nervous system in crustaceans. Several criteria including avoidance learning, changes in haemolymph metabolites indicating physiological stress and the effect of analgesics on the sensitivity to painful stimulus indicate that there is a strong possibility that crustaceans can experience and process painful stimuli (Elwood et al., 2009; Elwood, 2012). This has implications for current practices in food production and the treatment of crustaceans. The growing customer concern over animal welfare issues has led to an aim for improving the handling and husbandry of crustaceans during capture, farming and processing practices and the long-term sustainability of the industry.

As most behaviours are indicators of certain signals, either to another animal or of how the individual responds to a situation, it would be beneficial to interpret these signals. Validating known behaviours and correlating these to known physiological indicators of stress, is one method that can assist in the interpretation of animal behaviour. To date physiological stress indicators for *Nephrops* have been developed during transportation and capture activities, primarily focussed on the trawl industry. However, there are many other factors that could be taken into consideration that impacts how an individual behaves, these can include the genotype, the environment, an individual's experience, anatomy and behavioural propensity (Lehner, 1996). In addition, personality between individuals and sub-populations could be a further area of research. Including personality within aquaculture research could prove useful for the improvement in husbandry, reproduction success and release activities. For example, bold individuals have shown to be over represented within the broodstock and within recapture studies (Gherardi et al., 2012). Whether individuals are bold or shy may also influence upon the reproductive success and post release survival as shown in other decapods (Gherardi et al., 2012).

Behavioural analysis can be used to modify aquaculture practices. For example, defining how the larvae swim and catch prey could indicate ideal prey sizes, densities and feeding regimes (Cox and Bruce, 2003). Behavioural research on PL shelter preference has led to habitat modifications (boulders, stones and casitas) in stock enhancement programmes to increase the survival of hatchery reared *H. gammarus* and *Panulirus* spp. (Philips, 2013). There has

been limited research investigating the effects of long term captivity and the holding environment in *Nephrops*. Such investigations would help develop husbandry practices in *Nephrops* aquaculture.

7.2.2. Broodstock

Aquaculture systems can control only a few of the many factors that can influence egg quality, some of the factors that can be controlled are broodstock nutrition, environmental conditions, development process (temperature regimes), sanitation, and minimising stress. A future perspective for all crustacean aquaculture could be towards selecting and genetically engineering broodstock for specific traits such as disease resistance, higher growth and faster development to sexual maturity. The lack of information for best practices in *Nephrops* broodstock management and husbandry, in addition to the 3-4.5 years it takes for *Nephrops* to reach sexual maturity (Tuck et al., 2000) make it difficult for such genetic selectivity studies to take place. Intentional or un-intentional artificial selection would depend upon the aim of the hatchery and a thorough impact assessment should be undertaken. Ellis et al. (2017) have identified distinct genetic markers for *Homarus* spp. residing on the limit of their distribution, in heavy depleted, isolated and self-recruiting stocks. DNA profiling also indicates differences between the larvae produced from wild and hatchery bred broodstock (Jørstad et al., 2005). However, the techniques described in this thesis use local wild broodstock which is changed each season, and therefore, should maintain genetic variation. It is unclear whether or not the genetic mixing of wild and released hatchery individuals would or does have an impact upon the populations diversity or general fitness. As larval supply is determined in part by water currents, and *Nephrops* exhibit specific habitat preference, the PL could settle away from the area of the parent female, which could lead to isolation by distance, similar to some populations of *Homarus* spp. (Ellis et al., 2017). Measures have been put in place to minimize deleterious impacts of disrupting the spatial population structure and lowering the genetic diversity and fitness of the population within fisheries such as salmonids and a similar objective should be sought for crustacean aquaculture (Lorenzen et al., 2010). Knowledge of the stock boundaries, larval supply, genetic flow and local spawning stock biomass should be incorporated into future stock enhancement activities.

7.2.3. Aquaculture techniques

Homarus spp. larviculture techniques have been developed since the 19th century, although research dedicated in *Nephrops* aquaculture techniques has had a limited advancement in comparison. Currently there are research activities in the north of Wales to develop farming technology for the European spiny lobster, but the majority of aquaculture activities relate to the ongrowing of captured juveniles from the fishery. Several factors that are important for successful aquaculture / stock enhancement activities are shown in Table 7.2 and are compared between *Nephrops*, *Homarus* spp. and spiny lobsters. The comparison indicates that *Nephrops* larvae are less robust, less cannibalistic, have a longer development time, must be reared at lower densities and the broodstock are less fecund when compared to *Homarus* spp. The value of UK landings from pots and traps of *Nephrops* compared with that of *H. gammarus* indicates that the value (£) per tonne of landed *H. gammarus* is ~295% higher than *Nephrops* between the years of 2011-15 (average value per tonne (£) ± standard deviation; Lobster=£2,027.80 ± 59.41; *Nephrops*=£513.00 ± 18.77; MMO, 2017). However, in comparison to spiny lobsters, *Nephrops* larvae have shorter development time are more robust and require similar tank hydrodynamics and hygiene levels. The technology and knowledge for the culture of *Nephrops* PL for stock enhancement is available. However, like *Homarus* spp. and spiny lobsters, the continuation of market driven dedicated research is essential for development. Historically spiny lobster hatchery development has been conducted in Japan and Australia. Only a select few hatcheries currently produce PL in Australia which are transported for on-growing in Vietnam and South-East Asia (Shanks and Jones, 2015). Detailed information is commercially sensitive and therefore limited.

Table 7.2. Comparison between *Nephrops*, *Homarus* spp. and spiny lobster aquaculture.

Factor	*Nephrops norvegicus*	*Homarus* Spp.	*Palinurid* and *Scylllarid*
Broodstock sourcing and holding	Broodstock sourced locally. Broodstock are susceptible to stress and must be handled with care. Broodstock are held individually due to potential cannibalism. Broodstock should be kept in the dark. Difficult to overwinter broodstock. Fecundity is ~1400 – 4100 eggs with continual loss during incubation.	Broodstock sourced locally. Broodstock are held individually due to high incidence of cannibalism or are placed communally with banded chelae. Broodstock should be kept in the dark and have similar requirement to that of *Nephrops*. Broodstock can be held overwinter. *Homarus* is more fecund than *Nephrops*, however can be variable dependent upon location and size of the individual (4,000 – 44,000).	Broodstock locally sourced or reared from juvenile stages. Fecundity ranges between species. ~700,000 eggs per spawning.
Mass culture	Until recently *Nephrops* were not able to be reared at high densities. Improvements in tank design and diets mean that larviculture can take place at scale similar to that of *Homarus* spp. However, non-optimal tank hydrodynamics can result in high mortality especially between larval stages and before metamorphosis. Larvae are extremely delicate. The telson can become easily entwined and fouled. Larvae must be handled with care (water to water techniques) which is labour intensive.	High cannibalism makes it difficult for mass rearing especially at the juvenile stages. Larvae are robust and can withstand high aeration, turbulent water and periods of emersion (moist). Both *Nephrops* and *Homarus* spp. use similar larval rearing tank designs (cylindro-conical tanks).	Able to culture the intermediate staged larvae that have been caught from the sea to juvenile stages. Mass culture from the egg is extremely difficult due to the long development time and number of larval stages. Limited number of larvae reaching PL stages. Tank design in crucial. The use of tiny pores in the base of the tank has proven successful.
Diets	Best larval growth and survival are with larvae fed *Artemia* spp as the preferred live diet. No suitable artificial diet has been found. Further research is needed.	Larvae can be fed on artificial and inert diets (*i.e.* copepod paste). Best success (growth and survival) is with live diets such as *Artemia* spp. Further research is needed.	Some success using *Artemia* spp. and phytoplankton, however the requirements of the phyllosoma larvae are not fully understood. Larvae may prefer larger soft bodied prey. Further research is needed.

Larval stages	There has been little research or development since the 1980's in rearing *Nephrops* larvae. 3 zoeal stages. Larval survival to stage 3 is on average ~40%. Density is ~4 larvae per L. Duration is ~ 30 days. Difficulty remains in discerning optimal feeding requirements for each stage. Further investigation on improving metamorphosis success is required.	Research and development began on *Homarus* spp. larviculture since the 19th Century and has continued till present. 3 zoeal stages. Higher larval survival than *Nephrops* to stage 4 which is on average ~60 - 80%. Density is ~15 - 40 larvae per L. Duration is shorter than *Nephrops* (*i.e.* <20 days). Prior to metamorphosis the larvae are placed in individual cells such as Orkney cells, the Aquahive or individual beakers.	Research began in the late 1890's. Many different larval stages and usually long duration (4-22 months).
PL rearing	Limited information on PL requirements or growth. PL are held individually. Growth to plate size is expected to be ~3-5 years.	PL are placed in individual cells/ compartments due to high cannibalism rates. Can be fed with *Artemia* spp. Growth to plate size is ~2-3 years.	Requirements for the phyllosoma larvae are limited. Juveniles are caught and reared in sea cages or in on-shore tanks. Growth from puerulus stage to market size is ~1-2 years (with eyestalk ablation).
PL Release	There is no known release activity of reared *Nephrops* PL to date.	Several PL enhancement programmes using tagged lobsters indicate a return rate of ~4-9%. Several methods have been trialled in releasing PL into suitable habitat.	PL may be released in exchange for a proportion of the captured juveniles from wild stocks.

Information gathered from Cobb and Phillips, 1980; Phillips and Kittaka, 2000; Phillips, 2013; Powell and Eriksson, 2013; Wickins and Lee, 2002.

The market for *Nephrops* in the UK is mainly for scampi, favoured by the consumer for its convenience, and whole "langoustine" is shipped abroad to Spain, France and Italy. Global competitors that supply large quantities of tropical crustaceans imported to the UK could show consumer favour due to the low price and convenience. However, UK consumers may in the future start to favour less processed foods in favour of a more luxurious commodity. With current supply issues including quotas, exchange rates and decreasing stock, there may be an opportunity to re-market the *Nephrops* to the UK consumer. The objective of the EU to reduce discards in addition to a change in the market favouring smaller sized *Nephrops* (Catchpole et al., 2002b) could give the *Nephrops* fishery several options. Approximately 45% of the catch (by weight) in the *Nephrops* demersal trawl fisheries is discarded (Catchpole et al., 2002a). The undersized "discarded" individuals could be held for on-growing in tanks or sea cages, or alternatively used for ranching and stock enhancement. Alternatively trawl caught *Nephrops* could be held for rehabilitation, to then be sold in the more lucrative live market trade. Hatchery raised *Nephrops* could be sold at smaller sizes than the current fishery target. Research for developing *Homarus* spp. hatcheries in the UK continues and are currently investigating open-water on-growing technologies (sea cages) while continuing to produce *H. gammarus* PL for stock enhancement.

At the time of writing the author is unaware of any commercial *Nephrops* hatchery programmes. However, there are at the least five lobster (*H. gammarus*) hatcheries (National Lobster Hatchery, Cornwall; Firth of Forth Lobster hatchery, North Berwick; Orkney Lobster Hatchery, Lamb Holm; Seascope Lobster Hatchery, Kilkeel; Lobster Hatchery of Wales, Anglesey; Northumberland Seafood Centre, Amble that release juveniles around the UK.

It is clear that the limited information available on the nutritional requirements for many of the crustacean species hinder aquaculture development. Research for developing complete formulation (artificial) diets for spiny lobsters and clawed lobsters was prevalent in the 1970's and 1980's. Research in lobster diet development since 2010 has focussed on mainly two separate areas. One area has focused on PL on-growing techniques using open water cages in which the PL feed upon the natural plankton and fouling organisms (Perez et al., 2010; Beal, 2012; Gendron et al., 2013; Daniels et al., 2015). The other factor has focused upon larval feed development with the use of probiotics, mannan oligosaccharides and dry feed pellets (Daniels et al., 2010 & 2013; Huu and Jones, 2014; Powell et al., 2017). However, there is need

for species specific nutritional research to facilitate the development and viability of commercial scale crustacean aquaculture. Currently live feeds such as *Artemia* and/or zooplankton still provide the most promising results in larval survival and growth when compared to manufactured diets. Interestingly, using lobster flesh as a feed for later staged hatchery cultured lobsters promotes comparable growth when fed on *Artemia* sp. alone (Van Olst et al., 1975). Current research indicates that *H. gammarus* larvae fed dead conspecifics resulted in 80% survival of larvae reaching PL stages (Powell et al., 2017).

7.3. Summary

When comparing with current commercial crustacean farms it seems imperative that if *Nephrops* aquaculture is to be successful then mass rearing of larvae needs to be achieved. Current *Nephrops* larviculture techniques are still developing and this thesis has illustrated that survival could potentially increase to >40% to stage 3. Culture to the PL stage is now feasible, although whether this is at sufficient numbers remains to be answered. Most temperate operations are both labour and capital expensive, with the main on-going costs being feed, labour and overheads. A reduction in these factors will need to be confronted for *Nephrops* aquaculture to become more viable. Developments such as automated rearing and feeding systems have historically been trialled, and continue to be explored in commercial aquaculture operations. Based upon this information and taking into account the current market conditions facilitates the need for further research and the production of pilot scale programmes to critically assess the feasibility of commercial culture of this species. Larval density, optimising feed type and quantity, in addition to developing tank hydrodynamics play a crucial role in disease and pathogenic infections and a major factor for future development. Optimal larval conditions and biological information are now available, making the short-term production of larvae for stock enhancement potentially feasible. The longer-term feasibility of commercial production will need more information on the PL stages, through potentially raising them in grow-out cages at a reduced cost.

It is hoped that the suggestions made in this thesis will contribute towards the information needed to critically evaluate developing aquaculture techniques and stock enhancement research activities for *Nephrops* by investors, managers and fishers. This thesis has indicated that the techniques currently adopted from other crustacean larviculture techniques can be

used for the development of a *Nephrops* hatchery, with the aim of stock enhancement. In conclusion, the key findings indicate the following:

- The **broodstock** are sensitive to stress and that operations need to minimize stress from capture through to release activities. Such recommendations from the thesis include holding broodstock in separate compartments (minimises stress caused from conspecifics), in adequate environmental conditions (i.e. low light, minimal disturbance and appropriate water parameters). Sedation could be used during transport or handling.
- *Nephrops* physiology, especially stress parameters are complex. Elucidating behavioural patterns and social activity within *Nephrops* could be used to advance the knowledge in the aim of improving crustacean welfare and to test several game theory models.
- **Larviculture** could be further improved to increase survival. This could be achieved by enhancing feeding regimes, such as a continual feeding mechanism. The optimal diet is still unknown, however to date, *Artemia* are suitable for the growth of larvae to PL stages. The addition of enrichment and probiotic bacteria has shown promising results. There is a need for further research to optimise crustacean diets.
- **PL** show a range of distinct behavioural patterns when selecting suitable habitat for settlement. Any enhancement activities should release PL on suitable grounds, where a population is already evident. Alternative habitats, such as cobbles, could potentially be used for PL release, but requires further investigation.

8. References

Adey, J.M., 2007. Aspects of the sustainability of creel fishing for Norway lobster, *Nephrops norvegicus* (L.), on the west coast of Scotland. PhD thesis. University of Glasgow.

Agnalt, A.L., Jørstad, K.E., Kristiansen, T.S., Nøstvold, E., Farestveit, E., Næss, H., Paulsen, O.I., 2004. Enhancing the European lobster (*Homarus gammarus*) stock at Kvitsøy Islands; Perspectives of rebuilding Norwegian stocks, in: Leber, K. M., Kitada, S., Blankenship, H.L., Svåsand, T. (Eds), Stock Enhancement and Sea Ranching: Developments, Pitfalls and Opportunities. Blackwell Publishing Ltd, Oxford, pp. 415–426.

Agnalt, A.L., Van der Meeren, G.I., Jørstad, K.E., Næss, H., Farestveit, E., Nøstvold, E., Korsøen, E., Ydstebø, L., Svåsand, T., 1999. Stock enhancement of European Lobster (*Homarus gammarus*): a large-scale experiment off Souoth-western Norway (Kvitsøy), in: Howell, B., Moksness, E., Svåsand, T. (Eds.), Stock Enhancement and Sea Ranching. Blackwell Scientific Press, London, pp. 401–419.

Aiken, D.E., 1973. Proecdysis, Setal Development, and Molt Prediction in the American Lobster (*Homarus americanus*). Journal of the Fisheries Research Board of Canada 30, 1337–1344. doi:10.1139/f73-214

Akbari, S., Khoshnod, M.J., Rajaian, H., Afsharnasab, M., 2010. The Use of Eugenol as an Anesthetic in Transportation of With Indian Shrimp (*Fenneropenaeus indicus*) Post Larvae. Turkish Journal of Fisheries and Aquatic Sciences 10, 423–429. doi:10.4194/trjfas.2010.0317

Albalat, A., Gornik, S.G., Beevers, N., Atkinson, R.J.A., Miskin, D., Neil, D.M., 2012. Hematodinium sp. infection in Norway lobster *Nephrops norvegicus* and its effects on meat quality. Diseases of Aquatic Organisms 100, 105–112. doi:10.3354/dao02500

Albalat, A., Sinclair, S., Laurie, J., Taylor, A., Neil, D., 2010. Targeting the live market: Recovery of Norway lobsters *Nephrops norvegicus* (L.) from trawl-capture as assessed by stress-related parameters and nucleotide breakdown. Journal of Experimental Marine Biology and Ecology 395, 206–214. doi:10.1016/j.jembe.2010.09.002

Anderson, W.G., Mckinley, R.S., Colavecchia, M., 1997. The Use of Clove Oil as an Anesthetic for Rainbow Trout and Its Effects on Swimming Performance. North American Journal of Fisheries Management 17, 201–307. doi:10.1577/1548-8675(1997)017<0301

Anger, K., Puschel, C., 1986. Growth and exuviation of Norway Lobster (*Nephrops norvegicus*) larvae reared in the laboratory. Ophelia 25, 157–167.

Anger, K., Storch, V., Anger, V., Capuzzo, J.M., 1985. Effects of starvation on oult cycle and hepatopancreas of stage 1 lobster (*Homarus americanus*) larvae. Helgolander Meeresunters. 39, 107–116.

Ara, K., 2001. Length-weight relationships and chemical content of the planktonic copepods in the Cananeia Lagoon estuarine system, Sao Paulo, Brazil. Plankton Biology and Ecology 48, 121–127.

Aragão, C., Conceição, L.E.C., Dinis, M.T., Fyhn, H.J., 2004. Amino acid pools of rotifers and *Artemia* under different conditions: Nutritional implications for fish larvae. Aquaculture 234, 429–445. doi:10.1016/j.aquaculture.2004.01.025

Araujo, R., Remón, J.M., Moreno, D., Ramos, M.A., 1995. Relaxing Techniques for Freshwater Molluscs: Trials for Evaluation of Different Methods. Malacologia 36, 29–41.

Arnold, K.E., Findlay, H.S., Spicer, J.I., Daniels, C.L., Boothroyd, D., 2009. Effect of CO_2-related acidification on aspects of the larval development of the European lobster, *Homarus gammarus* (L.). Biogeosciences 6, 1747–1754. doi:10.5194/bgd-6-3087-2009

Arnott, G., Elwood, R.W., 2009. Assessment of fighting ability in animal contests. Animal Behaviour 77, 991–1004. doi:10.1016/j.anbehav.2009.02.010

Arnott, S., Neil, D., Ansell, A., 1999. Escape trajectories of the brown shrimp *Crangon crangon*, and a theoretical consideration of initial escape angles from predators. The Journal of Experimental Biology 202 (Pt 2), 193–209.

Atema, J., Voigt, R., 1995. Behavior and sensory biology, in: Factor, J.R. (Ed.), Biology of the Lobster *Homarus Americanus*. Academic Press, New York, pp. 313–348.

Avella, M.A., Gioacchini, G., Decamp, O., Makridis, P., Bracciatelli, C., Carnevali, O., 2010. Application of multi-species of Bacillus in sea bream larviculture. Aquaculture 305, 12–19. doi:10.1016/j.aquaculture.2010.03.029

Balasundaram, C., Pandian, T., 1982. Egg loss during incubation in *Macrobruchium nobilii*. Journal of Experimental Marine Biology and Ecology 59, 289–299.

Bannister, R.C.A., Addison, J.T., 1998. Enhancing lobster stocks: A review of recent European methods, results, and future prospects. Bulletin of Marine Science 62, 369-387.

Bannister, R.C.A., Addison, J.T., Lovewell, S.R.J., 1994. Growth, movement, recapture rate and survival of hatchery-reared lobsters (*Homarus gammarus*, L.) released into the wild on the English east coast. Crustaceana 67, 156–172.

Barclay, M.C., Dall, W., Smith, D.M., 1983. Changes in lipid and protein during starvation and the moulting cycle in the Tiger Prawn, *Penaeus Escullentus* Haswell. Journal of Experimental Marine Biology and Ecology 68, 229–244.

Barrento, S., Marques, A., Vaz-Pires, P., Leonor Nunes, M., 2011. *Cancer pagurus* (Linnaeus, 1758) physiological responses to simulated live transport: Influence of temperature, air exposure and AQUI-S. Journal of Thermal Biology 36, 128–137. doi:10.1016/j.jtherbio.2010.12.006

Barreto, E., Bailey, N., 2015. Fish and Shellfish Stocks: 2015. Marine Scotland Science. URL: http://www.gov.scot/Publications/2015/05/4861/downloads (accessed 01.12.2016).

Beal, B.F., 2012. Ocean-Based Nurseries for Cultured Lobster (*Homarus americanus* Milne Edwards) Postlarvae: Initial Field Experiments off the Coast of Eastern Maine to Examine Effects of Habitat and Container Type on Growth and Survival. Journal of Shellfish Research 31(1), 167-176.

Bell, J.G., McEvoy, L.A., Estevez, A., Shields, R.J., Sargent, J.R., 2003. Optimising lipid nutrition in first-feeding flatfish larvae. Aquaculture 227, 211–220. doi:10.1016/S0044-8486(03)00504-0

Bell, J.G., Sargent, J.R., 2003. Arachidonic acid in aquaculture feeds: current status and

future opportunities. Aquaculture 218, 491–499. doi:10.1016/S0044-8486(02)00370-8

Bell, M., Tuck, I., Dobby, H., 2013. Nephrops Species, in: Phillips, B. (Ed.), Lobsters: Biology, Management, Aquaculture and Fisheries. Wiley-Blackwell, Oxford, pp. 357–413.

Bengtson, D., 2003. Live feeds in marine aquaculture. Blackwell Publishing, Oxford.

Bergmann, M., Taylor, A.C., Geoffrey Moore, P., 2001. Physiological stress in decapod crustaceans (*Munida rugosa* and *Liocarcinus depurator*) discarded in the Clyde *Nephrops* fishery. Journal Of Experimental Marine Biology and Ecology 259, 215–229.

Bernardi, C., Baggiani, L., Tirloni, E., Stella, S., Colombo, F., Moretti, V.M., Cattaneo, P., 2015. Hemolymph parameters as physiological biomarkers in American lobster (*Homarus americanus*) for monitoring the effects of two commercial maintenance methods. Fisheries Research 161, 280–284. doi:10.1016/j.fishres.2014.08.013

Bernasconi, C.J., Uglow, R.F., 2008. Effects of emersion and re-immersion on physiological and immunological variables in creel-caught and trawled Norway lobster *Nephrops norvegicus*. Diseases of Aquatic Organisms 81, 241–247. doi:10.3354/dao01957

Berrill, M., 1974. The burrowing behviour of newly-settled lobsters, *Homarus vulgaris* (Crustacea - Decapoda). Journal of marine biological association UK 54, 797–801.

Binggeli, C., Waring, A., Mihuc, T., 2011. Methods for Determining New Biovolumes for Copepods and Cladocerans. Scientia Discipulorum, New York.

Bolton, J.C., Collins, S., Smith, R., Perkins, B., Bushway, R., Bayer, R., Vetelino, J., 2009. Spectroscopic Analysis of Hemolymph from the American Lobster (*Homarus americanus*). Journal of Shellfish Research 28, 905–912. doi:10.2983/035.028.0421

Boltz, D.F., Howell, J.A., 1978. Colorimetric determination of nonmetals, 2nd ed. John Wiley & Sons Inc, London.

Bradbury, J., Vehrencamp, S., 2011. Web Topic 11: Conflict Resolution. Principles of Animal Communication, Second Edition. URL http://sites.sinauer.com/animalcommunication2e-/chapter11.01.html (accessed 7.4.16).

Breithaupt, T., Thiel, M. (Eds.), 2010. Chemical Communication in Crustaceans. Springer, New York.

Briffa, M., Elwood, R.W., 2001. Decision rules, energy metabolism and vigour of hermit-crab fights. Proceedings of the Royal Society of London B 268, 1841–8. doi:10.1098/rspb.2001.1752

Briffa, M., Elwood, R.W., 2002. Power of shell-rapping signals influences physiological costs and subsequent decisions during hermit crab fights. Proceedings of the Royal Society of London B 269, 2331–6. doi:10.1098/rspb.2002.2158

Briffa, M., Sneddon, L.U., 2007. Physiological constraints on contest behaviour. Functional Ecology 21, 627–637. doi:10.1111/j.1365-2435.2006.01188.x

Briggs, R., 2002. The application of fecundity estimates to determine the spawning stock biomass of Irish Sea *Nephrops norvegicus* (L.) using the annual larval production method. ICES Journal of Marine Science 59, 109–119. doi:10.1006/jmsc.2001.1144

Brillon, S., Lambert, Y., Dodson, J., 2005. Egg survival, embryonic development, and larval characteristics of northern shrimp (*Pandalus borealis*) females subject to different temperature and feeding conditions. Marine Biology 147, 895–911. doi:10.1007/s00227-005-1633-6

Briones-Fourza, P., Dominguez-Gallegos, R., Lozano-Alvarez, E., 2015. Aggressive behaviour of spotted spiny lobsters (*Panulirus guttatus*) in different social contexts: the influence of sex, size, and missing limbs. ICES Journal of Marine Science 72, i155–i163. doi:10.1093/icesjms/fsu219 Contribution

Browne, R., Benavente, G.P., Uglem, I., Carlos, J., Balsa, M., 2009. An illustrated hatchery guide for the production of clawed lobsters *Homarus gammarus*, using a green water technique. Aquaculture Explained 23, 1-36.

Burdett-Coutts, V., Wahle, R., Snelgrove, P., Rochette, R., 2014. Spatial linkages between settling young-of-year and older juvenile lobsters. Marine Ecology Progress Series 499, 143–155. doi:10.3354/meps10625

Burton, C.A., 2001. The role of lobster (*Homarus* spp.) hatcheries in ranching, restoration and remediation programmes. Hydrobiologia 465, 45-48.

Burton, C.A., 2003. Lobster hatcheries and stocking programmes : An introductory manual. Seafish Report SR552. Seafish.

Burton, C.A., Adamson, K., 2002. A Preliminary Study of the Costs of Operating a Lobster Hatchery in Orkney and the Development of an Economic Model for Future Hatchery Programmes. Seafish Technical Report. Seafish.

Butler, M.J., Macdiarmid, A., Gnanalingam, G., 2015. The effect of parental size on spermatophore production, egg quality, fertilisation success, and larval characteristics in the Caribbean Spiny lobster, *Panulirus argus*. ICES Journal of Marine Science 72, i115-i123. doi:10.1093/icesjms/fsv015

Calado, R., Pimentel, T., Vitorino, A., Dionísio, G., Dinis, M.T., 2008. Technical improvements of a rearing system for the culture of decapod crustacean larvae, with emphasis on marine ornamental species. Aquaculture 285, 264–269. doi:10.1016/j.aquaculture.2008.08.019

Calliari, D., Tiselius, P., 2009. Organic carbon fluxes through the mesozooplankton and their variability at different time-scales in the Gullmarsfjord, Sweden. Estuarine, Coastal and Shelf Science 85, 107–117. doi:10.1016/j.ecss.2009.06.016

Campbell, N., Allan, L., Weetman, A., Dobby, H., 2009. Investigating the link between *Nephrops norvegicus* burrow density and sediment composition in Scottish waters. ICES Journal of Marine Science 66, 2052–2059.

Castro, K.M., Cobb, J.S., 2005. Behaviour of hatchery-reared and wild-caught 4th and 5th stage American lobsters, *Homarus americanus*. New Zealand Journal of Marine and Freshwater Research 39, 963–972. doi:10.1080/00288330.2005.9517366

Catchpole, T.L., Frid, C.L.J. and Gray, T.S. 2002a. Estimated catch and discard quantities for the English Northeast coast *Nephrops norvegicus* fishery. ICES 2002/V:33.

Catchpole, T.L., Frid, C.L.J. and Gray, T.S. 2002b. Factors influencing discarding patterns, a case study of the English Northeast coast *Nephrops norvegicus* fishery. ICES 2002/V:06.

Chakraborty, K., Chakraborty, R.D., Radhakrishnan, E. V, Vijayan, K.K., 2010. Fatty acid profiles of spiny lobster (*Panulirus homarus*) phyllosoma fed enriched *Artemia*. Aquaculture Research 41, 393–403. doi:10.1111/j.1365-2109.2009.02469.x

Chang, E.S., 1995. Physiological and biochemical changes during the molt cycle in decapod crustacens : an overview. Journal of Experimental Marine Biology and Ecology 193, 1–14. doi:10.1016/0022-0981(95)00106-9

Chapman, C.J., 1979. Some observations on populations of Norway lobster, *Nephrops norvegicus* (L.) using diving, television and photography. Rapports et Procés Verbaux des Reunions 175, 127-133.

Chapman, C.J., 1980. Ecology of juvenile and adult *Nephrops*, in: Cobb, J.S., Phillips, B.F. (Eds.), The Biology and Management of Lobsters. Voll II. Academic Press, New York, pp. 143–175.

Chapman, C.J., Rice, A.L., 1971. Some direct observations on the ecology and behaviour of the Norway lobster *Nephrops norvegicus*. Marine Biology 10, 321–329. doi:10.1007/BF00368092

Cheng, W., Liu, C.H., Kuo, C.M., 2003. Effects of dissolved oxygen on hemolymph parameters of freshwater giant prawn, *Macrobrachium rosenbergii* (de Man). Aquaculture 220, 843–856. doi:10.1016/S0044-8486(02)00534-3

Clarke, K., Gorley, R., 2006. PRIMER v5: User Manual/Tutorial. PRIMER-E, Plymouth.

Cobb, J.S., Wahle, R.A.W., 1993. Early Life History and Recruitment Processes of Clawed Lobsters. Crustaceana 67, 1–25.

Conklin, D.E., 1995. Digestive physiology and nutrition, in: Factor, J.R. (Ed.), Biology of the Lobster *Homarus americanus*. Academic Press, New York, pp. 441–463.

Cowing, D., Powell, A., Johnson, M., 2015. Evaluation of different concentration doses of eugenol on the behaviour of *Nephrops norvegicus*. Aquaculture 442, 78–85.

doi:10.1016/j.aquaculture.2015.02.039

Cox, S.L., Bruce, M.P., 2003. Feeding behaviour and associated sensory mechanisms of stage I - III phyllosoma of *Jasus edwardsii* and *Jasus verreauxi*. Journal of Marine Biological Association UK 83, 465–468.

Coyle, S.D., Dasgupta, S., Tidwell, J.H., Beavers, T., Bright, L.A., Yasharian, D.K., 2005. Comparative Efficacy of Anesthetics for the Freshwater Prawn *Macrobrachiurn rosenbergii*. Journal of the World Aquaculture Society 36, 282–290.

Coyle, S.D., Durborow, R.M., Tidwell, J.H., 2004. Anesthetics in Aquaculture. SRAC Publcation No. 3900.

Cromarty, S.I., Mello, J., Kass-Simon, G., 2000. Molt-related and size-dependent differences in the escape response and post-threat behavior of the American lobster, *Homarus americanus*. Biological Bulletin 199, 265–277.

CrustaSea, 2006. Development of best practice, grading & transportation technology in the crustacean fishery sector. Project no: COLL-CT-2006-030421. National Institute of Technology, Norway.

Cushing, D.H., Humphrey, G.H., Banse, K., Laevastu, T., 1958. Report of the committee on terms and equivalents. Rapports et Proces-verbaux des Réunions. Conseil International pour l'Éxploration de la Mer 144, 15–16.

D`Abramo, L.R., 1989. Lipid requirements of shrimp. Advances in tropical aquaculture 9, 277–285. doi:10.1046/j.1365-2095.2002.00221.x

Dagá, P., Feijoo, G., Moreira, M.T., Costas, D., Villanueva, a. G., Lema, J.M., 2012. Bioencapsulated probiotics increased survival, growth and improved gut flora of turbot (*Psetta maxima*) larvae. Aquaculture International 21, 337–345. doi:10.1007/s10499-012-9556-y

Dall, W., Smith, D.M., 1987. Changes in protein-bound and free amino acids in the muscle of the Tiger Prawn *Penaeus esculentus* during starvation. Marine Biology 95, 509–520.

Danford, A.R., Uglow, R.F., Garland, J., 2001. Effect of long-haul international transport on

lobster hemolymph constituents and nitrogen metabolism, in: Paust, B.C., Rice, A.A. (Eds.), Marketing and Shipping Live Aquatic Products. Proceedings of the Second International Conference and Exhibition, Seattle, WA. Fairbanks: University of Alaska Sea Grant, AK-SG-01-03, pp. 9–18.

Daniels, C.L., Merrifield, D.L., Boothroyd, D.P., Davies, S.J., Factor, J.R., Arnold, K.E., 2010. Effect of dietary Bacillus spp. and mannan oligosaccharides (MOS) on European lobster (*Homarus gammarus* L.) larvae growth performance, gut morphology and gut microbiota. Aquaculture 304, 49–57. doi:10.1016/j.aquaculture.2010.03.018

Daniels, C.L., Merrifield, D.L., Ringø, E., Davies, S.J., 2013. Probiotic, prebiotic and synbiotic applications for the improvement of larval European lobster (*Homarus gammarus*) culture. Aquaculture 416–417, 396–406. doi:10.1016/j.aquaculture.2013.08.001

Daniels, C.L., Wills, B., Ruiz-Perez, M., Miles, E., Wilson, RW., Boothroyd, D., 2015. Development of sea based container culture for rearing European lobster (*Homarus gammarus*) around South West England. Aquaculture 448, 189-195.

Dawkins, MS., 2004. Using behaviour to assess animal welfare. Animal Welfare: 13, S3-7.

de Figueiredo, M.J., 1979. Artificial culture of *Nephrops norvegicus* (L.). I —further studies on larval culture of *Nephrops norvegicus* (L.) reared from the egg. Bio. Inst. Nac. Invest. Pescas 1, 5–12.

De Souza, D.M., Suita, S.M., Leite, F.P.L., Romano, L.A., Wasielesky, W., Ballester, E.L.C., 2012. The use of probiotics during the nursery rearing of the pink shrimp *Farfantepenaeus brasiliensis* (Latreille, 1817) in a zero exchange system. Aquaculture Research 43, 1828–1837. doi:10.1111/j.1365-2109.2011.02992.x

Dhont, J., Van Stappen, G., 2003. Biology, Tank Production and Nutritional Value of *Artemia*, in: Stottrup, J.G., McEvoy, L.A. (Eds.), Live Feeds in Marine Aquaculture. Blackwell Publishing, Oxford, pp.318.

Dickey-Collas, M., McQuaid, N., Armstrong, M.J., Allen, M., Briggs, R.P., 2000. Temperature-dependent stage durations of Irish Sea *Nephrops* larvae. Journal of Plankton Research 22, 749–760. doi:10.1093/plankt/22.4.749

Dissanayake, A., Galloway, T.S., Jones, M.B., 2009. Physiological condition and intraspecific agonistic behaviour in *Carcinus maenas* (Crustacea: Decapoda). Journal of Experimental Marine Biology and Ecology 375, 57–63. doi:10.1016/j.jembe.2009.05.007

Drillet, G., Frouël, S., Sichlau, M.H., Jepsen, P.M., Højgaard, J.K., Joarder, A.K., Hansen, B.W., 2011. Status and recommendations on marine copepod cultivation for use as live feed. Aquaculture 315, 155–166. doi:10.1016/j.aquaculture.2011.02.027

Dunthorn, A., 1967. Some observations on the Behaviour and Development of the Norway Lobster. International Council for the Exploration of the Sea 1967/K:5 (mimeo)

Durand, F., Chausson, F., Regnault, M., 1999. Increases in tissue free amino acid levels in response to prolonged emersion in marine crabs: an ammonia-detoxifying process efficient in the intertidal *Carcinus maenas* but not in the subtidal *Necora puber*. The Journal of experimental biology 202, 2191–2202.

Dytham, C., 2003. Choosing and Using Statistics: A Biologists Guide, 2nd Edition. Blackwell Publishing, Oxford.

Ellis, C.D., Hodgson, D.J., Daniels, C.L., Boothroyd, D.P., Bannister, R.C.A., Griffiths, A.G.F., 2015. European lobster stocking requires comprehensive impact assessment to determine fishery benefits. ICES Journal of Marine Science 72, 35–48.

Ellis, C.D., Hodgson, D.J., Daniels, C.L., Collins, M., Griffiths, A.G.F., 2017. Population genetic structure in European lobsters: implications for connectivity, diversity and hatchery stocking. Marine Ecology Progress Series 563:123–137. doi: 10.3354/meps11957

Ellis, T., North, B., Scott, A.P., Bromage, N.R., Porter, M., Gadd, D., 2002. The relationships between stocking density and welfare in farmed rainbow trout. Journal of Fish Biology 61, 493–531. doi:10.1006/jfbi.2002.2057

Elwood, R.W., 2012. Evidence for pain in decapod crustaceans. Animal Welfare 21(2), 23-27.

Elwood, R.W., Barr, S. and Patterson, L. 2009. Pain and stress in crustaceans? Applied Animal Behaviour Science 118; 128–136.

Epelbaum, A., Borisov, R., 2006. Feeding behaviour and functional morphology of the

feeding appendages of red king crab *Paralithodes camtschaticus* larvae. Marine Biology Research 2, 77–88. doi:10.1080/17451000600672529

Eriksson, S.P., Baden, S.P., 1997. Behaviour and tolerance to hypoxia in juvenile Norway lobster (*Nephrops norvegicus*) of different ages. Marine Biology 128, 49–54. doi:10.1007/s002270050067

Eriksson, S.P., Hernroth, B., Baden, S.P., 2012. Stress biology and immunology in *Nephrops norvegicus*, in: Johnson, M.L., Johnson, M.P. (Eds.), The Ecology and Biology of *Nephrops norvegicus*. Advances in Marine Biology 64, 149-200.

Eriksson, S.P., Nabbing, M., Sjöman, E., 2006. Is brood care in *Nephrops norvegicus* during hypoxia adaptive or a waste of energy? Functional Ecology 20, 1097–1104. doi:10.1111/j.1365-2435.2006.01182.x

Factor, J.R., 1995. Biology of the Lobster *Homarus americanus*. Academic Press, London.

FAO, 2010. No Title [WWW Document]. Landing data for *Nephrops norvegicus* in 1955–2010 using FAO programme and database FishStatJ. URL http://www.fao.org/fishery/statistics/software/fis, [accessed 15/10/2013]

FAO, 2015. No Title [WWW Document]. Capture Production for Nephrops norvegicus 1950-2013 using FAO Dataset. URL http://www.fao.org/fishery/statistics/global-capture-production/query/en, [accessed 15/10/2013]

Farhadi, A., Jensen, M.A., 2015. Effects of photoperiod and stocking density on survival, growth and physiological responses of narrow clawed crayfish (*Astacus leptodactylus*). Aquaculture Research 46, In Press. doi:10.1111/are.12700

Farmer, A.S., 1974a. Reproduction in *Nephrops norvegicus* (Decapoda: Nephropidae). Journal of Zoology 174, 161–183. doi:10.1111/j.1469-7998.1974.tb03150.x

Farmer, A.S., 1974b. The development of the external sexual characters of *Nephrops norvegicus* (L.) (Decapoda: Nephropidae). Journal of Natural History 8, 241–255. doi:10.1080/00222937400770231

Farmer, A.S.D., 1975. Synopsis of the biological data on the Norway lobster *Nephrops*

norvegicus (Linnaeus, 1758). FAO Fisheries Synopsis 112, 1–97.

Figueiredo, M.J.D.E., Vilela, M.H., 1972. On the artificial culture of *Nephrops norvegicus* reared from the egg. Aquaculture 1, 173–180.

Figueiredo, M.J. de, 1979. Artificial culture of *Nephrops norvegicus* (L.). II - some studies on the growth of early post-larvae of *Nephrops norvegicus* (L.) reared from the egg. Bol. Inst. Nac. Invest. Pescas, Lisboa 1, 13–23.

Figueredo, J., van Woesik, R., Lin, J., Narciso, L., 2009. *Artemia* enrichment model how to keep them small rich and alive. Aquaculture 294, 212–220.

Fiore, D.R., Tlusty, M.F., 2005. Use of commercial *Artemia* replacement diets in culturing larval American lobsters (*Homarus americanus*). Aquaculture 243, 291–303. doi:10.1016/j.aquaculture.2004.10.009

Fitzgibbon, Q.P., Battaglene, S.C., 2012. Effect of photoperiod on the culture of early-stage phyllosoma and metamorphosis of spiny lobster (*Sagmariasus verreauxi*). Aquaculture 368–369, 48–54. doi:10.1016/j.aquaculture.2012.09.018

Fletcher, R.J., 2006. Emergent properties of conspecific attraction in fragmented landscapes. The American naturalist 168, 207–219. doi:10.1086/505764

Forward, R.B., Cronin, T.W., Stearns, D.E., 1984. Control of diel vertical migration: Photoresponses of a larval crustacean. Limnology and Oceanography 29, 146–154. doi:10.4319/lo.1984.29.1.0146

Fotedar, S., Evans, L., 2011. Health management during handling and live transport of Crustaceans: a review. Journal of Invertebrate Pathology 106, 143–52. doi:10.1016/j.jip.2010.09.011

Fürtbauer, I., 2015. Consistent individual differences in haemolymph density reflect risk propensity in a marine invertebrate. Royal Society Open Science 2, 140482. dio:10.1098/rsos.140482

Galindo, C., Gaxiola, G., Cuzon, G., Chiappa-Carrara, X., 2009. Physiological and Biochemical Variations during the Molt Cycle in Juvenile *Litopenaeus vannamei* Under Laboratory

Conditions. Journal of Crustacean Biology 29, 544–549. doi:10.1651/08-3094.1

García-Guerrero, M., Villarreal, H., Racotta, I.S., 2003. Effect of temperature on lipids, proteins, and carbohydrates levels during development from egg extrusion to juvenile stage of *Cherax quadricarinatus* (Decapoda: Parastacidae). Comparative biochemistry and physiology. Part A, Molecular & integrative physiology 135, 147–54.

Gaten, E., Moss, S., Johnson, M., 2013. The reniform reflecting superposition compound eyes of *Nephrops norvegicus*: Optics, susceptibility to light induced damage, electrophysiology and a ray tracing model. Advances in Marine Biology 64, 107-148.

Gear, D., Shelmerdine, R.L., Leslie, B., 2010. The effects of storage and diet on the condition and flesh quality of European lobsters. (Report). NAFC Marine Centre, Shetland, pp.25.

Gendron, L., Tremblay, R., Belvin, S., Génard, B., Motnikar, S., Côté, J., 2013. Condition, survival and growth in situ of hatchery-reared stage IV lobster (*Homarus americanus*) fed *Artemia* and lipid-rich wild zooplankton. Aquaculture 416–417, 380–389. doi:10.1016/j.aquaculture.2013.08.003

Gerhardt, L., Baden, S.P., 1998. Gender- and oxygen-related irrigation behaviour of the decapod *Nephrops norvegicus*. Marine Biology 131, 553–558.

Gherardi, F., Aquiloni, L., Tricarico, E., 2012. Behavioral plasticity, behavioral syndromes and animal personality in crustacean decapods: An imperfect map is better than no map. Current Zoology 58, 567-579.

Gilmour, K.M., DiBattista, J.D., Thomas, J.B., 2005. Physiological Causes and Consequences of Social Status in Salmonid Fish. Integrative and Comparative Biology 45, 263–273. doi:10.1093/icb/45.2.263

Giomi, F., Raicevich, S., Giovanardi, O., Pranovi, F., Muro, P. Di, Beltramini, M., 2008. Catch me in winter! Seasonal variation in air temperature severely enhances physiological stress and mortality of species subjected to sorting operations and discarded during annual fishing activities. Hydrobiologia 606, 195–202. doi:10.1007/s10750-008-9336-x

Goddard, S., 1996. Feed Managment in Intensive Aquaculture. Chapman & Hall, London.

Gogoi, B., Safi, V., Das, D.N., 2016. The Cladoceran as live Feed in Fish Culture : A Brief Review. Research Journal of Animal Veterinary and Fishery Sciences 4, 7–12.

Gomez-Jimenez, S., Uglow, R.F., Gollas-Galvan, T., 2000. The effects of cooling and emersion on total haemocyte count and phenoloxidase activity of the spiny lobster *Panulirus interruptus*. Fish & shellfish immunology 10, 631–635. doi:10.1006/fsim.2000.0277

Goodwin, T.W., 1951. Carotenoid Metabolism during Development of Lobster Eggs. Nature 167, 559–559. doi:10.1038/167559a0

Gowland, D., 2016. Shellfish Hatchery Systems Ltd [WWW Document]. URL www.aquahive.co.uk/ (accessed 10.17.16).

Guerin, A.J., Neil, D.M., 2015. Escape trajectories of the rockpool prawn (*Palaemon elegans*) in response to visual and mechanosensory stimuli. Marine and Freshwater Behaviour and Physiology 48, 145–161. doi:10.1080/10236244.2015.1027541

Haché, R., Daoud, D., Hébert Y., Dumas, A., 2014. New developments in lobster resource enhancement in Atlantic Canada through stage IV larvae release. In: Proceedings of the 10th International Conference and Workshop on the Biology and Management of Lobsters, Cancún, Mexico. May 18–23, 2014. Puerto Morelos, Quintana Roo, Mexico, Institute of Ocean Sciences and Limnology, p.60.

Hai, N. Van, Buller, N., Fotedar, R., 2009. Effects of probiotics (*Pseudomonas synxantha* and *Pseudomonas aeruginosa*) on the growth, survival and immune parameters of juvenile western king prawns (*Penaeus latisulcatus* kishinouye, 1896). Aquaculture Research 40, 590–602. doi:10.1111/j.1365-2109.2008.02135.x

Hamre, K., Opstad, I., Espe, M., Solbakken, J., Hemre, G.-I., Pittman, K., 2002. Nutrient composition and metamorphosis success of Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae fed natural zooplankton or *Artemia*. Aquaculture Nutrition 8, 139–148. doi:10.1046/j.1365-2095.2002.00201.x

Harmon, T.S., 2009. Methods for reducing stressors and maintaining water quality associated with live fish transport in tanks: a review of the basics. Reviews in Aquaculture 1, 58–66. doi:10.1111/j.1753-5131.2008.01003.x

Harris, R.P., Wiebe, P.H., Lenz, J., Skjoldal, H.R., Huntley, M., 2000. Zooplankton Methodology Manual. Elsevier Academic Press, London.

Harris, R.R., Andrews, M.B., 2005. Physiological changes in the Norway lobster *Nephrops norvegicus* (L.) escaping and discarded from commercial trawls on the West Coast of Scotland: II. Disturbances in haemolymph respiratory gases, tissue metabolites and swimming performance after capture a. Journal of Experimental Marine Biology and Ecology 320, 195–210. doi:10.1016/j.jembe.2004.12.028

Hennig, C.W., Dunlap, W.P., 1978. Tonic immobility in *Anolis carolinensis*: Effects of time and conditions of captivity. Behavioral Biology 23, 75–86. doi:10.1016/S0091-6773(78)91180-X

Hernández-Falcón, J., Basu, A.C., Govindasamy, S., Kravitz, E. A., 2005. Changes in heart rate associated with contest outcome in agonistic encounters in lobsters. Cellular and Molecular Neurobiology 25, 329–343. doi:10.1007/s10571-005-3063-x

Hills, J., 1975. Captive rearing of larvae of the Dublin Bay Prawn *Nephrops norvegicus* (L.). Irish Fisheries Investigations (Series B) 16, 3–12.

Hinojosa, I.A., Green, B.S., Gardner, C., Jeffs, A., 2014. Settlement and early survival of southern rock lobster, *Jasus edwardsii*, under climate-driven decline of kelp habitats. ICES Journal of Marine Science 72, i59–i68. doi:10.1093/icesjms/fst034

Hoskonen, P., Heikkinen, J., Eskelinen, P., Pirhonen, J., 2013. Efficacy of clove oil and ethanol against *Saprolegnia* sp. and usability as antifungal agents during incubation of rainbow trout *Oncorhynchus mykiss* (Walbaum) eggs. Aquaculture Research 10, 1–9. doi:10.1111/are.12200

Houlihan, D.F., Mathers, E., 1985. Effects of Captivity and Exercise on the Energetics of Locomotion and Muscle of *Carcinus maenas* (L.). Journal of Experimental Marine Biology and Ecology 92, 125–142.

Hunter, D.A., Uglow, R.F., 1993. Handling-induced changes in haemolymph ammonia concentration and ammonia excretion rate of *Crangon crangon* (L.). Ophelia 38, 137–147. doi:10.1080/00785326.1993.10429895

Huntingford, F.A., Taylor, A.C., Smith, I.P., Thorpe, K.E., 1995. Behavioural and physiological studies of aggression in swimming crabs. Journal of Experimental Marine Biology and Ecology 193, 21–39. doi:10.1016/0022-0981(95)00108-5

Huntsberger, C., 2012. Use of Injectable Eugenol for Euthanasia and Anesthesia of American Lobsters (*Homarus americanus*) and Similar Species. Masters Thesis. Roger Williams University.

Huu, HD., Jones, CM., 2014. Effects of dietary mannan oligosaccharide supplementation on juvenile spiny lobster *Panulirus homarus* (Palinuridae). Aquaculture 432, 258-264.

ICES., 2016a. Norway lobster (*Nephrops norvegicus*) in divisions 4.b and 4.c, Functional Unit 5 (central and southern North Sea, Botney Cut-Silver Pit) In: ICES Advice on fishing opportunities, catch, and effort Greater North Sea Ecoregion. p.1-10.

ICES., 2016b. Norway lobster (*Nephrops norvegicus*) in Division 4.a, Functional Unit 10 (northern North Sea, Noup). In: ICES Advice on fishing opportunities, catch, and effort Celtic Seas Ecoregion and Greater North Sea Ecoregions. p.1-9.

ICES., 2017c. Norway lobster (*Nephrops norvegicus*) in Division 4.b, Functional Unit 9 (central North Sea, Moray Firth). In: ICES Advice on fishing opportunities, catch, and effort Greater North Sea Ecoregion. p.1-10. DOI: 10.17895/ices.pub.3195.

ICES., 2017d. Norway lobster (*Nephrops norvegicus*) in Division 4.b, Functional Unit 8 (central North Sea, Firth of Forth). In: ICES Advice on fishing opportunities, catch, and effort Greater North Sea Ecoregion. p.1-11.

ICES., 2017e. Norway lobster (*Nephrops norvegicus*) in Division 4.b, Functional Unit 6 (central North Sea, Farn Deeps). In: ICES Advice on fishing opportunities, catch, and effort Greater North Sea Ecoregion. p.1-8.

ICES., 2016f. Norway lobster (*Nephrops norvegicus*) in Division 4.b, Functional Unit 34 (central North Sea, Devil's Hole). In: ICES Advice on fishing opportunities, catch, and effort Greater North Sea Ecoregion. p.1-9.

ICES., 2017g. Norway lobster (*Nephrops norvegicus*) in Division 4.a, Functional Unit 7

(northern North Sea, Fladen Ground). In: ICES Advice on fishing opportunities, catch, and effort Greater North Sea Ecoregion. p.1-11. DOI: 10.17895/ices.pub.3527

ICES., 2017h. Norway lobster (*Nephrops norvegicus*) in Division 6.a, Functional Unit 11 (West of Scotland, North Minch). In: ICES Advice on fishing opportunities, catch, and effort Celtic Seas Ecoregion. p.1-8. DOI: 10.17895/ices.pub.3386

ICESi., 2017i. Norway lobster (*Nephrops norvegicus*) in Division 6.a, Functional Unit 11 (West of Scotland, South Minch). In: ICES Advice on fishing opportunities, catch, and effort Celtic Seas Ecoregion. p.1-8. DOI: 10.17895/ices.pub.3385

ICES., 2017j. Norway lobster (*Nephrops norvegicus*) in Division 6.a, Functional Unit 13 (West of Scotland, the Firth of Clyde, and the Sound of Jura). In: ICES Advice on fishing opportunities, catch, and effort Celtic Seas Ecoregion. p.1-10. DOI: 10.17895/ices.pub.3390

ICES., 2017k. Norway lobster (*Nephrops norvegicus*) in Division 7.a, Functional Unit 14 (Irish Sea, East). In: ICES Advice on fishing opportunities, catch, and effort Celtic Seas Ecoregion. p.1-7. DOI: 10.17895/ices.pub.3395

ICESl., 2017l. Norway lobster (*Nephrops norvegicus*) in Division 7.a, Functional Unit 15 (Irish Sea, West). In: ICES Advice on fishing opportunities, catch, and effort Celtic Seas Ecoregion. p.1-8. DOI: 10.17895/ices.pub.3400

ICES., 2017m. Norway lobster (*Nephrops norvegicus*) in divisions 7.g and 7.f, Functional Unit 22 (Celtic Sea, Bristol Channel). In: ICES Advice on fishing opportunities, catch, and effort Celtic Seas Ecoregion. p.1-7. DOI: 10.17895/ices.pub.3398

ICES., 2017n. Norway lobster (*Nephrops norvegicus*) in divisions 7.g and 7.h, functional units 20 and 21 (Celtic Sea). In: ICES Advice on fishing opportunities, catch, and effort Celtic Seas Ecoregion. nep.fu.2021. p.1-7. DOI: 10.17895/ices.pub.3402

ICESo., 2017o. Norway lobster (*Nephrops norvegicus*) in divisions 7.a, 7.g, and 7.j, Functional Unit 19 (Irish Sea, Celtic Sea, eastern part of southwest of Ireland). In: ICES Advice on fishing opportunities, catch, and effort Celtic Seas Ecoregion. p.1-7. DOI: 10.17895/ices.pub.3397

ICES., 2017p. Norway lobster (*Nephrops norvegicus*) in Division 7.b, Functional Unit 17 (west of Ireland, Aran grounds). In: ICES Advice on fishing opportunities, catch, and effort Celtic Seas Ecoregion. p.1-8. DOI: 10.17895/ices.pub.3396

ICES., 2017q. Norway lobster (*Nephrops norvegicus*) in divisions 7.b–c and 7.j–k, Functional Unit 16 (west and southwest of Ireland, Porcupine Bank). In: ICES Advice on fishing opportunities, catch, and effort Celtic Seas and Oceanic Northeast Atlantic Ecoregions. p.1-8. DOI: 10.17895/ices.pub.3401

Jacklin, M., 2005. Assessment of Stress and Mortality of the Prawn (*Nephrops norvegicus*) During Live Handling from Vessel to Market. Seafish SR424, Seafish.

Jee, J.-H., Koo, J.-G., Keum, Y.-H., Kang, J.-S., Park, K.H. A, Kang, J.-C., 2007. Effects of diet and sediment type on survival, growth and moulting of juvenile Tiger crab, *Orithyia sinica* L. Aquaculture Research 38, 26–35. doi:10.1111/j.1365-2109.2006.01620.x

Johns, P.M., Mann, K.H., 1987. An experimental investigation of juvenile lobster habitat preference and mortality among habitats of varying structural complexity. Journal of Experimental Marine Biology and Ecology 109, 275–285. doi:10.1016/0022-0981(87)90058-X

Johnson, M., Johnson, M.P., Aguzzi, J., Baden, S.P., Bell, E., Breithaupt, T., Bublitz, R., Clarke, J.W., Cowing, D., Dobson, N., Eriksson, S.P., Gaten, E., Hernroth, B., Katoh, E., Lordan, C., Moss, S., Powell, A., Power, A.M., Sandell, J., Sbragaglia, V., Tshudy, D., Ungfors, A., 2013. The Ecology and Biology of *Nephrops norvegicus*, 64th edition. Advances in Marine Biology. Academic Press, London.

Johnson, M.P., Lordan, C., Power, A.M., 2013. Habitat and Ecology of *Nephrops norvegicus*, in: Johnson, M.L., Johnson, M.P. (Eds.), The Ecology and Biology of *Nephrops norvegicus*. Academic Press, London, pp. 27–54.

Johnson, S., 1995. Handbook of Shrimp Diseases. Aquaculture. Department of Wildlife and Fisheries Sciences: A&M University, Byan, Texas.

Jones, D. A., Kumlu, M., Le Vay, L., Fletcher, D.J., 1997. The digestive physiology of herbivorous, omnivorous and carnivorous Crustacean larvae: a review. Aquaculture

155, 285–295. doi:10.1016/S0044-8486(97)00129-4

Jorgensen, O.M., 1923. The Early Stages of *Nephrops norvegicus*, from the Northumberland Plankton , together with a note on the post-larval development of *Homarus vulgaris*. Journal of the Marine Biological Association of the United Kingdom 13 (4), 870-879.

Jørstad, K.E., Prodöhl, P. A., Kristiansen, T.S., Hughes, M., Farestveit, E., Taggart, J.B., Agnalt, A.-L., Ferguson, A., 2005. Communal larval rearing of European lobster (*Homarus gammarus*): Family identification by microsatellite DNA profiling and offspring fitness comparisons. Aquaculture 247, 275–285. doi:10.1016/j.aquaculture.2005.02.025

Juanes, F., Smith, L.D., 1995. The ecological consequences of limb damage and loss in decapod Crustaceans: a review and prospectus. Journal of Experimental Marine Biology and Ecology 193, 197–223. doi:10.1016/0022-0981(95)00118-2

Jussila, J., Jago, J., Tsvetnenko, E., Dunstan, B., Evans, L.H., 1997. Total and differential haemocyte counts in Western Rock Lobsters (*Panulirus cygnus*) under post-harvest stress. Marine and Freshwater Research 48, 863–867.

Jussila, J., Jago, J., Tsvetnenko, E., Evans, L.H., 2001. Effects of handling or injury disturbance on total hemocyte counts in western rock lobster (*Panulirus cygnus* George), in: Evans, L., Jones, B. (Eds.), International Symposium on Lobster Health Management. Adelaide, pp. 52–63.

Jussila, J., Paganini, M., Mansfield, S., Evans, L.H., 1999. On physiological responses, plasma glucose, total hemocyte counts and dehydration, of Marron *Cheraax tenuimanus* (Smith) to handling and transport under simulated conditions. Freshwater Crayfish 12, 154–167.

Kallen, J.L., Abrahamse, S.L., Vanherp, F., 1990. Circadian rhythmicity of the Crustacean hyperglycemic hormone (CHH) in the hemolymph of the crayfish. Biological Bulletin 179, 351–357. doi:10.2307/1542327

Kamarudin, M.S., Jones, D.A., le Vay, L., Abidin, A.Z., 1994. Ontogenetic change in digestive enzyme activity during larval development of *Macrobrachium rosenbergii*. Aquaculture 123, 323–333. doi:10.1016/0044-8486(94)90068-X

Katan, T., Nash, G.W., Rise, M.L., Hall, J.R., Fernandes, J.M.O., Boyce, D., Johnsen, C.A., Gamperl, A.K., 2016. A Little Goes a Long Way: Improved growth in Atlantic cod (*Gadus morhua*) fed small amounts of wild zooplankton. Aquaculture 451, 271-282.

Katoh, E., 2011. Sex , Pheromone and Aggression in Norway lobster (*Nephrops norvegicus*). PhD Thesis. University of Hull, UK.

Katoh, E., Johnson, M., Breithaupt, T., 2008. Fighting behaviour and the role of urinary signals in dominance assessment of Norway lobsters, *Nephrops norvegicus*. Behaviour 145, 1447–1464. doi:10.1163/156853908785765917

Katoh, E., Sbragaglia, V., Aguzzi, J., Breithaupt, T., 2013. Sensory Biology and Behaviour of *Nephrops norvegicus*, in: Johnson, M.L., Johnson, M.P. (Eds.), The Ecology and Biology of *Nephrops norvegicus*. Academic Press, London, pp. 65–107.

Kitada, S., 2018. Economic, ecological and genetic impacts of marine stock enhancemnt and sea ranching: A systematic review. Fish and Fisheries 19, 511-532. doi:10.1111/faf.12271

KT Prawns., 2018. Juvenile Prawns. URL: https://www.ktprawns.com/ [accessed 21.05.2018].

Kurmaly, K., Jones, D.A., Yule, A.B., 1990. Acceptability and digestion of diets fed to larval stages of *Homarus gammarus* and the role of dietary conditioning behaviour. Marine Biology 106, 181–190.

Ladhar, C., Ayadi, H., Denis, F., Tastard, E., Sellami, I., 2014. The effect of environmental factors on the fatty acid composition of Copepods and *Artemia* in the Sfax solar saltern (Tunisia). Biochemical Systematics and Ecology 56, 237–245. doi:10.1016/j.bse.2014.06.005

Latrouite, D., Lorec, J., 1991. L'experience Francaise de forcage du recruitement du homard Europeen (*Homarus gammarus*) resultats preliminaries. ICES Marine Science Symposia 192, 93–98.

Le Moullac, G., Haffner, P., 2000. Environmental factors affecting immune responses in

Crustacea. Aquaculture 191, 121–131. doi:10.1016/S0044-8486(00)00422-1

Le Vay, L., Jones, D., Puello-Cruz, C., Sangha, R.S., Ngamphongsai, C., 2001. Digestion in relation to feeding strategies exhibited by Crustacean larvae. Comparative Biochemistry and Physiology. Part A, Molecular & integrative physiology 128, 623–630. doi:10.1016/S1095-6433(00)00339-1

Leavitt, D.F., Bayer, R.C., 1977. A refractometric method of determining serum protein concentration in the American lobster. Aquaculture 12, 169–171. doi:10.1016/0044-8486(77)90184-3

Léger, P., Bengtson, D.., Simpson, K.., Sorgeloos, P., 1986. The use and nutritional value of *Artemia* as a food source. Oceanography and Marine Biology: An Annual Review 24, 521–623.

Lehner, P.N., 1996. Handbook of Ethological Methods, Second. ed. Cambridge University Press, Cambridge.

Leocádio, A.M., Whitmarsh, D., Castro, M., 2012. Comparing trawl and creel fishing for Norway lobster (*Nephrops norvegicus*): biological and economic considerations. PloS one 7, e39567. doi:10.1371/journal.pone.0039567

Liu, C.H., Chiu, C.S., Ho, P.L., Wang, S.W., 2009. Improvement in the growth performance of white shrimp, *Litopenaeus vannamei*, by a protease-producing probiotic, *Bacillus subtilis* E20, from natto. Journal of Applied Microbiology 107, 1031–1041. doi:10.1111/j.1365-2672.2009.04284.x

Loew, E.R., 1976. Light, and photoreceptor degeneration in the Norway lobster, *Nephrops norvegicus* (L.). Proceedings of the Royal Society of London. Series B, Containing papers of a Biological Character. Royal Society (Great Britain) 193, 31–44.

Logan, D.T., Epifanio, C.E., 1978. A laboratory energy balance for the larvae and juveniles of the American Lobster *Homarus americanus*. Marine Biology 47, 381–389. doi:10.1007/BF00388930

Lorenzen, K., Leber, K.M., Blankenship, H.L., 2010. Responsible Approach to Marine Stock

Enhancement: An Update. Reviews in Fisheries Science 18, 189–210. doi:10.1080/10641262.2010.491564

Lorenzon, S., 2005. Hyperglycemic stress response in Crustacea. Invertebrate Survival Journal 2, 132–141.

Lorenzon, S., Giulianini, P.G., Libralato, S., Martinis, M., Ferrero, E., 2008. Stress effect of two different transport systems on the physiological profiles of the crab *Cancer pagurus*. Aquaculture 278, 156–163. doi:10.1016/j.aquaculture.2008.03.011

Lorenzon, S., Giulianini, P.G., Martinis, M., Ferrero, E., 2007. Stress effect of different temperatures and air exposure during transport on physiological profiles in the American lobster *Homarus americanus*. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 147, 94–102. doi:10.1016/j.cbpa.2006.11.028

Lorenzon, S., Martinis, M., Ferrero, E.A., 2011. Ecological Relevance of Hemolymph Total Protein Concentration in Seven Unrelated Crustacean Species from Different Habitats Measured Predictively by a Density-Salinity Refractometer. Journal of Marine Biology 2011, 1–7. doi:10.1155/2011/153654

Lovell, T., 1989. Nutrition and feeding of fish. Van Nostrand Reinhold International Company Limited, New York.

Lund, H.S., Wang, T., Chang, E.S., Pedersen, L.F., Taylor, E.W., Pedersen, P.B., McKenzie, D.J., 2009. Recovery by the Norway lobster *Nephrops norvegicus* (L.) from the physiological stresses of trawling: Influence of season and live-storage position. Journal of Experimental Marine Biology and Ecology 373, 124–132. doi:10.1016/j.jembe.2009.04.004

Maar, M., Nielsen, T.G., Gooding, S., Tönnesson, K., Tiselius, P., Zervoudaki, S., Christou, E., Sell, A., Richardson, K., 2004. Trophodynamic function of Copepods, appendicularians and protozooplankton in the late summer zooplankton community in the Skagerrak. Marine Biology 144, 917–933. doi:10.1007/s00227-003-1263-9

Maciel, C.R., New, M.B., Valenti, W.C., 2012. The Predation of *Artemia* nauplii by the Larvae of the Amazon River Prawn, *Macrobrachium amazonicum* (Heller, 1862), is Affected by

Prey Density, Time of Day, and Ontogenetic Development. Journal of the World Aquaculture Society 43, 659–669. doi:10.1111/j.1749-7345.2012.00599.x

Manley, C.B., Rakocinski, C.F., Lee, P.G., Blaylock, R.B., 2015. Feeding frequency mediates aggression and cannibalism in larval hatchery-reared spotted seatrout, *Cynoscion nebulosus*. Aquaculture 437, 155–160. doi:10.1016/j.aquaculture.2014.11.012

Marine Institute, 2014. The Stock Book 2014: Annual Review of Fish Stocks in 2014 with Management Advice for 2015. Marine Institute, Galway, Ireland.

Marine Management Organsation, 2014. UK Sea Fisheries Statistics. Office for National Statistics, Newport, UK.

Marsden, G., Mather, P., Richardson, N., 2007. Captivity, ablation and starvation of the prawn *Penaeus monodon* affects protein and lipid content in ovary and hepatopancreas tissues. Aquaculture 271, 507–515. doi:10.1016/j.aquaculture.2007.04.076

Mauchline, J., 1998. The Biology of Calanoid Copepods, Advances in Marine Biology. Academic Press, California.

Maynou, F., Sardà, F., 1997. *Nephrops norvegicus* population and morphometrical characteristics in relation to substrate heterogeneity. Fisheries Research 30, 139–149.

McEvoy, L.A., Naess, T., Bell, J.G., Lie, O., 1998. Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (*Hippoglossus hippoglossus*) fed enriched *Artemia*: A comparison with fry fed wild copepods. Aquaculture 163, 237–250. doi:10.1016/S0044-8486(98)00237-3

Meager, J.J., Williamson, I., Loneragan, N.R., Vance, D.J., 2005. Habitat selection of juvenile banana prawns, *Penaeus merguiensis* de Man: Testing the roles of habitat structure, predators, light phase and prawn size. Journal of Experimental Marine Biology and Ecology 324, 89–98. doi:10.1016/j.jembe.2005.04.012

Mente, E., 2010. Survival, food consumption and growth of Norway lobster (*Nephrops norvegicus*) kept in laboratory conditions. Integrative Zoology 5, 256–263.

doi:10.1111/j.1749-4877.2010.00211.x

Merrifield, D.L., Harper, G.M., Dimitroglou, A., RingÃ¸, E., Davies, S.J., 2009. Possible influence of probiotic adhesion to intestinal mucosa on the activity and morphology of rainbow trout (*Oncorhynchus mykiss*) enterocytes. Aquaculture Research 1268–1272. doi:10.1111/j.1365-2109.2009.02397.x

Miami Aqua-culture Inc., 2018. Livestock. URL: http://www.miami-aquaculture.com/marine%20shrimp%20culture.htm [accessed: 21.05.2018].

Middlemiss, K.L., Daniels, C.L., Urbina, M. A., Wilson, R.W., 2015. Combined effects of UV irradiation, ozonation, and the probiotic Bacillus spp. on growth, survival, and general fitness in European lobster (*Homarus gammarus*). Aquaculture 444, 99–107. doi:10.1016/j.aquaculture.2015.03.028

Mikami, S., 1993. Effects of Starvation upon Survival, Growth and Molting Interval of the Larvae of the Spiny Lobster *Panulirus japonicus* (Decapoda , Palinuridea). Crustaceana 64, 137–142.

Millikin, M.R., 1978. Blue Crab Larval Culture Methods and Management. Marine Fisheries Review 40, 1–17.

MMO, 2012. UK Sea Fisheries Statistics 2012 [WWW Document]. National Statistics. URL www.marinemanagement.org.uk/fisheries/statistics, [accessed 19/05/2013].

MMO, 2017. UK Sea Fisheries Statistics 2017 [WWW Document]. National Statistics. URL www.marinemanagement.org.uk/fisheries/statistics, [accessed 12/05/2017]

Moksness, E., Roald, S., van der Meeren, G., 1998. Profitability analysis of sea ranching with Atlantic salmon (*Salmo salar*), Arctic charr (*Salvelinus alpinus*), and European lobster (*Homarus gammarus*) in Norway. Bulletin of Marine Science 62, 689-699.

Moore, P.A., 2007. Agonistic behaviour in freshwater crayfish: the influence of intrinsic and extrinsic factors on aggressive encounters and dominance, in: Duffy, J.E., Thiel, M. (Eds.), Evolutionary Ecology of Social and Sexual Systems: Crustacean as Model Organisms. Oxford University Press, New York, pp. 90–114.

Morris, S., Airries, C.N., 1998. Integration of physiological responses of crustaceans to environmental challenge. South African Journal of Zoology 33:2, 87-106. doi:10.1080/02541858.1998.11448458

Mouat, B., 2002. Reproductive dynamics of the male Norway lobster, *Nephrops norvegicus* (L.). PhD Thesis. University of Glasgow.

Naess, T., Germain-Henry, M., Naas, K.E., 1995. First feeding of Atlantic Halibut (*Hippoglossus hippoglossus*) using different combinations of *Artemia* and wild Zooplankton. Aquaculture 130, 235–250.

Nakayama, C.L., Peixoto, S., Bianchini, A., Robaldo, R.B., Cavalli, R.O., 2008. Performance of *Farfantepenaeus paulensis* (Pérez-Farfante, 1967) broodstock in tanks with sand and hard substrate. Aquaculture Research 39, 398–405. doi:10.1111/j.1365-2109.2007.01800.x

Neil, D.M., 2012. Ensuring Crustacean product quality in the post-harvest phase. Journal of Invertebrate Pathology 110, 267–75. doi:10.1016/j.jip.2012.03.009

Neil, D.M., Macmillan, D.L., Robertson, R.M., Laverack, M.S., 1976. The structure and function of thoracic expodites in the larvae of the lobster *Homarus gammarus* (L.). Philosophical Transactions of the Royal Society London B 274, 53–68.

Nelson, M.M., Mooney, B.D., Nichols, P.D., Phleger, C.E., Smith, G.G., Hart, P., Ritar, A.J., 2002. The Effect of Diet on the Biochemical Composition of Juvenile *Artemia*: Potential Formulations for Rock Lobster Aquaculture. Journal of the World Aquaculture Society 33, 146–157. doi:10.1111/j.1749-7345.2002.tb00489.x

Newland, P.L., Chapman, C.J., 1985. Some observations on the behaviour of *Nephrops* in relation to trawling. Internation Council for the Exploration of the Sea Committee Meeting. (Shellfish and Benthos Commitee) K:7:1-8 (mimeo)

Nhan, D.T., Wille, M., Hung, L.T., Sorgeloos, P., 2010. Effects of larval stocking density and feeding regime on larval rearing of giant freshwater prawn (*Macrobrachium rosenbergii*). Aquaculture 3, 80–86. doi:10.1016/j.aquaculture.2009.12.012

Nichols, J.H., Bennett, D.B., Symonds, D.J., Grainger, R., 1987. Estimation of the stock size of adult *Nephrops norvegicus* (L.) from larvae surveys in the western Irish Sea in 1982. Journal of Natural History 21, 1433–1450. doi:10.1080/00222938700770901

Nicosia, F., Lavalli, K., 1999. Homarid Lobster Hatcheries : Their History and Role in Research, Management, and Aquaculture. Marine Fisheries Review 61, 1–57.

Øie, G., Galloway, T., Sørøy, M., Holmvaag Hansen, M., Norheim, I.A., Halseth, C.K., Almli, M., Berg, M., Gagnat, M.R., Wold, P.A., Attramadal, K., Hagemann, A., Evjemo, J.O., Kjørsvik, E., 2015. Effect of cultivated copepods (*Acartia tonsa*) in first-feeding of Atlantic cod (*Gadus morhua*) and ballan wrasse (*Labrus bergylta*) larvae. Aquaculture Nutrition 23, 3-17. doi:10.1111/anu.12352

Ozbay, G., Riley, J.G., 2002. An analysis of refractometry as a method of determining blood total protein concentration in the American lobster *Homarus americanus* (Milne Edwards). Aquaculture Research 33, 557–562. doi:10.1046/j.1365-2109.2002.00675.x

Palacios, E., Ibarra, A.M., Ramirez, J.L., Portillo, G., Racotta, I.S., 1998. Biochemical composition of eggs and nauplii in White Pacific Shrimp, *Penaeus vannamei* (Boone), in relation to the physiological condition of spawners in a commercial hatchery. Aquaculture Research 29, 183–189.

Palma, J., Bureau, D.P., Andrade, J.P., 2014. The effect of diet on ontogenic development of the digestive tract in juvenile reared long snout seahorse *Hippocampus guttulatus*. Fish Physiology and Biochemistry 40, 739–50. doi:10.1007/s10695-013-9881-8.

Pandian, T., 1970. Ecophysiological studies on the developing eggs and embryos of the European lobster *Homarus gammarus*. Marine Biology 5, 154–167.

Parker, G., 1974. Assesment strategy and the evolution of fighting behaviour. Journal of Theoretical Biology 47, 223–243.

Parodi, T. V, Cunha, M., Heldwein, C.G., de Souza, D.M., Martins, Á.C., Garcia, L.D.O., Wasielesky, W., Monserrat, J.M., Schmidt, D., Caron, B.O., Heinzmann, B., Baldisserotto, B., 2012. The anesthetic efficacy of eugenol and the essential oils of *Lippia alba* and *Aloysia triphylla* in post-larvae and sub-adults of *Litopenaeus vannamei*

(Crustacea, Penaeidae). Comparative Biochemistry and Physiology. Part C: Toxicology & Pharmacology 155, 462–468. doi:10.1016/j.cbpc.2011.12.003

Peeke, H. V, Blank, G.S., Figler, M.H., Chang, E.S., 2000. Effects of exogenous serotonin on a motor behavior and shelter competition in juvenile lobsters (*Homarus americanus*). Journal of Comparative Physiology A 186, 575–582.

Perez Benavente, G., Uglem, I., Browne, R. and Balsa, C.M., 2010. Culture of juvenile European lobster (*Homarus gammarus* L.) in submerged cages. Aquaculture International 18, 1177-1189. doi:10.1007/s10499-010-9332-9

Perkins, H., 1971. Egg loss during incubation from offshore northern lobsters (Decapoda: Homaridae). Fishery Bulletin 69, 451–453.

Persson, M., Cerenius, L., Soderhall, K., 1987. The Influence of Hemocyte Number on the Resistance of the Fresh-Water Crayfish, *Pacifastacus Leniusculus* Dana, to the Parasitic Fungus *Aphanomyces astaci*. Journal of Fish Diseases 10, 471–477. doi:10.1111/j.1365-2761.1987.tb01098.x.

Peyton, K.A., Hanisak, M.D., Lin, J., 2004. Marine algal symbionts benefit benthic invertebrate embryos deposited in gelatinous egg masses. Journal of Experimental Marine Biology and Ecology 307, 139–164. doi:10.1016/j.jembe.2004.01.022.

Philips., 2013. Lobsters: Biology, Management, Aquaculture and Fisheries. Second Edition. Wiley-Blackwell, Oxford.

Pochelon, P.N., 2011. Embyonic and larval ecology and biochemistry of offshore decapods. PhD Thesis. Universidade de Aveiro.

Pochelon, P.N., Calado, R., Dos Santos, A., Queiroga, H., 2009. Feeding ability of early zoeal stages of the Norway lobster *Nephrops norvegicus* (L.). The Biological bulletin 216, 335–43.

Pochelon, P.N., da Silva, T.L., Reis, A., dos Santos, A., Queiroga, H., Calado, R., 2011. Inter-individual and within-brood variability in the fatty acid profiles of Norway lobster, *Nephrops norvegicus* (L.) embryos. Marine Biology 158, 2825–2833.

doi:10.1007/s00227-011-1781-9.

Powell, A., Cowing, D.M., Scolding, J.W.S., Shepherd-Waring, T., Eriksson, S.P., Lupatsch, I., Johnson, M.L., Shields, R.J., Gowland, D. 2015. The NEPHROPS Manual. Patent application number GB1509666.

Powell, A., Cowing, D.M., Eriksson, S.P. and Johnson, M.L., 2017. Stress response according to transport protocol in Norway lobster, *Nephrops norvegicus*. Crustacean Research 46, 17-24.

Powell, A., Eriksson, S.P., 2013. Reproduction: Life Cycle, Larvae and Larviculture., in: Johnson, M.L., Johnson, M.P. (Eds.), The Biology and Ecology of *Nephrops norvegicus*. Academic Press, London, pp. 201–240. doi:10.1016/B978-0-12-410466-2.00006-6.

Powell, A., Hinchcliffe, J., Sundell, K., Carlsson, N.G. and Eriksson, S.P., 2017. Comparative survival and growth performance of European lobster larvae, *Homarus gammarus*, reared on dry feed and conspecifics. Aquaculture Research 2017, 1–11.

Powell, A., Rowley, A.F., 2008. Tissue changes in the shore crab *Carcinus maenas* as a result of infection by the parasitic barnacle *Sacculina carcini*. Diseases of Aquatic Organisms 80, 75–79. doi:10.3354/dao01930.

Pramod, P.K., Ramachandran, A., Sajeevan, T.P., Thampy, S., Pai, S.S., 2010. Comparative efficacy of MS-222 and benzocaine as anaesthetics under simulated transport conditions of a tropical ornamental fish *Puntius filamentosus* (Valenciennes). Aquaculture Research 41, 309–314. doi:10.1111/j.1365-2109.2009.02333.x.

R Core Team 3.1.2, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-90051-07-0, URL http://www.R-project.org/.

Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1995. The significance of lipids at early stage of marine fish: a review. Aquaculture 155, 103–115. doi:10.1016/S0044-8486(97)00121-X.

Rajkumar, M., Kumaraguru vasagam, K.P., 2006. Suitability of the copepod, *Acartia clausi* as a live feed for Seabass larvae (*Lates calcarifer* Bloch): Compared to traditional live-food

organisms with special emphasis on the nutritional value. Aquaculture 261, 649–658. doi:10.1016/j.aquaculture.2006.08.043.

Ramallo, R., Birba, A., Honji, R.M., Moran-, L., Moreira, R.G., Somoza, G.M., 2015. A multidisciplinary study on social status and the relationship between inter-individual variation in hormone levels and agonistic behavior in a Neotropical cichlid fish. Hormones and Behavior 69, 139–151. doi:10.1016/j.yhbeh.2015.01.008.

Reaney, L.T., Backwell, P.R.Y., 2007. Risk-taking behavior predicts aggression and mating success in a fiddler crab. Behavioral Ecology 18, 521–525.

Regnault, M., 1987. Nitrogen excretion in marine and freshwater Crustacea. Biological Reviews 62, 1-24.

Reis, D.B., Acosta, N.G., Almansa, E., Navarro, J.C., Tocher, D.R., Andrade, J.P., Sykes, A. V., Rodríguez, C., 2016. Comparative study on fatty acid metabolism of early stages of two crustacean species: *Artemia* sp. metanauplii and *Grapsus adscensionis* zoeae, as live prey for marine animals. Comparative Biochemistry and Physiology Part B 204, 53-60. doi:10.1016/j.cbpb.2016.11.002

Rhodes, C., 1981. Artificial incubation of the eggs of the Crayfish *Austropotamobius pallipes* (Lereboullet). Aquaculture 25, 129–140.

Rice, A.L., Chapman, C.J., 1971. Observations on the burrows and burrowing behaviour of two mud-dwelling decapod crustaceans, *Nephrops norvegicus* and *Goneplax rhomboides*. Marine Biology 10, 330–342.

Ridgway, I.D., 2005. Post capture physiology and pathology of the Norway lobster. PhD Thesis. University of Glasgow.

Ridgway, I.D., Taylor, A.C., Atkinson, R., Chang, E.S., Neil, D.M., 2006a. Impact of capture method and trawl duration on the health status of the Norway lobster, *Nephrops norvegicus*. Journal of Experimental Marine Biology and Ecology 339, 135–147. doi:10.1016/j.jembe.2006.07.008

Ridgway, I.D., Taylor, A.C., Atkinson, R.J.A., Stentiford, G.D., Chang, E.S., Chang, S.A., Neil,

D.M., 2006b. Morbidity and mortality in Norway lobsters, *Nephrops norvegicus*: physiological, immunological and pathological effects of aerial exposure. Journal of Experimental Marine Biology and Ecology 328, 251–264. doi:10.1016/j.jembe.2005.07.015

Ritar, A.J., Dunstan, G. A., Crear, B.J., Brown, M.R., 2003. Biochemical composition during growth and starvation of early larval stages of cultured spiny lobster (*Jasus edwardsii*) phyllosoma. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 136, 353–370. doi:10.1016/S1095-6433(03)00167-3

Robertson, L., Lawrence, A.L., Castille, F.L., 1993. Effect of feeding frequency and feeding time on growth of *Penaeus vannamei* (Boone). Aquaculture and Fisheries Management 24, 1–6.

Rocha, G.S., Katan, T., Parrish, C.C., Gamperl, A.K., 2017. Effects of wild zooplankton versus enriched rotifers and *Artemia* on the biochemical composition of Atlantic cod (*Gadus morhua*) larvae. Aquaculture 479, 100-113.

Rollo, A., Sulpizio, R., Nardi, M., Silvi, S., Orpianesi, C., Caggiano, M., Cresci, A., Carnevali, O., 2006. Live microbial feed supplement in aquaculture for improvement of stress tolerance. Fish Physiology and Biochemistry 32, 167–177.

Rosa, R., Morais, S., Calado, R., Narciso, L., Nunes, M., 2003. Biochemical changes during the embryonic development of Norway lobster, *Nephrops norvegicus*. Aquaculture 221, 507–522. doi:10.1016/S0044-8486(03)00117-0

Rosa, R., Nunes, M.L., 2003. Seasonal changes in nucleic acids, amino acids and protein content in juvenile Norway lobster (*Nephrops norvegicus*). Marine Biology 143, 565–572. doi:10.1007/s00227-003-1096-6

Ross, L.G., Ross, B., 2008. Anaesthetic and Sedative Techniques for Aquatic Animals, Thrid edition. Blackwell Publishing, Oxford.

Rotllant, G., Anger, K., Durfort, M., Sarda, F., 2004. Elemental and biochemical composition of *Nephrops norvegicus* (Linnaeus 1758) larvae from the Mediterranean and Irish Seas. Helgoland Marine Research 58, 206–210. doi:10.1007/s10152-004-0186-2

Rotllant, G., Charmantier-Daures, M Charmantier, G., Anger, K., Sarda, F., 2001. Effects of diet on *Nephrops norvegicus* (L.) larval and postlarval development, growth, and elemental composition. Journal of Shellfish Research 20, 347–352.

Sánchez-Paz, A., García-Carreño, F., Muhlia-Almazán, A., Peregrino-Uriarte, A.B., Hernández-López, J., Yepiz-Plascencia, G., 2006. Usage of energy reserves in crustaceans during starvation: status and future directions. Insect biochemistry and molecular biology 36, 241–9. doi:10.1016/j.ibmb.2006.01.002

Santucci, R., 1926. Lo sviluppo el' ecologia post-embrionali dello "Scampo" *Nephrops norvegicus* (L.) nel Tirreno e nei mari nordici. Mem. R. Com. Talassoger. Ital. 126, 1–36.

Sardà, F., 1995. A review (1967-1990) of some aspects of the life history of *Nephrops norvegicus*. ICES Marine Science Symposia 199, 78–88.

Sardà, F., Aguzzi, J., 2011. A review of burrow counting as an alternative to other typical methods of assessment of Norway lobster populations. Reviews in Fish Biology and Fisheries 22, 409–422. doi:10.1007/s11160-011-9242-6

Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999a. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191–199. doi:10.1016/S0044-8486(99)00083-6

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., Tocher, D., 1999b. Lipid nutrition of marine fish during early development: Current status and future directions. Aquaculture 179, 217–229. doi:10.1016/S0044-8486(99)00191-X

Sargent, J.R., 1995. Origins and functions of egg lipids: nutritional implications, in: Bromage, N.R., Roberts, R.J. (Eds.), Broodstock Management and Egg and Larval Quality. Blackwell Science Ltd, Oxford, pp. 353-373.

Sars, G.O., 1884. Bidrag til Kundskaben am Dekapodornes Forvandlinger 1. Arc. Math. Naturv. 9, 155–204.

Saydmohammed, M., Pal, A.K., 2009. Anesthetic effect of eugenol and menthol on handling stress in *Macrobrachium rosenbergii*. Aquaculture 298, 162–167.

doi:10.1016/j.aquaculture.2009.10.020

Schmalenbach, I., Mehrtens, F., Janke, M., Buchholz, F., 2011. A mark-recapture study of hatchery-reared juvenile European lobsters, *Homarus gammarus*, released at the rocky island of Helgoland (German Bight, North Sea) from 2000 to 2009. Fisheries Research 108, 22–30.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. ImageJ Software, National Institutes of Health, Bethesda, Maryland, USA. http://imagej.nih.gov/ij/, 1997-2016.

Schoo, K.L., Aberle, N., Malzahn, A.M., Schmalenbach, I., Boersma, M., 2014. The reaction of European lobster larvae (*Homarus gammarus*) to different quality food: effects of ontogenetic shifts and pre-feeding history. Oecologia 174, 581–94. doi:10.1007/s00442-013-2786-5

Shanks, S., Jones, C., 2015. Status of lobster hatchery technology development. In: Jones, C.M. Spiny lobster aquaculture development in Indonesia, Vietnam and Australia: Proceedings of the International lobster aquaculture symposium held in Lombok, Indonesia, 22-25 April 2014. ACIAR Proceedings No. 145. Australian Centre for International Agricultural Research, Canberra. pp. 154-158.

Shields, J.D., 1994. The parasitic dinoflagellates of marine Crustaceans. Annual Review of Fish Diseases 4, 241–271. doi:10.1016/0959-8030(94)90031-0

Sibert, V., Ouellet, P., Brêthes, J.-C., 2004. Changes in yolk total proteins and lipid components and embryonic growth rates during lobster (*Homarus americanus*) egg development under a simulated seasonal temperature cycle. Marine Biology 144, 1075–1086. doi:10.1007/s00227-003-1287-1

Sieburth, J.M., Smetacek, V., Lenz, J., 1978. Pelagic ecosystem structure: Heterotrophic. Limnology and Oceanography 23, 1256–1263.

Sloman, K.A., Armstrong, J.D., 2002. Physiological effects of dominance hierarchies: Laboratory artefacts or natural phenomena? Journal of Fish Biology 61, 1–23. doi:10.1111/j.1095-8649.2002.tb01733.x

Smith, G.G., Ritar, A.J., 2010. Effect of physical disturbance on reproductive performance in the spiny lobster, *Jasus edwardsii*. New Zealand Journal of Marine and Freshwater Research 39, 317–324. doi:10.1080/00288330.2005.9517312

Smith, G.G., Ritar, A.J., Dunstan, G., 2003. An activity test to evaluate larval competency in spiny lobsters (*Jasus edwardsii*) from wild and captive ovigerous broodstock held under different environmental conditions. Aquaculture 218, 293–307. doi:10.1016/S0044-8486(02)00526-4

Smith, G.G., Ritar, A.J., Thompson, P., Dunstan, G., Brown, M.R., 2002. The effect of embryo incubation temperature on indicators of larval viability in Stage I phyllosoma of the spiny lobster, *Jasus edwardsii*. Aquaculture 209, 157–167. doi:10.1016/S0044-8486(01)00758-X

Smith, R.S.M., 1987. The Biology of larval and juvenile *Nephrops norvegicus* (L.) in the Firth of Clyde. PhD Thesis. Glasgow University.

Sneddon, L.U., Taylor, A.C., Huntingford, F.A., 1999. Metabolic consequences of agonistic behaviour: crab fights in declining oxygen tensions. Animal behaviour 57, 353–363. doi:10.1006/anbe.1998.0982

Steneck, R.S., 2006. Possible Demographic Consequences of intraspecific shelter competition among American Lobsters. Journal of Crustacean Biology 26 (4), 628-638.

Stentiford, G.D., Neil, D.M., 2011. Diseases of *Nephrops* and *Metanephrops*: A review. Journal of Invertebrate Pathology 106, 92–109. doi:10.1016/j.jip.2010.09.017

Sterk, W., Redant, F., 1989. Further evidence on the biennial spawning of the Norway lobster, *Nephrops norvegicus*, in the Central North Sea. International Council for the Exploration of the Sea CM1989/K2.

Stoner, A.W., 2012. Assessing Stress and Predicting Mortality in Economically Significant Crustaceans. Reviews in Fisheries Science 20, 111–135. doi:10.1080/10641262.2012.689025

Støttrup, J.G., 2003. Production and nutritional value of Copepods, in: Støttrup, J.G.,

McEvoy, L.A. (Eds.), Live Feeds in Marine Aquaculture. Blackwell Science Ltd, Oxford, pp. 318.

Streiff, R., Mira, S., Castro, M., Cancela, M.L., 2004. Multiple paternity in Norway lobster (*Nephrops norvegicus* L.) assessed with microsatellite markers. Marine Biotechnology 6, 60–66. doi:10.1007/s10126-003-0015-7

Subramoniam, T., 2011. Mechanisms and control of vitellogenesis in Crustaceans. Fisheries Science 77, 1–21. doi:10.1007/s12562-010-0301-z

Sumpter, D.J.T., 2006. The principles of collective animal behaviour. Philosophical Transactions of the Royal Society B 361, 5–22. doi:10.1098/rstb.2005.1733

Svetlichny, L., 1983. Calculation of planktonic copepod biomass by means of coefficients of proportionality between volume and linear dimensions of the body. Ecologia Morja 15, 46–58.

Tarling, G. A., Johnson, M.L., 2006. Satiation gives krill that sinking feeling. Current Biology 16, 83–84. doi:10.1016/j.cub.2006.01.044

Taylor, H.H., Paterson, B.D., Wong, R.J., Wells, R.M., 1997. Physiology and live transport of lobsters: report from a workshop. Marine and Freshwater Research 48, 817–822.

Taylor, S., Landman, M.J., Ling, N., 2009. Flow cytometric characterization of freshwater crayfish hemocytes for the examination of physiological status in wild and captive animals. Journal of Aquatic Animal Health 21, 195–203. doi:10.1577/H09-003.1

Telsnig, J.D., 2011. The feasibility of aquaculture, aquaponics and a lobster hatchery in Amble. Amble Development Trust.

Thiel, M., Baeza, J. A., 2001. Factors affecting the social behaviour of crustaceans living symbiotically with other marine invertebrates: a modelling approach. Symbiosis 30, 163–190.

Thomas, C.W., Carter, C.G., Crear, B.J., 2003. Feed availability and its relationship to survival, growth, dominance and the agonistic behaviour of the southern rock lobster, *Jasus edwardsii* in captivity. Aquaculture 215, 45–65. doi:10.1016/S0044-8486(01)00899-7

Thomas, H.J., 1965. The white fish communities associated with *Nephrops norvegicus* (L.) and the by-catch of white fish in the Norway lobster fishery, together with notes on Norway lobster predators. Rapports et Procés - Verbaux Des Réunions Du Conseil International Pour l'Exploration de La Mer 156, 155–160.

Thompson, B.M., Ayers, R.A., 1989. Laboratory studies on the development of *Nephrops norvegicus* larvae. Journal of Marine Biological Association UK 69, 795–801.

Tlusty, M.F., Myers, A., Metzler, A., 2008. Short- and long-term dietary effects on disease and mortality in American lobster *Homarus americanus*. Diseases of Aquatic Organisms 78, 249–253. doi:10.3354/dao01867

Tönnesson, K., Nielsen, T.G., Tiselius, P., 2006. Feeding and production of the carnivorous copepod *Pareuchaeta norvegica* in the Skagerrak. Marine Ecology Progress Series 314, 213–225. doi:10.3354/meps314213

Trushenski, J.T., Bowker, J.D., Cooke, S.J., Erdahl, D., Bell, T., MacMillan, J.R., Yanong, R.P., Hill, J.E., Fabrizio, M.C., Garvey, J.E., Sharon, S., 2013. Issues Regarding the Use of Sedatives in Fisheries and the Need for Immediate-Release Options. Transactions of the American Fisheries Society 142, 156–170. doi:10.1080/00028487.2012.732651

Tuck, I.D., Atkinson, R.J.A., Chapman, C.J., 1994. The structure and seasonal variability in the spatial distribution of *Nephrops norvegicus* burrows. Ophelia 40, 13–25. doi:10.1080/00785326.1994.10429547

Tuck, I.D., Atkinson, R.J.A., Chapman, C.J., 2000. Population biology of the Norway lobster, *Nephrops norvegicus* (L.) in the firth of Clyde, Scotland II: fecundity and size at onset of sexual maturity. ICES Journal of Marine Science 57, 1227-1239. doi:10.1006/jmsc.2000.0809

Tuck, I.D., Chapman, C.J., Atkinson, R.J.A., 1997. Population biology of the Norway lobster , *Nephrops norvegicus* (L.) in the Firth of Clyde, Scotland – I: Growth and density. ICES Journal of Marine Science 54, 125–135.

Uglow, R.F., Hosie, D.A., Johnson, I.T., MacMullen, P.H., 1986. Live Handling and Transport of Crustacean Shellfish : An Investigation of Mortalities., Seafish Technology SR280,

MAFF R&D Commission, London. 24pp.

Ungfors, A., Bell, E., Johnson, M.L., Cowing, D., Dobson, N.C., Bublitz, R., Sandell, J., 2013. *Nephrops* fisheries in European waters., in: Johnson, M.L., Johnson, M.P. (Eds.), The Ecology and Biology of *Nephrops norvegicus*. Academic Press, London, pp. 248–306. doi:10.1016/B978-0-12-410466-2.00007-8

Usta, J., Kreydiyyeh, S., Bajakian, K., Nakkash-Chmaisse, H., 2002. In vitro effect of eugenol and cinnamaldehyde on membrane potential and respiratory chain complexes in isolated rat liver mitochondria. Food and Chemical Toxicology 40, 935–40.

van der Meeren, G.I., 2000. Predation on hatchery-reared lobsters *Homarus gammarus* released in the wild. Canadian Journal of Fisheries and Aquatic Sciences 57, 1794–1803.

Van Der Meeren, G.I., 2005. Potential of ecological studies of cultivated and released European lobsters, *Homarus gammarus*. New Zealand Journal of Marine and Freshwater Research 39, 399–424. doi:10.1080/00288330.2005.9517321

Van Olst, J.C., Carlberg, J.M. and Ford, R.F., 1975. Effects of substrate type and other factors on the growth survival, and cannibalism of juvenile *Homarus americanus* in mass rearing systems. Proc Annu Meet- World Marine Society, 8, 271-292.

Vanhaecke, P., Sorgeloos, P., 1980. International study on *Artemia*. The biometric of *Artemia* strains from different geographical origin, in: Persoone, G., Sorgeloos, P., Roels, O., Jaspers, E. (Eds.), The Brine Shrimp *Artemia*. Universa Press, Wetteren, Belgium, pp. 393–406.

Vartak, V., Singh, R., 2006. Anesthetic effects of clove oil during handling and transportation of the freshwater prawn, *Macrobrachium rosenbergii* (De Man). The Israeli Journal of Aquaculture - Bamidgeh 58, 46–54.

Vijayakumaran, M., Radhakrishnan, E. V, 1986. Effects of food density on feeding and moulting of phyllosoma larvae of the Spiny Lobster *Panulirus homarus* (Linnaeus). Proceedings of the symposium on coastal aquaculture. Proceedings of the symposium on coastal aquaculture. Marine Biological Association of India 4, 1281–1285.

Waddy, S.L., Aiken, D.E., De Kleijn, D., 1995. Control of growth and reproduction, in: Factor, J.R. (Ed.), Biology of the Lobster *Homarus americanus*. Academic Press, New York, pp. 217–266. doi:10.1016/b978-012247570-2/50032-3

Wagner, E., Arndt, R., Hilton, B., 2002. Physiological stress responses, egg survival and sperm motility for rainbow trout broodstock anesthetized with clove oil, tricaine methanesulfonate or carbon dioxide. Aquaculture 211, 353–366. doi:10.1016/S0044-8486(01)00878-X

Wahle, R.A., Steneck, R.S., 1991. Recruitment habitats and nursery grounds of the American lobster *Homarus americanus*: a demographic bottleneck? Marine Ecology Progress Series 69, 231–243.

Wahle, R.A., Steneck, R.S., 1992. Habitat restrictions in early benthic life: experiments on habitat selection and *in situ* predation with the American lobster. Journal of Experimental Marine Biology and Ecology 157, 91–114.

Walter, G.M., van Uitregt, V.O., Wilson, R.S., 2011. Social control of unreliable signals of strength in male but not female crayfish, *Cherax destructor*. Journal of Experimental Biology 214, 3294–3299. doi:10.1242/jeb.056754

Wang, M., O'Rorke, R., Waite, M., Beckley, L.E., Thompson, P., Jeffs, G., 2014. Fatty acid profiles of phyllosoma larvae of western rock lobster (*Panulirus cygnus*) in cyclonic and anticyclonic eddies of the Leeuwin Current off Western Australia. Progress in Oceanography 122, 153–162. doi:10.1016/j.pocean.2014.01.003

Wang, Y.B., 2007. Effect of probiotics on growth performance and digestive enzyme activity of the shrimp *Penaeus vannamei*. Aquaculture 269, 259–264. doi:10.1016/j.aquaculture.2007.05.035

Wang, Y.B., Xu, Z.R., Xia, M.S., 2005. The effectiveness of commercial probiotics in northern white shrimp *Penaeus vannamei* ponds. Fisheries Science 71, 1036–1041. doi:10.1111/j.1444-2906.2005.01061.x

Waterstrat, P., Pinkham, L., 2005. Evaluation of Eugenol as an Anesthetic for the American Lobster *Homerus americanus*. Journal of the World Aquaculture Society 36, 420–424.

Wear, R.G., 1974. Incubation in British Decapod Crustacea, and the effects of temperature on the rate and success of embryonic development. Journal of the Marine Biological Association of the United Kingdom 54, 745–762.

Wickins, J.F., Barry, J., 1996. The effect of previous experience on the motivation to burrow in early benthic phase lobsters (*Homarus gammarus* (L.)). Marine and Freshwater Behaviour and Physiology 28, 211–228. doi:10.1080/10236249609378993

Wickins, J.F., Lee, D.O., 2002. Crustacean farming, ranching and culture, 2nd edition. Blackwell Science Ltd, Oxford.

Wickins, J.F., Roberts, J.C., Heasman, M.S., 1996. Within-burrow behaviour of juvenile European lobsters *Homarus gammarus* (L.). Marine and Freshwater Behaviour and Physiology 28, 229–253. doi:10.1080/10236249609378994

Wilcox, J.A., Tracy, P.L., Marcus, N.H., 2006. Improving live feeds: Effect of a mixed diet of copepod nauplii (*Acartia tonsa*) and rotifers on the survival and growth of first-feeding larvae of the southern flounder, *Paralichthys lethostigma*. Journal of the World Aquaculture Society 37, 113–120. doi:10.1111/j.1749-7345.2006.00014.x

Wood, H.L., Eriksson, S.P., Nordborg, M., Styf, H.K., 2015. The effect of environmental stressors on the early development of the Norway lobster *Nephrops norvegicus* (L.). Journal of Experimental Marine Biology and Ecology 473, 35–42. doi:10.1016/j.jembe.2015.08.009

Yamamoto, T., Teruya, K., Hara, T., Hokazono, H., Hashimoto, H., Suzuki, N., Iwashita, Y., Matsunari, H., Furuita, H., Mushiake, K., 2008. Nutritional evaluation of live food organisms and commercial dry feeds used for seed production of amberjack *Seriola dumerili*. Fisheries Science 74, 1096–1108. doi:10.1111/j.1444-2906.2008.01629.x

Yasuda, C., Takeshita, F., Wada, S., 2012. Assessment strategy in male-male contests of the hermit crab *Pagurus middendorffii*. Animal Behaviour 84, 385–390. doi:10.1016/j.anbehav.2012.05.007

Yasuda, C.I., Koga, T., 2016. Do weaponless males of the hermit crab *Pagurus minutus* give up contests without escalation? Behavior of intruders that lack their major cheliped in

male–male contests. Journal of Ethology. doi:10.1007/s10164-016-0470-z

Zahl, I.H., Kiessling, A., Samuelsen, O.B., Hansen, M.K., 2011. Anaesthesia of Atlantic halibut (*Hippoglossus hippoglossus*) Effect of pre-anaesthetic sedation, and importance of body weight and water temperature. Aquaculture Research 42, 1235–1245. doi:10.1111/j.1365-2109.2010.02711.x

Zhang, Q., Tan, B., Mai, K., Zhang, W., Ma, H., Ai, Q., Wang, X., Liufu, Z., 2011. Dietary administration of Bacillus (*B. licheniformis* and *B. subtilis*) and isomaltooligosaccharide influences the intestinal microflora, immunological parameters and resistance against Vibrio alginolyticus in shrimp, *Penaeus japonicus* (Decapoda: Penaeidae). Aquaculture Research 42, 943–952. doi:10.1111/j.1365-2109.2010.02677.x

Zhou, S., Shirley, T.C., 1997. Behavioural responses of red king crab to crab pots. Fisheries Research 30(3), 117-189.

Zhou, X., Wang, Y., Li, W., 2009. Effect of probiotic on larvae shrimp (*Penaeus vannamei*) based on water quality, survival rate and digestive enzyme activities. Aquaculture 287, 349–353. doi:10.1016/j.aquaculture.2008.10.046

Ziegler, F., Valentinsson, D., 2008. Environmental life cycle assessment of Norway lobster (*Nephrops norvegicus*) caught along the Swedish west coast by creels and conventional trawls - LCA methodology with case study. International Journal of Life Cycle Assessment 13, 487–497. doi:10.1007/s11367-008-0024-x

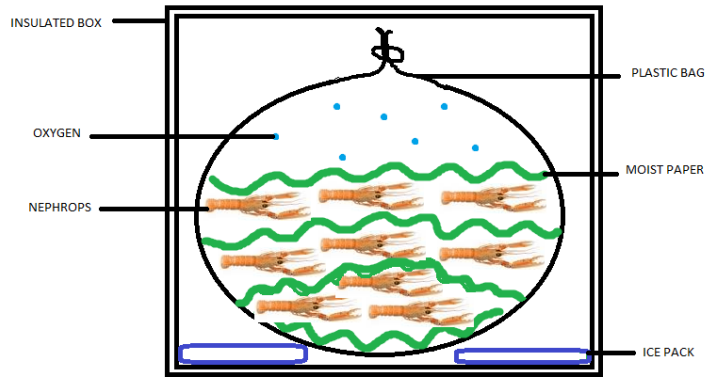
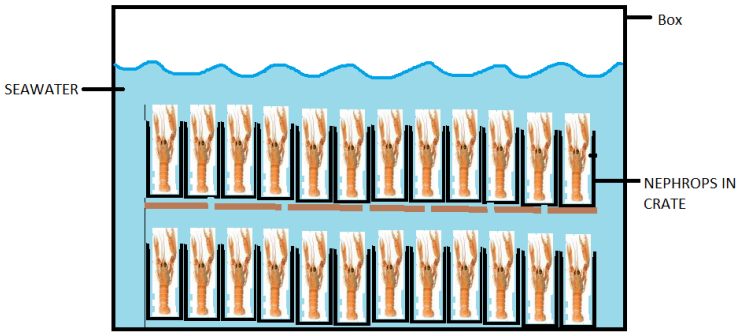
Appendix 1 Broodstock Procurement and Transport

Reducing mortality during and post transportation is an important part of the commercial processing of *Nephrops* as high mortality results in low monetary values for the dead individuals.

Mortality arises from poor handling and storage increasing the levels of stress within the individuals (Fotedar and Evans, 2011; Stoner, 2012). Stress in *Nephrops* has been found to arise from aerial exposure, physical damage and stimulation, thermal shock and reduced water quality (Ridgway et al., 2006b). One of the most likely periods to increase stress in the broodstock and embryos is during and shortly after transportation, since it is challenging to maintain adequate life support systems for long time periods.

There are currently several methods of transporting live Crustacea, and the duration of transport and financial implications typically determines which method is used. If the journey time is below 30 minutes, the dry/damp transport method would be satisfactory with minimal mortality. The dry transport method comprises wet layers of seaweed or paper, placed between individual *Nephrops*, which are placed over ice packs within a sealed polystyrene box (table A1.1). If transportation duration is expected to be longer than 30 minutes, then a moist or wet transport method is preferred (table A1.1). Vivier trucks are commonly used for the transportation of live shellfish over long distances (>2hours), which uses life support systems, to maintain optimal water parameters.

Table A1.1. Comparison of the dry and wet transport methods

Method	Illustration and Description	Pros & Cons
DRY	 Usually *Nephrops* are placed in a sealed plastic bag and placed in a polystyrene fish box. Occasionally within the plastic bag some material such as wet newspaper or seaweed is placed in-between several layers to keep the individuals moist and separate. Ice packs can be used to lower the temperature.	• Cheap • Easy to pack • No added weight of water or expensive equipment. • Unable to respire efficiently • Abrasion upon eggs • No control over temperature • High mortality for prolonged journey times • Stress of handling • Can crush juveniles at the bottom
WET	 Can be as simple as a box/tank filled with clean seawater. *Nephrops* can be placed within the 'tubes', 'free' or 'free and banded'. Vivier systems aerate and filter the water similar to recirculation aquaculture system (RAS) and control factors such as temperature and water quality.	• Able to fully respire and excrete metabolic waste • Protection from impacts • The potential to control temperature and water parameters • Individuals separated • Require a short acclimation period compared to dry and moist methods • Good survival for longer transportation periods • More expensive than the dry method • Heavy due to the amount of water • Requires more equipment and space • More complicated than other methods

The transport methods used during this thesis were dependent upon the locality, the transport duration and the preferred holding technology used by the fishers. The experiments took place in a number of locations including the Orkney Lobster Hatchery, Scotland (OLH), the Centre for Environmental and Marine Sciences (CEMS), University of Hull, Scarborough, and the Sven Lovén Centre for Marine Sciences culture facility (SLC), Gothenburg University, Kristineberg, Sweden (figure A1.1).

Broodstock sourced from Stromness (Orkney, Scotland) were creel caught individuals that were transported using the "modified vivier" box system (see below). However, the supply of broodstock was not reliable and the individuals had low vitality. Broodstock sourced from Eyemouth (D.R. Collins Ltd) were transported by the "modified vivier" to Scarborough (University of Hull). The supply of broodstock were found to be both constant in number and acceptable in female survival and egg quality. However, the method of fishing could not be defined, and may have included trawled fishing techniques. Broodstock acquired in Sweden were creel-caught by a local fisherman and of high vitality. The caught broodstock were held in cool boxes surrounded in damp newspaper and transported directly to Kristineberg (Gothenburg University).

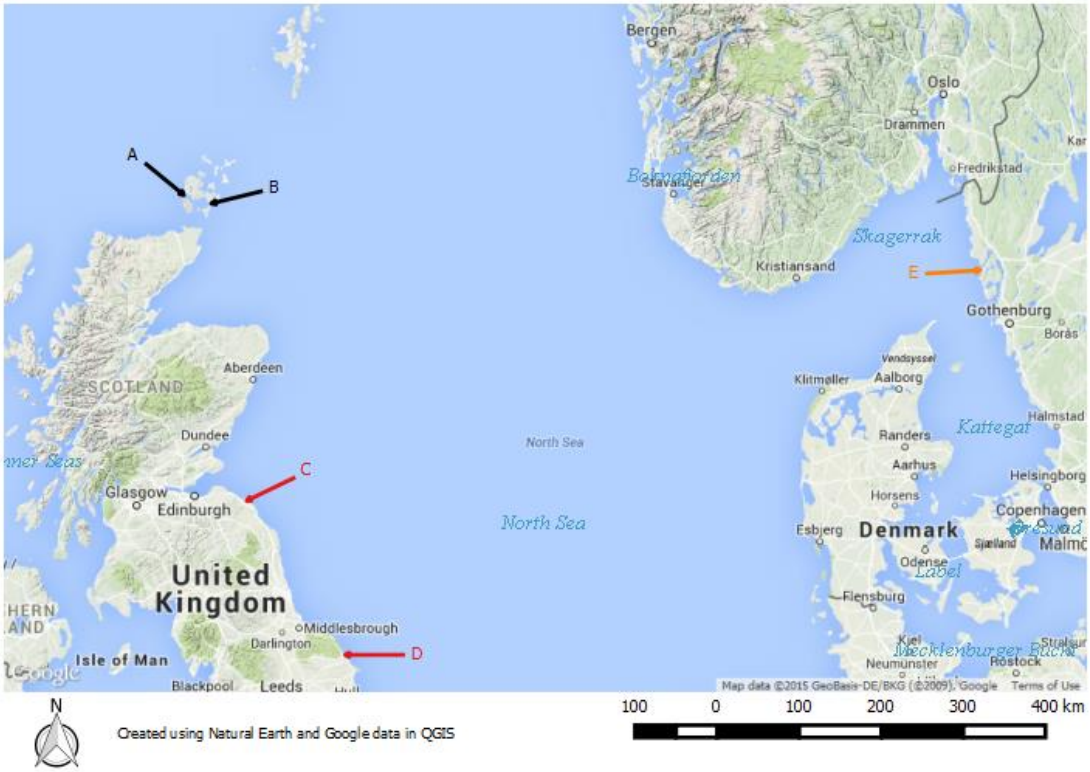


Figure A1.1. Locations of broodstock procurement and the destinations. (A) Stromness harbour to (B) OLH, Lambs Holm; (C) DR Collin & Son Ltd, Eyemouth to (D) CEMS, Scarborough; (E) SLC, Kristenberg. Similar coloured arrows represent transport links.

A "modified vivier" transport box was developed and used which mimics that of commercial vivier systems. The design (detailed below) could be used to transport live *Nephrops* for use within an aquaculture or experimental application.

The transport box consisted of a 250L straight sided storage tank (389E043N, Key Industries, UK) which accommodated two crates of *Nephrops* tubes, so approximately 300 individuals could be placed within the box. The tubes were typical of those used in commercial live storage operations, made from light perforated PVC to create vertically set individual chambers (approximate dimension of each chamber which can be altered to the size category of individuals, H 150 x W 50 x L 50 mm, Lund et al., 2009). The transport box was further developed by the addition of an aquarium pump (Ocean Runner 1200, Aqua Medic, DE) which passed water through mechanical and bio filtration media (Eheim EHFISubstrat, Eheim MECH, Eheim EHFISYNTH wool, UK) held in a plastic housing. The water pump was connected to a soft start DC to AC inverter (240Vac, 300W, 12V, 651.663UK, AVSL Group Ltd, UK) and a leisure

battery (85 $A^{/hr}$ 12 volt). Battery operated aerators were also added to maintain oxygen levels. The box was set upon a wooden pallet for easy transport. Additional acrylic sections around the corners provided protection and maintained structure. Insulation (Celotex 50mm, UK) was fixed to the box to maintain water temperature.

Water temperature during the transporting of *Nephrops* needs to be controlled, especially during the warmer summer months. Transport for over five hours will incur a significant water temperature rise. The insulation around the box does reduce the rate of temperate change, but is dependent upon the outside air temperature (figure A1.2). A water chiller may be needed for transport durations over 5 hours in duration, as decreasing the sea water temperature to below 10°C reduces metabolic rates and excretion, therefore decreasing the amount of ammonia in the system (Farmer, 1995).

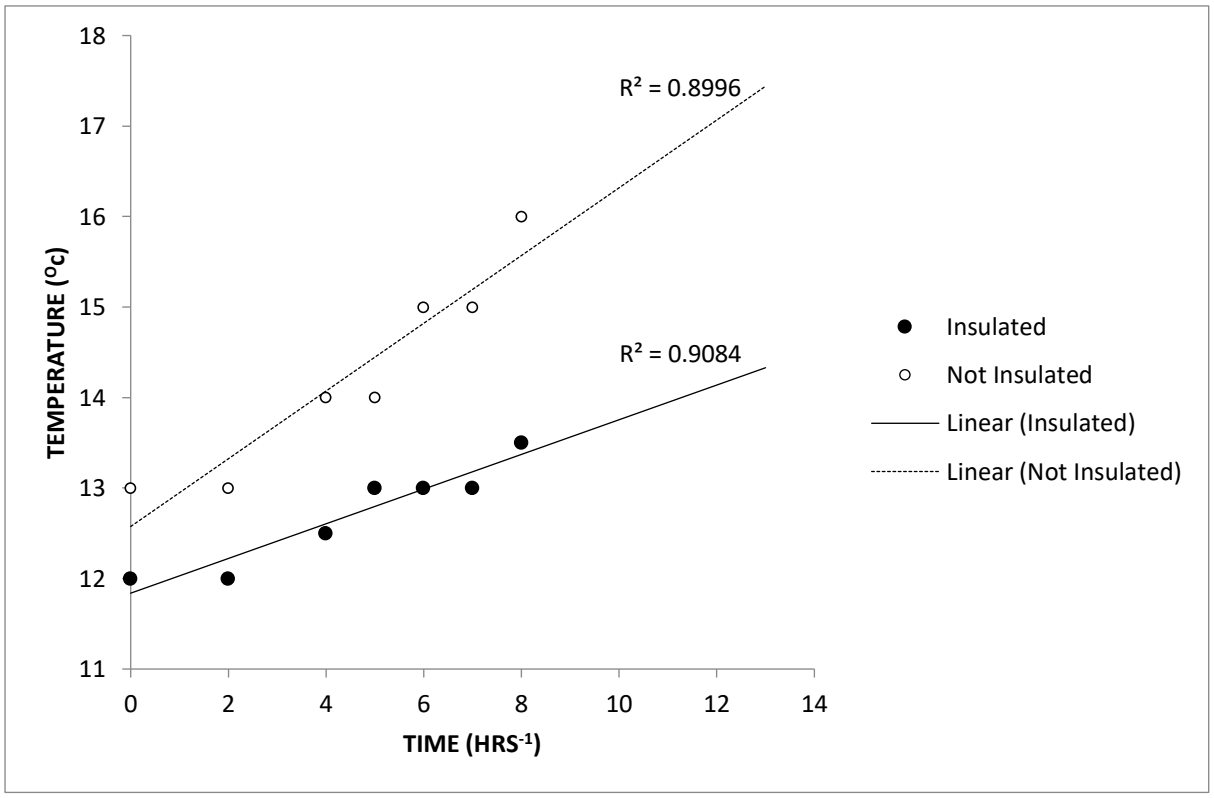


Figure A1.2. The inside water temperature between and insulated and un-insulated transport box over time with external air temperatures of 20°C.

Appendix 2 *Nephrops* Fisheries Around the UK

Table A2.1. Stock abundance, density and harvest rate information of the different *Nephrops* fishing grounds around the UK.

Fishing Grounds	ICES sub-division	FU	Stock Abundance	Density (*Nephrops* per m^2)	Harvest Rate (%)	Reference
North Sea, Botney Cut-Silver Pit	4.b & 4.c	5	Data limited, and the state of the stock is unknown.	High (0.70).	Data limited. A precautionary approach for 2017-18 was advised by ICES.	ICES, 2016a
North Sea, Noup	4.a	10	Data limited.	Low (0.13).	Only fished by a few vessels, and landings are relatively low compared to other FU. A precautionary approach for 2017-18 was advised by ICES.	ICES, 2016b
North Sea, Moray Firth	4.a	9	Stock abundance remains above the MSY $B_{trigger}$.	Not defined.	The harvest rate (%) is currently above the F_{MSY} but has historically been variable, fluctuating around the F_{MSY} level.	ICES, 2017c
North Sea, Firth of Forth	4.b	8	Stock abundance remains above the MSY $B_{trigger}$.	Not defined.	The harvest rate (%) is currently below F_{MSY} but has been variable over recent years, frequently above F_{MSY} levels.	ICES, 2017d
North Sea, Farn Deeps	4.b	6	Stock abundance remained below the MSY $B_{trigger}$ since 2012 but since 2017 it has	Not defined.	Harvest rates (%) continue to be above the F_{MSY} since 2008.	ICES, 2017e

			increased above the MSY $B_{trigger}$.			
North Sea, Devils Hole	4.b	34	Data limited.	Low (0.15).	Data limited therefore a precautionary approach has been advised by ICES.	ICES, 2016f
North Sea, Fladen Ground	4.a	7	Stock abundance dropped to below the MSY $B_{trigger}$ in 2015. Since 2016 the stock abundance has increased and is above MSY $B_{trigger}$.	Not defined.	The harvest rate (%) is below the F_{MSY} and has continued to decrease since 2010. Landings have decreased by ~80% since 2010 but are currently stable.	ICES, 2017g
West of Scotland, North & South Minch	6.a	11 & 12	Stock abundance remains above the MSY $B_{trigger}$ although showing a drop compared to the previous 3 years.	Medium (0.60) in FU11 and medium (0.44) in FU 12.	The harvest rate (%) has fluctuated around the F_{MSY} and is currently just below.	ICSES, 2017h & ICES, 2017i
West of Scotland, Forth of Clyde and Sound of Jura	6.a	13	Stock abundance remains to fluctuate above the MSY $B_{trigger}$ since 1995.	High (0.80).	Harvest rate (%) fluctuates around the F_{MSY} increasing above F_{MSY} since 2015.	ICES, 2017j
Celtic Seas, Irish Sea - East	7.a	14	Stock abundance remains above MSY $B_{trigger}$ since 2010.	Medium (0.48).	Harvest rates (%) remain considerably below the F_{MSY}.	ICES, 2017k
Celtic Seas, Irish Sea - West	7.a	15	Stock abundance remains steadily above MSY $B_{trigger}$.	High (0.90).	Harvest rates (%) fluctuate around the F_{MSY} and dropped below in 2016.	ICES, 2017l

Celtic Seas, The Smalls and Bristol Channel	7.g and 7.f	22	Stock abundance has remained above the MSY $B_{trigger}$ except for in 2016, but it looks to have increased again above the trigger.	Medium (0.40).	After 5 years of remaining under the F_{MSY} the harvest rates (%) increased well above the F_{MSY} in 2016.	ICES, 2017m
Celtic Seas, Labadle, Jones and Cockburn	7.g & 7.h	20-21	Stock abundance has almost doubled since 2016. The MSY $B_{trigger}$ has not been defined.	Not defined.	The harvest rates (%) remain below the F_{MSY} from 2013 to 2016.	ICES, 2017n
Celtic Seas, Ireland SW and SE Coast	7.a, 7.g & 7.j	19	Stock abundance shows a steady decline since 2011 and is currently above the MSY $B_{trigger}$.	Medium (0.30).	Harvest rates (%) are currently below the F_{MSY}.	ICES, 2017o
Celtic Seas, Aran grounds, Galway Bay & Slyne Head	7.b	17	Stock abundance indicates an overall decreasing trend since 2004 and is currently below the MSY $B_{trigger}$.	Medium (~0.30). Declined from >0.80.	Harvest rates (%) are currently above F_{MSY} with high fluctuation.	ICES, 2017p
Celtic Seas, Porcupine Bank	7.b-c & 7.j-k	16	Limited data for stock abundance but has declined from 2016. The MSY $B_{trigger}$ has not been defined.	Low (0.10).	Harvest rate (%) has an increasing trend from 2014 and remains below the F_{MSY} level.	ICES, 2017q

FU= Functional Unit; Green indicates stock abundance or harvest rates are in acceptable limits, Red indicates stock abundance or harvest rates are beyond either the MSY $B_{trigger}$ and F_{MSY} trigger point.