Aspects of the Biology and Husbandry of the New Zealand Scampi (*Metanephrops challengeri*)

By

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Abstract

The overall purpose of the research presented in this thesis was to examine some key aspects of the biology of New Zealand scampi, *Metanephrops challengeri*, that are relevant to the assessment for the potential development of this species for aquaculture. Currently, the biology of this species is poorly understood outside fishery management research due to the significant constraints the offshore and deep benthic natural habitat of this species places on conducting any detailed biological research. This study overcame these constraints by firstly developing novel methods for capturing live wild scampi from their natural habitat, then transporting and holding them long-term in a laboratory aquaria facility on land. Captive animals enabled the subsequent detailed studies of fecundity, characteristics of egg and embryo development at various temperatures, the rearing and description of the early stages of their life cycle and the influence of temperature on respiration in adult scampi.

Scampi were found to have a low fecundity with a mean of 367 ± 133 (n = 314) eggs per female. Eggs were successfully hatched and raised through all the larval stages to first instar juveniles enabling a detailed description of their larval development through all of the sequential larval stages. Temperature was found to have a significant effect on the embryo size and development rate. The Perkins Index method was used to estimate that at 7 °C the eggs would take 318 days to hatch while at 13 °C it would take 226 days. The volume of the egg and the size of the embryo eye, and therefore the embryo, increased with increasing temperature. The adults were found to be stenothermal within a range of <7 to 14 °C, while 15 °C was lethal. The respiration rate of *M. challengeri* is not markedly different from other temperate water crustacean species but this species has a low respiratory quotient (Q_{10}), indicating slow somatic growth.

As a potential aquaculture species New Zealand scampi has some favorable traits, such as robust larval stages of short duration with high survival, and a willingness for all stages of the life cycle to consume prepared feed. The species also has some unfavorable traits for aquaculture development, such as low fecundity, long egg incubation periods, slow growth, and a tolerance of only a narrow temperature range. Further research on diets and the collection and/or selection of broodstock that are tolerant of higher temperatures may increase the aquaculture potential of this species. Although this species is of high value and the issues of aquaculture production could be addressed through further research, the time required to fully domesticate the species will probably be prohibitive.

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The transport and holding of the scampi, as well as the experimental procedures, were approved by the Animal Ethics Committee of the Nelson Marlborough Institute of Technology (AEC2014-CAW-02).

Table of Contents

List of Figures

List of Tables

Chapter 1 : General Introduction

Figure 1.1 Photograph of an adult (± 50g) New Zealand scampi (*Metanephrops challengeri*).

1.1 New Zealand scampi

The New Zealand scampi, *Metanephrops challengeri*, is a clawed reptant marine lobster from the family Nephropridae (Crustacea: Decapoda) (Tshudy, 2013; Tshudy et al., 2007). The species is one of 18 extant and three extinct species found in this genus (Bell et al., 2013; Okamoto, 2008b), although some researchers believe there are only 17 extant species (Chan et al., 2009). The extant *Metanephrops* species are found in parts of the Indo West Pacific, East Australia, East and Central Pacific, Caribbean, eastern coast of South America, Antarctica and Madagascar (Chan et al., 2009; Tshudy et al., 2007). *Metanephrops challengeri* is endemic to New Zealand and is the only scampi species found in these waters (Figure 1-1). The current hypothesis is that the *Metanephrops* genus originated in Antarctica in the Cretaceous period (Tshudy, 2013) and diversified as it dispersed northward into new habitats.

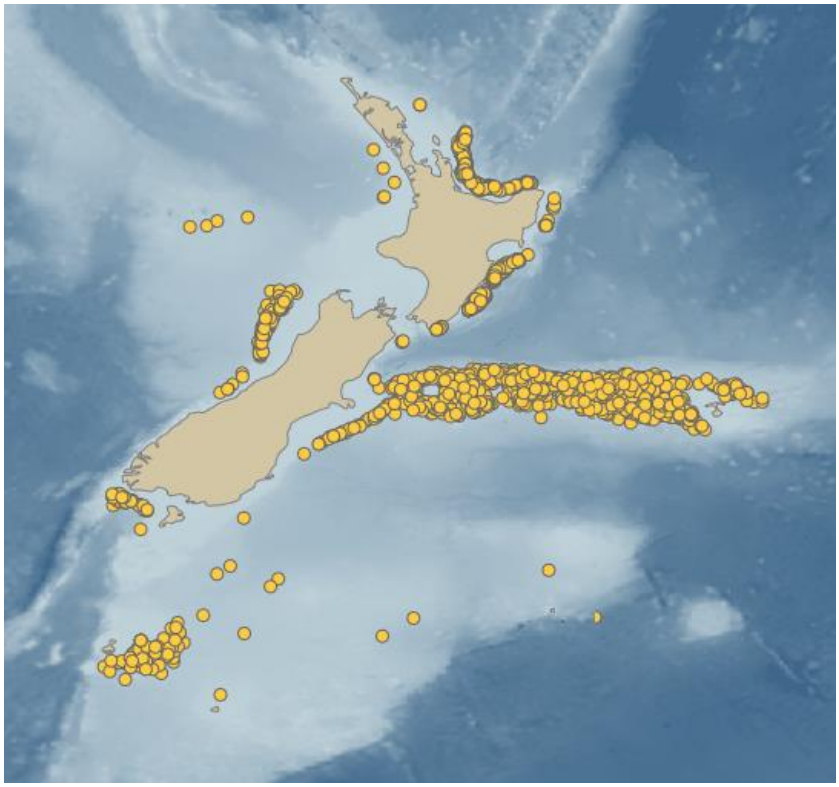


Figure 1.2 Map of the distribution of New Zealand scampi, *Metanephrops challengeri*, around New Zealand based on fisheries capture data. Each dot indicates a fishing trawl. (Generated at www.iobis.org)

Metanephrops challengeri is morphologically most closely related to the Norway lobster, *Nephrops norvegicus*. However, the results of DNA analysis indicate that *N. norvegicus* is more closely related to members of the genus *Homarus* (i.e. American and European clawed lobsters, *H. americanus* and *H. gammarus*, respectively) than to those from *Metanephrops*. It is considered that the morphological similarities display an occurrence of convergent evolution (Tshudy, 2013). This is supported by the fact that *N. norvegicus* inhabits similar habitats and has remarkably similar behaviour and autecology to *M. challengeri*.

1.2 The New Zealand scampi fishery

Metanephrops challengeri is an economically important species in New Zealand where it is harvested by ground trawling over extensive areas in deep water (i.e., >200 m) plateaus. The main fishing areas are the Bay of Plenty, Hawkes Bay, Wairarapa Coast, Chatham Rise and Sub-Antarctic Islands (MPI 2013) (Figures 1-1 & 1-2). Since the 1991/1992 fishing season, this species has been managed through a quota management system (QMA) (Tuck, 2014). Approximately 900 t of scampi was landed in 1991-92 (Bell et al., 2013), this declined to approximately 600 t in 2008-2009 (Anderson, 2012) but has since increased to 877 t by 2021 (Fisheries-New-Zealand, 2021; Wahle et al., 2020). The total allowable catch in all the fishing

areas in New Zealand is 1272 t (Fisheries-New-Zealand, 2021; Tuck, 2014). The wholesale landed value of scampi in 2009 was a mean of $18.30/kg (Anderson, 2012), with a retail value of US$51/kg (Wahle et al., 2020).

As a result of its economic importance, the research on the New Zealand scampi to date has been largely restricted to topics that are relevant to fisheries (Cryer & Coburn, 2000; Cryer et al., 2005; Cryer et al., 2001; Cryer et al., 2004; Cryer & Stotter, 1999; Thrush & Dayton, 2002; Tuck, 2009; Tuck, 2010; Tuck, 2014; Tuck & Dunn, 2012; Tuck & Spong, 2013), genetics (Smith, 1999), disease (Stentiford & Neil, 2011), body composition (Meynier et al., 2008), natural diets (van der Reis et al., 2018), foraging behaviour and potting (Major & Jeffs, 2018; Major et al., 2017) and cladistics (Chan et al., 2009; Chan & Yu, 1991; Feldmann, 1989; Jenkins, 1972; Tshudy, 2013; Tshudy et al., 2007).

The environmental effects and sustainability of the New Zealand fishery practices are of concern. For instance, fishing vessels have high fuel use getting to the offshore fishing grounds and while trawling, resulting in high carbon emissions per unit of catch (Tyedmers, 2004). The trawls are destructive to the benthic habitat for which there is increasing evidence of negative ecosystem impacts (Kaiser et al., 2002; Thrush & Dayton, 2002). The disturbance of the seabed by trawling has also recently been implicated in releasing large amounts of otherwise sequestered carbon from the seabed (Sala et al., 2021). Trawling for scampi also produces the highest bycatch of any New Zealand fishery where the harvesting of 893 t of scampi resulted in an estimated 2130 - 2761 t of bycatch (Anderson, 2012; Anderson & Edwards, 2018; Ballara, 2009; Hartill et al., 2006). While marketable bycatch is processed where possible, the remainder of the bycatch is discarded. In addition, crustacean fisheries are suggested to have a high CO_2 production per tonne of harvest compared to other fisheries (Boenish et al., 2021). New trawl methods are also mitigating bycatch to some extent (Hartill et al., 2006), but it remains significant. As a result of these impacts there is increasing interest in alternative methods of production of scampi. Attempts at catching scampi using pots to eliminate bycatch and seabed damage have been ineffective (Major & Jeffs, 2018), leaving aquaculture as a potential alternative route to the sustainable production of this species worthy of further investigation.

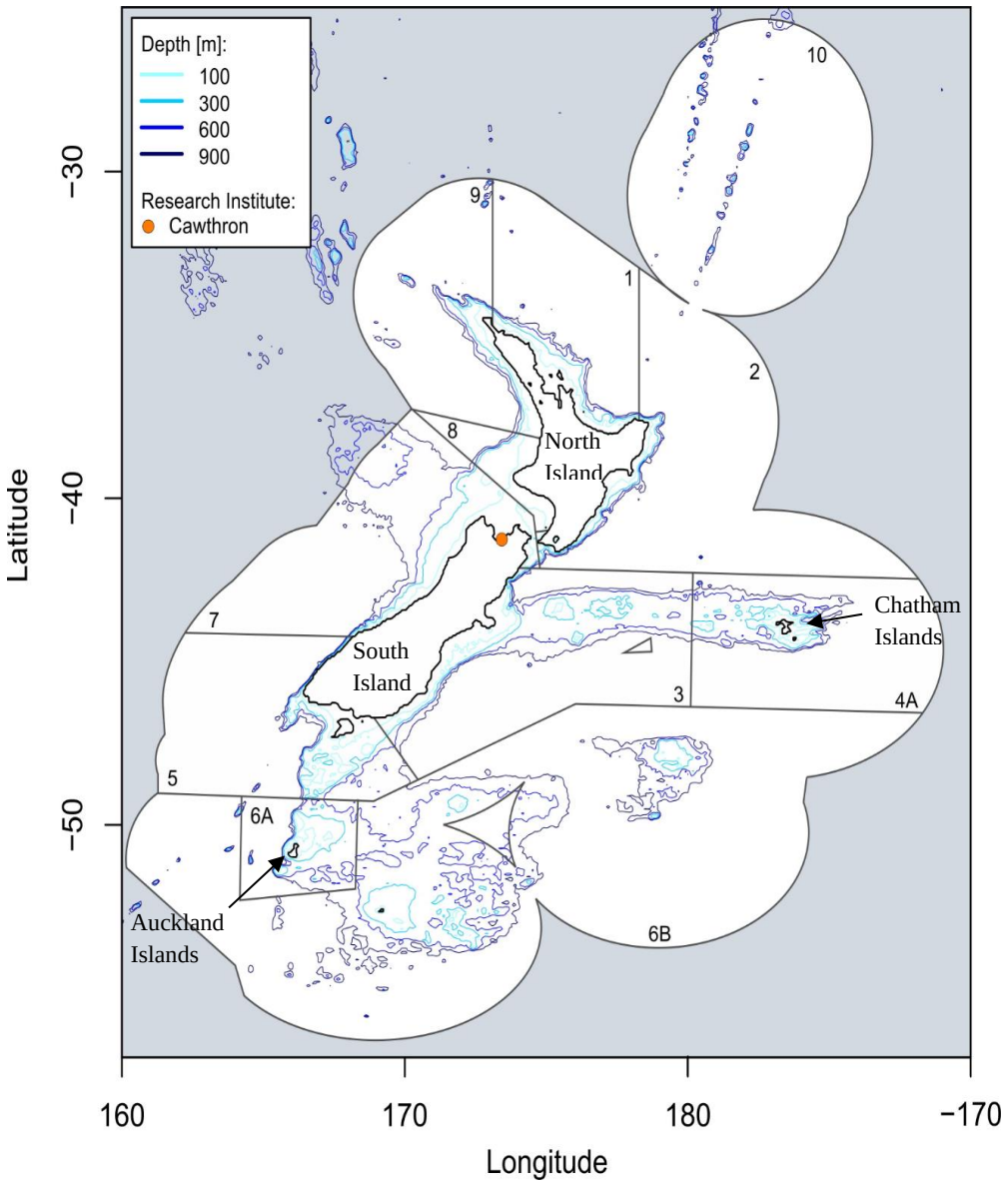


Figure 1.3 Map of the 11 New Zealand scampi commercial fisheries management areas. Live scampi used for this study were captured in 3 (SCI3) and transferred to aquarium facilities at the Cawthron Institute's Aquaculture Park near Nelson (orange dot).

1.3 Assessing new species for aquaculture

Many factors need to be investigated to identify whether the key biological attributes of a target organism for aquaculture development are consistent with requirements of efficient commercial cultivation and to help determine the optimum conditions under which any aquatic animal can effectively be cultivated (Azra et al., 2021; Duarte et al., 2007; Fotedar & Phillips, 2011; Leung et al., 2007). Biological attributes include important aspects of reproductive biology that are critical to efficiently generating a seed supply for aquaculture production, such as fecundity and the larval development pathway. Determining environmental requirements for

new species targeted for aquaculture is critical so that ideal culture conditions can be achieved for all stages of the life cycle, and knowledge of environmental tolerances can assist in defining the range of controlled conditions under which the target animal can be cultured. An understanding of factors which can affect the physiology of cultivated species can significantly enhance their productivity, health and survival in an aquaculture setting. For example, temperature has a fundamental influence on many aspects of the production of poikilothermic aquaculture species, affecting growth, respiratory needs, feed requirements, food conversion, larval development and survival.

1.4 Aspects of the biology of scampi relevant to aquaculture

Given the relatively recent commercial fisheries development of New Zealand scampi and its deep-sea habitat, which is difficult to access in situ for more detailed autecological studies, there is little information available on the biology of scampi on which to begin to assess its potential for commercial aquaculture development. It is a burrowing species in its natural habitat (Cryer & Coburn, 2000) but remote underwater video observations have recorded scampi also being associated with seabed trenches rather than burrows in SCI 6 (Tuck & Dunn, 2012). The burrowing behaviour appears to be important for avoiding predators such as skate and ling (Dunnet al., 2010; Forman & Dunn, 2012) and therefore the preference for a muddy and/or silt covered substrate is important and likely to influence their distribution on the seabed (Cryer et al., 2001). Sediment samples taken from the otter boards of bottom trawlers show the fine composition of the sediment (Table 1-1).

Table 1-1 The sediment fraction collected from trawl nets (otter boards) from two *M. challengeri* fishing regions (SCI 7 and SCI 2) (see Figure 1-3).

Area	Region SCI 7: West Coast	Region SCI 2: Wairarapa Coast
Date	Sep-2015	Oct-2015
Total Organic Carbon fraction	0.33	0.57
Fraction < 63 µm	91.5	73
Fraction 63 - 125 µm	3.9	15.4
Fraction 125 - 250 µm	2.7	8.9
Fraction 250 - 500 µm	1.3	1.8
Fraction 500 µm - 1 mm	0.4	0.7
Fraction 1 - 2 mm	0.2	0.2
Fraction ≥ 2 mm	< 0.1	< 0.1

1.5 Life cycle

There is only one published report on any aspect of the lifecycle of the New Zealand scampi. Wear (1976) reported the presence of a pre-zoea and a single larval stage and made assumptions on the characteristics of the following larval stage based on observations of the developing larvae beneath the cuticle of the preceding larval stage. Wear (1976) was unable to rear the hatched larvae beyond this initial stage. There are few reports of the life stages of other *Metanephrops* species, which may otherwise help to guide the formulation of some hypotheses around what to expect for the remaining larval development of New Zealand scampi. Of the 18 extant *Metanephrops* species, the larval stages of only five species (*M. japonicus, M. thomsoni, M. andamanicus, M. sagamiensis, and M. challengeri*) have been investigated and described in part or in full (Berry, 1969; Iwata et al., 1992; Okamoto, 2008a; Uchida & Dotsu, 1973; Wear, 1976). Therefore, there is a dearth of information on techniques for the successful rearing of eggs and larvae for any *Metanephrops* species.

McCarthy et al., (2018) showed that the size of scampi that had 50% of females with mature gonads ($L_{50gonads}$) for *M. challengeri* is around 30.0 – 30.1 mm orbital carapace length (OCL) while the size of *M. challengeri* at the L_{50eggs} stage is between 39.8 and 48.8 mm OCL. It is suggested that temperature appears to influence the size of scampi at the L_{50eggs} stage with the

scampi increasing in size relative to decreasing temperature. The $L_{50gonads}$ for male scampi was estimated to occur at 52.5 mm CL (McCarthy et al., 2018).

From casual observations, female New Zealand scampi are known to carry relatively small clutches of blue coloured eggs under their tails for some time. However, there is no information with regards to clutch sizes, egg sizes, embryo development duration and the influence of the environment on eggs and embryos. Indeed, there is no published information available regarding embryo development and duration within the *Metanephrops* genera, which would otherwise help to inform the likely pattern of embryo development in New Zealand scampi.

1.6 Diet

Typically, the taxonomic identification of the fauna found within the diet of crustacea has been through the use of visual identification of gut contents (Choi et al., 2008; Wassenberg & Hill 1989). This is obviously a difficult and often inaccurate method of diet analysis. The advent of DNA metabarcoding has enabled these constraints to be overcome as shown by van der Reis et al. (2018) who found DNA traces of over 150 species in the gut contents of six scampi. Crabs, prawns and fish appear to be the dominant types of food, indicating that the scampi are opportunistic benthic scavengers and predators. Santana et al., (2020) suggest that *N. norvegicus* diet is composed of up to 47% suspended particulate organic matter (POM). Although not tested in this study it is possible that *M. challengeri* could also gain nutrition from POM, although it seems unlikely based on the DNA profile recovered from the gut contents of scampi (van der Reis et al., 2018).

1.7 Temperature

The New Zealand scampi is suggested to tolerate a water temperature range of 6.7 to 13.5 °C based on benthic temperature measures during commercial fishing and from modelling of the areas commercially fished for scampi (Chiswell 2002; Cryer et al., 2005; Hadfield et al., 2007; Heath, 1985; Morris et al., 2001; Sutton, 2003). It is not clear from the literature whether this species has a relatively narrow range of temperature tolerances that may restrict its natural habitat range and would also limit its resilience to fluctuating temperatures in aquaculture conditions.

In marine poikilotherms, body temperature and metabolism vary according to ambient temperature (Daoud et al., 2007; Eckert 1988; Perera et al., 2007). Temperature is considered

the most important environmental factor affecting poikilotherms (Pörtner & Lannig 2009; Yamamoto et al., 2017). The metabolic rates of ectotherms and many higher-level processes are greatly influenced by temperature (Brown et al., 2004; Clarke, 2017b, 2017c, 2017d). Temperature also affects the development rate of poikilothermic embryos, as well as the size and success of the resultant larvae (Smith et al., 2002).

The determination of metabolism (the biological processing of energy and materials (Brown et al., 2004)) in response to temperature is an effective initial step for investigating the thermal range without the need for extensive replicated temperature growth experiments, which have the potential to be confounded by other factors, such as food quality (Froehlich et al., 2016; Horodysky et al., 2015; Yuen et al., 2019). Knowledge of the thermal range of aquaculture organisms can also guide inferences on other important attributes for aquaculture, such as hypoxia tolerance (Froehlich et al., 2016).

The respiration rate, measured as the rate of oxygen consumption over time at a set temperature, is frequently used as a proxy for the metabolic rate (Daoud et al., 2007). This is important in aquaculture because respiration provides a useful indicator of metabolic expenditure for maintaining vital functions and growth (Brett & Groves, 1979) and assists with understanding the animal's environmental requirements in an aquaculture production setting, especially temperature (Fitzgibbon & Battaglene, 2012). Crustaceans are suggested to have a high thermal sensitivity (Lagerspetz & Vainio, 2006), especially decapods (Matsuda & Yamakawa, 1997). Many other factors influence oxygen consumption in organisms, including body mass (Jensen et al., 2013; Villarreal & Ocampo, 1993), the extent of physical activity, as well as the intake and processing of food (Perera et al., 2007), all of which need to be controlled for when measuring respiration in organisms.

Associated with the respiration rate is the Q_{10} or temperature coefficient which measures the velocity of chemical reactions in relation to a 10 °C difference in temperature, i.e., effectively a measure of temperature sensitivity. In general, among crustaceans the "normal" Q_{10} range is 1.73 to 4.97 (Daoud et al., 2007). Any Q_{10} below 2.2 would be considered to indicate a slow growing species.

1.8 Aims and objectives

Undertaking an initial assessment of the potential for developing the sustainable aquaculture production of New Zealand scampi relies on obtaining further information about aspects of the

basic biology of the species, such as characteristics of early stages of their life cycle and their temperature requirements. Such new knowledge will not only contribute to a better understanding, of New Zealand scampi, but also for other deep-water lobster species, for which there is a dearth of such information to contribute toward managing fisheries and providing a broader ecological understanding.

Researching the biology of a deep-water lobster species poses a number of challenges, with the greatest obstacle being the logistical impossibility of in situ studies. This over-riding constraint required the prior successful development of methods to capture and transfer live New Zealand scampi from their deep-sea offshore habitat into land-based captive holding facilities where they could be maintained for long term observation and experimental studies. The development of these novel methods comprised a significant component of this study, however, the description of these methods are only provided as background to the research results that were made possible through their successful development prior to the research being undertaken.

Therefore, the overall purpose of this research was to examine some key aspects of the biology of New Zealand scampi that are relevant to the assessment of this species for potential aquaculture development. This was addressed through these objectives examining key aspects of the biology of *M. challengeri*:

1.8.1 Characterisation of the fecundity of *M. challengeri*

This objective was achieved by assessing the number of eggs on females and describing the techniques used for hatching and raising larvae through to settled juveniles in captivity for the first time. This research is presented in **Chapter 2** and has been published as:

Heasman, K.G., Jeffs A.G. 2019. Fecundity and potential juvenile production for aquaculture of the New Zealand scampi, *Metanephrops challengeri* (Balss, 1914) (Decapoda: Nephropidae). Aquaculture, 511, 634184

And

Jeffs, A., Daniels, C., Heasman, K. 2020. Aquaculture of Marine Lobsters. In: Lovrich, G. and M. Thiel, editors. Fisheries and Aquaculture: Natural History of Crustacea Vol. 9. Oxford University Press, New York, USA. pp. 285-311.

1.8.2 Investigation of the effects of temperature on the morphometrics of eggs and embryo development in *M. challengeri*.

The the aim of this study was to characterize the effects of temperature on embryo development using the Perkins eye index and other metrics of the embryo advancement.

This research is presented in **Chapter Three -**

Heasman, K.G., Tremblay, L.A., Jeffs, A. The effect of temperature on rate of embryo development and egg volume in New Zealand scampi (*Metanephrops challengeri*). (In preparation for submission).

1.8.3 A description of the larval stages of *M. challengeri*.

The objective of this study was to provide a detailed description of the larval development of *M. challengeri* based on samples of sequential developmental stages taken from the successful hatching of scampi eggs and larval rearing in captivity.

This research is presented in **Chapter Four** –

Heasman, K.G., Webber, R., Jeffs, A. The larval development of the New Zealand deepwater scampi, *Metanephrops challengeri* (Balss, 1914) (Decapoda: Nephropidae). (In preparation for submission).

Estimation of the metabolic scope and most advantageous thermal conditions for growth of the New Zealand scampi (*Metanephrops challengeri*) .The aim of this study was to investigate the influence of temperature on the oxygen consumption of adult *M. challengeri* at rest and when active with a view to establishing an optimal rearing temperature and establishing the Q_{10} of the species.

This research is presented in **Chapter Five** –

Heasman, K.G., Tremblay, L.A., Jeffs, A. Estimation of the metabolic scope and most advantageous thermal conditions for growth of the New Zealand scampi (Metanephrops challengeri). (In preparation for submission).

1.8.4 General Discussion

Chapter Six is a general discussion which provides an overview and synthesis of the collective body of research presented in this thesis. It also provides a preliminary assessment of the aquaculture potential of the species and some recommendations on future directions for research and aquaculture for New Zealand scampi.

1.8.5 Research Hypotheses

Several hypotheses were suggested at the initiation of this study. They were:

- The fecundity of New Zealand scampi would be low with the high survival rate of advanced hatching larvae.
- Temperature has a significant influence on the pace of development and morphometrics of eggs and embryo development in New Zealand scampi.
- An optimal husbandry temperature can be established for the cultivation of the adult New Zealand scampi.
- The growth potential of New Zealand scampi can be estimated via initial measurement of the temperature coefficient Q_{10}.
- The egg incubation and larval development of New Zealand scampi would be consistent with the extended incubation period and truncated pelagic larval development observed in other members of the *Metanephrops* genus.

Chapter 2 : Fecundity and potential juvenile production for aquaculture of the New Zealand Scampi, *Metanephrops challengeri* (Balss, 1914) (Decapoda: Nephropidae).

2.1 Introduction

The New Zealand scampi, *Metanephrops challengeri* (Balss, 1914), is an endemic species of lobster that is distributed in waters from 200 - 600 m deep around most of New Zealand in densities ranging from 0.02 to 0.1 m^{-2} (Cryer et al., 2005). *Metanephrops challengeri* lives in water temperatures of 7 – 13.5 °C on the east coast of the North Island of New Zealand (Cryer et al., 2005), 7 – 10 °C on the Chatham Rise (Hadfield et al., 2007) and 6 – 9 °C in areas further south (Morris et al., 2001; Sutton, 2003). It is an important commercial species that is harvested by bottom-trawling with landings of around 800 t a year valued at over US$20.4 million (MPI, 2018). An understanding of the reproduction and larval development of this high value species is a critical first step towards realising an artificially managed 'seed' supply for aquaculture or fisheries enhancement. One previous study attempted to rear *M. challengeri* larvae in captivity resulting in a first, free-swimming zoeal stage (Wear, 1976). This early success gave an inkling of husbandry potential but there is almost no information on the aquaculture potential of this species.

The larval development of five of the 18 extant *Metanephrops* species (Chan et al., 2009), (i.e., *M. japonicus, M. thomsoni, M. andamanicus, M. sagamiensis, and M. challengeri*) have been described in part or in full, based on larval rearing in captivity (Berry, 1969; Iwata et al., 1992; Okamoto 2008a; Uchida & Dotsu, 1973). The eggs of these five species were hatched from clutches of late-stage embryos carried by captured adult females. Larvae of these species were not all successfully raised to metamorphosis and therefore assumptions regarding later larval stages were often made by the researchers. Four species (*M. japonicus, M. thomsoni, M. sagamiensis, and M. challengeri* were found, or suggested to have, an abbreviated pelagic larval development which lasted 4 – 9 days (Anger, 2001). On this basis the fifth species, *M. andamanicus* is also assumed to have a short larval duration. Therefore, larval rearing studies from other species in the genus, make it difficult to draw any conclusions about the likely larval development in the New Zealand scampi species.

The overall aim of this study was to describe attributes of the early stages of the life cycle of *M. challengeri,* especially fecundity and larval development, in terms of their potential for

future aquaculture development. There were four research objectives to achieve this aim. The first was an assessment of fecundity from captured female scampi. The second involved the hatching and culturing of eggs. The third objective was larval rearing, and the fourth was behavioural observations to assist with future husbandry of this species in captivity.

For the purposes of this manuscript, for a species to have aquaculture potential it is suggested that it needs to include the following parameters: be resilient in captivity; consume a prepared food preferably over its full life history; be able to mate and spawn in captivity; have a high fecundity or high larval rearing success rate; have a short larval period; have low mortality in production; be easily accommodated in captivity; should not show intra specific-aggression, and show an economically viable growth rate (Leung et al., 2007). While this study was largely focused on the first detailed descriptions of reproductive and attributes of the larval biology for this species, where possible useful observations of aspects relating to these criteria for aquaculture suitability have been provided.

2.2 Material and Methods

2.2.1 Collection of adult males and ovigerous females

Live adult male and ovigerous adult female scampi (OCL = 35 – 52 mm) were collected on six occasions from 2014 to 2016 using bottom trawling at a depth of around 350 m on the Chatham Rise, some 280 km off the east coast of the South Island of New Zealand (within 100 km of 42.924394° S, 177.260249° E). During each fishing trip, up to 75 lively ovigerous females were retrieved from the trawl net, placed in lidded plastic holding trays (60 × 40 × 12 cm), with all surfaces perforated including the lid, to allow water flow. The trays were stacked inside a 1000 l insulated transport bin containing seawater. The seawater in the insulated container was circulated and chilled to the ambient temperature from which the adult scampi were captured (i.e., 8 - 9 $^{\circ}$C) and aerated to maintain dissolved oxygen above 95% saturation. A third of the seawater was replaced every 12 h during the subsequent 48 h vessel trip back to port. The water replacement regime resulted in a temperature range of 7 to 12 $^{\circ}$C during transit back to port. On reaching port the insulated container was lifted onto a truck and transported for 2 hours to the Cawthron Aquaculture Park (CAP) near Nelson, New Zealand.

2.2.2 Holding adult males and ovigerous females

Upon arrival at the CAP, the scampi were transferred into a purpose-built holding system which provided high quality recirculated seawater at a constant 10.5 °C and a pH of 8.2. Seawater to supply the rearing system was drawn from a shallow water pipe extending 100 m from the boulder beach adjacent to the facility. The raw seawater is filtered through a 100 µm filter and UV sterilised prior to being introduced to the facility. This seawater had a pH of 8.1 (± 0.1). The same source of water used for all experiments referred to in this research. Ammonia, nitrite and nitrate in the recirculated seawater were assessed with a Hach DR890 colorimeter and kept at trace levels and carbonate levels were maintained by dosing with sodium carbonate. Dissolved oxygen was maintained at > 95% at all times through high water exchange and aeration. All seawater went through a full water recycling system. Seawater returning from the scampi containers went through 100 µm bag filters, 3 m^3 activated filter media, moving bed sand filters, a protein skimmer where ozone was introduced, UV treated, chilled, oxygenated and returned to the scampi containers. The recycling system was designed to accommodate a 3 kg loading of fed out protein a day, however, the protein levels seldom exceeded 500 g per day.

An attempt was made to provide a custom-made diet designed by fish nutritionist (Dr. I. Lupatsch) to provide all the scampi's requirements. Animals were fed to satiation with the custom-made pellets (55% protein, 10.2% lipid, 15.1 MJ/kg energy – refer to Table 6.1), however, there were consistency issues which appeared to cause the scampi difficulties for consumption. Therefore, fresh fish, squid and bivalve tissues were fed to the adult scampi. Females did not moult or grow during this period as moulting would have resulted in discarded eggs. Excess food was removed from the tanks after 6 h and all holding tanks were cleaned at least once every 3 days. Dim blue light (10 lumens) was provided in the facility at the local daylight photoperiod at the facility as a diurnal cue for the animals. The requirement for this was uncertain particularly with regard to blue light penetration of water, but it was added to cover all possibilities and may have been unnecessary. Dim red (915 nm) 5 w LED lighting was used to allow staff to work in the facility but not interfere with the scampi, as decapod crustaceans are thought to be generally insensitive to red light (Meyer-Rochow, 1994).

The female scampi carrying eggs were housed individually in plastic trays (50 × 20 × 20 cm) to avoid any stress or damage to clutches of eggs that might otherwise be caused by

antagonistic interactions. Clean seawater from the recycling system header tank was introduced from one end of the scampi holding tank (at 0.6 l min^{-1}) and exited through holes in the floor of the tank to create a down-current designed to remove any hatching larvae from the chamber. This was designed to reduce their possible consumption of the hatched larvae by the females. The exiting water from the trays flowed slowly through a screened (1 mm mesh) larval catching sump where they could be recovered and transferred to larval rearing units.

Mortality of scampi may vary according to their handling during capture and transport. Extended trawl duration (over 1 hour), delayed transfer from the deck of the vessel to the holding tank, and equipment issues would result in increased mortality (immediate or delayed over the next 10 days). In this instance mortality may be <5% if all developed procedures were followed, however, mortality can be as high > 50% if procedures were not followed or equipment failed. Once settled into the system (10 days +), mortalities of the broodstock were below 5% over the following six months.

Adults are not inherently aggressive and can tolerate being in a communal setting, however, there are occasional instances of aggressive activity. The aggression is generally limited to pushing and pulling but since the chelae are not strong, the result may be the occasional lost limb but this is seldom fatal. However, moulting adults were attacked if they moulted in the presence of other adults. In these instances, the moulting animals generally died. For these reasons the adults were housed separately

2.2.3 Egg counts

For the assessment of fecundity, a total of 314 berried female scampi (mean orbital carapace length 39.88 mm (SD ± 4.50) and mass 47.72 g (SD ± 15.26) were collected over a period of two years from trawling events on the Chatham Rise also used for catching live scampi for captive holding. The scampi were euthanised, weighed, measured and frozen. Eggs were subsequently stripped from the frozen females, placed in slightly acidified seawater (0.012M HCl to assist with egg de-clumping) and counted.

2.2.4 Mating activity

Attempts to establish if scampi would mate in captivity was assessed by video recording male – female pairs of scampi housed together in a single tank and monitored with an overhead video

system using infrared lighting (Major & Jeffs, 2018, 2017a, 2017b). The first system was a 1 m^2 tank with 40 cm deep sand ranging from 50 - 750 µm grain size. The water in the tank was 50 cm deep. A second system was a black polyethylene tank (180 × 56 × 54 cm) with a water depth of 25 cm. Water conditions were 10.5 °C, water flow 5 l min^{-1}, pH of 8.2 and no detectable ammonia, nitrate or nitrite, oxygen held at > 95% saturation. Seawater was UV and ozone treated. Video recordings were reviewed for signs of agnostic or mating behaviour. The majority of the time the scampi were observed they were generally inactive. Total video duration was approximately 710 hours, of which approximately 151 hours of that period the scampi pairs were active in close visual proximity to each other. 193 pairs of scampi were observed.

2.2.5 Larval rearing

Larvae were derived from over 1000 wild caught females held captive over the 2014 to 2016 period. Larvae hatched on any one day, often from multiple females, were mixed into a single larval cohort. Cohorts (mean = 91 SD = 31 larvae per day) of larvae were cultured with increasing success as observations of the larvae were used to improve the larval holding and handling methods (mean survival 25% SD = 16.5). Larvae were cultured in ten modified 9 l kreisel tanks (Serfling, Van Olst & Ford, 1974). Initial observations found that scampi larvae were susceptible to shear forces resulting from water injected into prototype kreisel tank upwelling units that was intended to encourage suspension of the larvae in the water column. Modifications to acrylic tanks were built to reduce the shear forces from water inflows. The tanks were supplied with UV and ozone sterilised, 10 µm filtered seawater at 10.5 °C. The water was introduced at a rate of 0.3-0.5 l min^{-1} across the width of each kreisel and was drained from the centre of each tank encouraging a flow of water from the kreisel tank walls to the centre of the tank. This setup created an even, rotating flow of water that maintained larvae in suspension. Larvae were kept at a density of 60 individuals l^{-1}. Larvae were susceptible to fungal and bacterial infection with any lapses in the seawater hygiene regime which increased with density.

2.2.6 Post-larval rearing

Post-larvae were initially held in tanks (10 × 10 × 12 cm) with a sandy bottom (sand grains from 250 - 750 µm) at a density of two per tank. Water flowed into the tank at a rate of 40 ml min^{-1}. However, it was found that as they increased in size in the tanks that included sand,

they became more prone to bacterial and fungal infection. The post-larvae were therefore transferred to Aquahive™ trays one day after moulting. Aquahive™ trays were held in the complete Aquahive™ system initially but since daily checking was required the trays were subsequently floated in a tank (1 × 1 × 1 m) with the same recycled water as used for the larval rearing which was exchanging at 2 l hr^{-1}. Post-larvae were fed three ProChaete™ semi moist pellets each day. The feeding rate was designed to provide excess feed. The point of satiation is plainly observed as the post larvae (and adults) will actively push the remaining food away and move in a way to distance themselves from the food if sufficient space allows. Post-larvae were moved to clean trays every two days. Up to five Aquahive™ trays were used at any one time to accommodate different cohorts.

2.2.7 Larval feeding

The ability of scampi pre-zoea and stage I zoea to feed was assessed by offering them live brine shrimp (*Artemia salina*) stage II nauplii (from Brine Shrimp Direct, USA) (5 ml^{-1}), and rotifers (*Brachionus plicatus*) (10 ml^{-1}) and monitoring the feeding behaviour of larvae. It was found that the larvae will also accept frozen zooplankton and prepared feed. To improve the veracity of these visual observations, 4 µm diameter luminous polystyrene beads were fed to the brine shrimp and rotifers prior to them being offered to the pre-zoea and stage I zoea. Separately, the fluorescent beads were also released into the water column to determine if the pre-zoea and stage I zoea were filter feeding.

Stage II zoea were fed live rotifers (*Brachionus plicatus*), and brine shrimp stage II nauplii (*A. salina*). To reduce the bacterial load of the brine shrimp being transferred into the larval scampi cultures, the stage II brine shrimp were fed axenically cultured microalgae (*Tisochrysis lutea* and *Chaetoceros calcitrans*) for 6 h to purge their intestine, then enriched with Selco® (0.3 g Selco l^{-1}) for 12 h. Then to reduce external bacteria the *Artemia* were held in a 0.4% formaldehyde solution for 30 min, rinsed in fresh seawater and then fed to the scampi larvae. A method to prepare artificial feeds was developed to a commercial proprietary formula used for white shrimp aquaculture. This diet contained 55% protein and 10% lipid.

2.2.8 Larvae preservation and analysis

Samples of scampi zoea at different stages of development were either photographed live or euthanized and preserved in 10% seawater buffered formaldehyde for dissection and morphological examination and measurement.

2.3 Results

2.3.1 Egg count

A mean egg count of extruded eggs on captured scampi with mean orbital carapace length (OCL) of 39.88 mm (SD ± 4.50) and mass of 47.72 g (SD ± 15.26) was 337 (SD ± 130.92) (*n* = 314) per clutch, with a range of 57 – 804 eggs per adult female. The number of eggs in the scampi clutch increased in proportion to the orbital length (Pearson's r = 0.690) and the mass of the female (Pearson's r = 0.703) (Figs. 2-1 and 2-2)

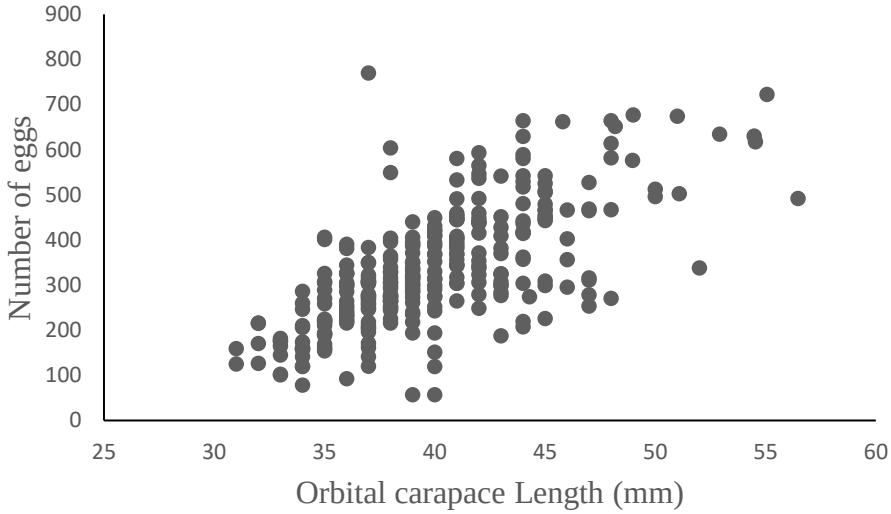


Figure 2.1 Comparison of clutch size (number of eggs) versus orbital carapace length of berried female *Metanephrops challengeri*.

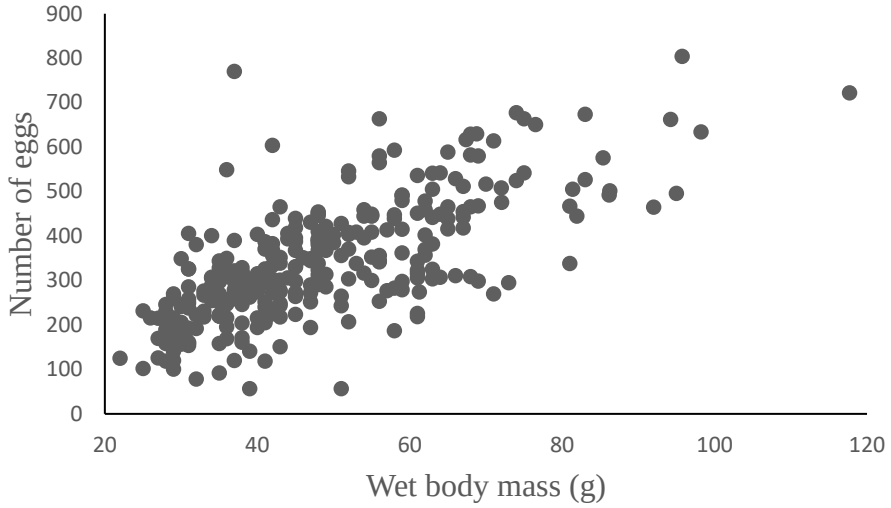


Figure 2.2 Comparison of clutch size (number of eggs) versus wet body mass of berried female *Metanephrops challengeri*.

2.3.2 Mating activity

Mating interaction was observed on 14 occasions of which successful mating was only observed on three of those occasions in over 151 hours of video observations where the scampi were in visual proximity to each other. All the successful mating interactions occurred when the male was larger or similar in size to the female. The three observed copulation events were short in duration ranging from 1 minute and 12 seconds to 4 minutes 51 seconds.

Of the three females that were observed completing a mating event, only one female produced eggs (~ 190) three days after copulation. These eggs were viable and developed normally.

The unsuccessful mating attempts occurred when: the male was smaller than the female and the male appeared to lack the strength to turn her over for copulation; or the male only had one cheliped and could not control the female, or the female escaped from the male's grasp.

2.3.3 Hatching, larval survival and rearing

Only one batch of eggs was tracked from spawning to hatch and that took ~225[1] days at ~10.5 °C. Larvae generally hatched at night. Larvae were assessed for health and vitality. The larvae in poor health were defined as those that did not moult past the pre-zoeal stage or spent

[1] Post-publication research has refined this duration (see Chapter 3).

extended time as a pre-zoea and remained on the bottom of the culture chamber where they perished.

Over 10,000 high quality larvae were hatched from ovigerous wild-caught females carrying newly-laid eggs, and from females that had extruded eggs while in captivity. From these larvae over 1600 post-larvae were raised in total. Up to 55% of the larvae hatching from these eggs advanced past the pre-zoea stage. The pre-zoea stage of healthy larvae was short-lived, moulting to stage I zoea occurring within minutes to hours. The pre-zoea did not swim as they had no setae on the exopods of the pereiopods (a defining character of pre-zoea stages). Pre-zoea and stage I zoea appeared to be lecithotrophic as luminous polystyrene beads fed to rotifers and brine shrimp stage II nauplii as a dietary marker were not observed in the alimentary canal of these scampi stages. Luminous beads released into the water column were also not filtered out and consumed by these larval scampi stages.

The stage I zoea, which bear natatory setae on the exopods of the pereiopods, were predominantly pelagic. They maintained a vertical head-up attitude except when encountering water eddies or solid objects, such as the side of the tank, at which time they flicked their abdomen as an escape response. It took a mean of 5.8 days (SD ± 3.15) for the stage I zoea to moult to stage II zoea.

Stage II zoea were primarily pelagic, only descending to the floor of kreisel tanks periodically (Figure 2-3). The stage II zoea were observed manipulating food (i.e., *Artemia* nauplii, rotifers and prepared feeds) with the chelae on the pereiopods whilst in the water column. Food material was clearly observed in the gut of the semi-transparent larvae. No predatory lunges at live prey items (as has been seen in *N. norvegicus* larvae – pers. obs.) or other types of prey capture events were ever seen over more than 20 h of observations.

The moult to the first post-larval stage took a mean of 8.3 (SD ± 1.01) days after hatching. Up to 50% of the remaining larvae were observed to progress to this stage. The survival of the zoea declined rapidly if they had not moulted after day 11. No stage II zoea survived longer than 23 days.

The first post-larval stage takes an average of 23.9 (SD 8.45) days to moult to the second post larvae stage. The highest survival of a cohort of pre-zoea to post-larvae during this study has been 26%[2].

The mode of swimming changed from stage II zoea in which the natatory exopods of legs 1 to 5 were used for propulsion, compared to the post-larvae at metamorphosis in which pleopods were used. At metamorphosis the 1st post-larvae could elect to swim or walk on the kreisel tank floor. They were held in the kreisel until a day before being transferred to an Aquahive™ chamber. When in the Aquahive™ chamber they raised their tails and the pleopods were used to draw water under the body posteriorly. Feeding in 1st stage post-larvae is active and both live food and prepared pellets were seen to be collected by the chelae and passed to the mouth. Pereiopods were observed to be sensitive to the presence of food as their interaction with a food item resulted in the larvae orienting themselves to face the item, grasp it with chelae and consume it by passing it into the region of the mouth (Figure 2-4).

Second post-larvae in rearing tanks remained fully benthic, swimming only as an escape response. The first pigment was seen to appear over much of the exoskeleton at this stage of development. If placed in a container with sand (grains ranging from 200 µm to 1 mm) they were vigorous landscapers (i.e., burrowing and moving sand) of benthic sediment at this stage of development.

[2]Post-publication research has increased this to 76%, mean 25.3% (SD 16.5) (n = 42 cohorts).

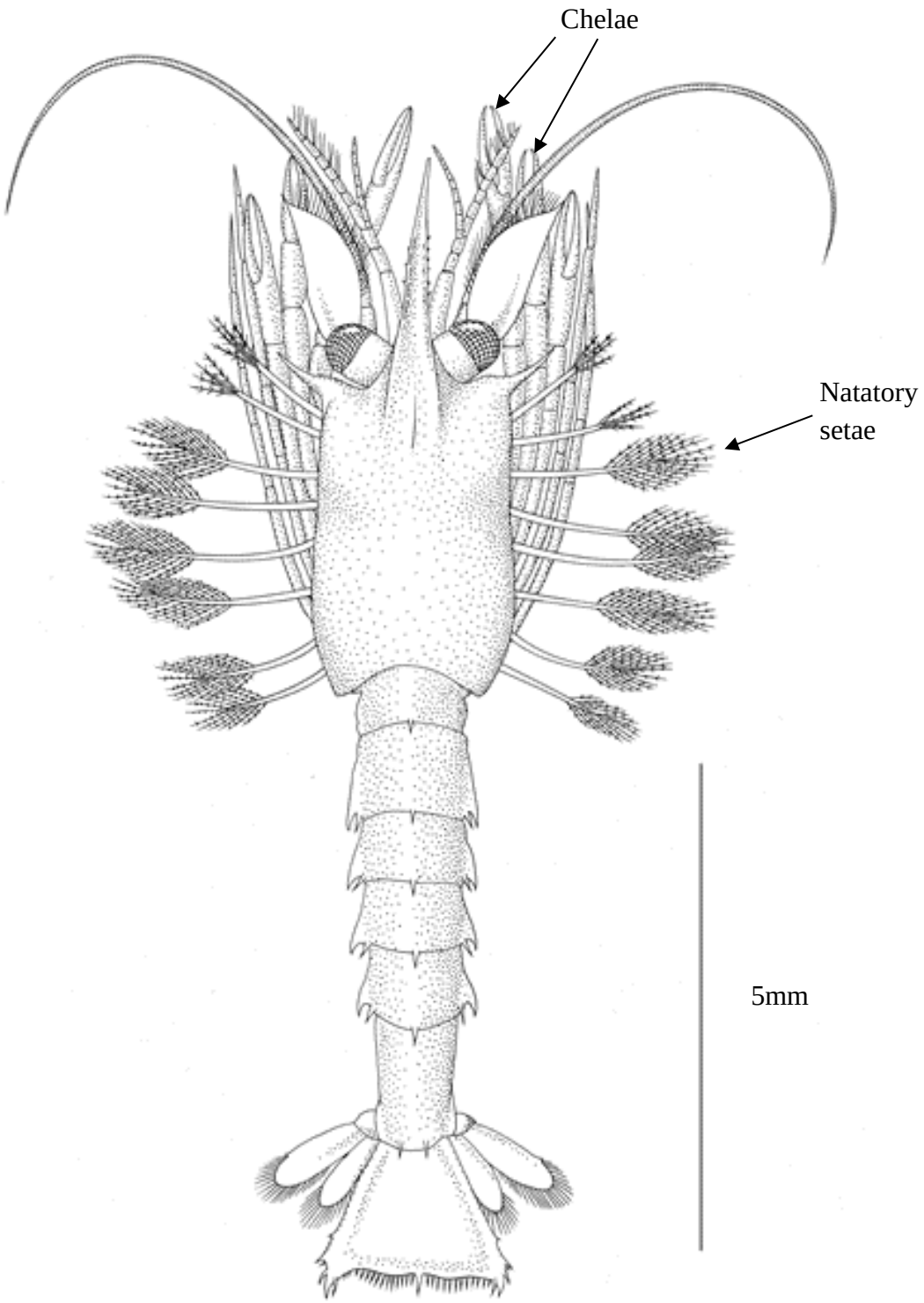


Figure 2.3 The stage II zoea of *Metanephrops challengeri* (diagram R. Webber). Natatory exopods used for locomotion and chelae utilised in the manipulation of food are indicated.

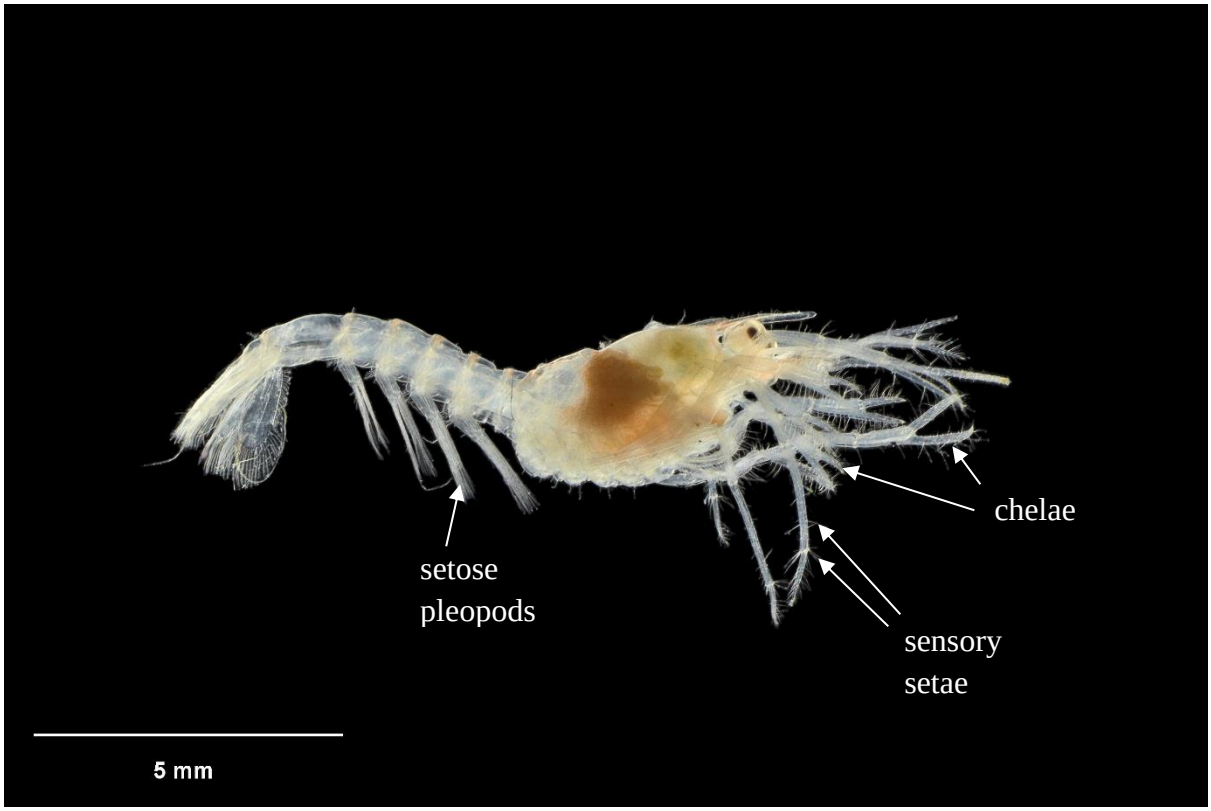


Figure 2.4 The 1st stage post-larva of *Metanephrops challengeri* showing abundant sensory setae and setose pleopods. Chelae used for the manipulation of food are also shown

2.4 Discussion

2.4.1 Fecundity

In this study, *M. challengeri* was found to have a maximum of 804 eggs and a mean of 337 (SD ± 130.92) eggs per clutch (mean female OCL 39.88 (SD ± 4.50) mm and mass 47.72 g (SD ± 15.26), n = 314).

When compared with three northern hemisphere representatives of the Nephropidae: *Nephrops norvegicus; Homarus gammarus* and *H. americanus*, members of the *Metanephrops* genera have significantly lower egg numbers. *Nephrops norvegicus* is estimated to have 900 – 6000 eggs per clutch (de Figueiredo & Vilela, 1972b). *Homarus americanus* is reported to have over 60,000 eggs (Campbell & Robinson, 1983), although a more recent study only found a maximum of 48,000 eggs (Koopman et al., 2014). *Homarus gammarus* is reported to have up to 40,000 eggs (Agnalt, 2008). However, both *Homarus* species can get much larger in size than both *Nephrops* and *Metanephrops* species. The number of eggs in *Homarus* is influenced by geographical and environmental conditions (Ellis et al., 2015; Koopman et al., 2014) and

eggs are known to be lost during incubation (Campbell & Brattey, 1986; Eiríksson, 2014; Matsuura & Hamasaki, 1987; McQuaid et al., 2009) or as a result of capture (Briggs et al., 2002; Tuck et al., 2000). It is estimated that 25% of eggs are lost from females caught in creels and as much as 75% of the eggs may be lost as a result of trawling (Powell & Eriksson, 2013). Natural egg loss has also been recorded and estimated to be between 30 and 50% in *H. americanus* (Campbell & Brattey, 1986; Perkins, 1972). The egg losses are typically attributed to reasons such as incomplete formation of egg envelope during oocyte maturation (Talbot & Harper, 1984) and poor attachment and/or interference or disturbance during egg laying (Talbot et al., 1984). From an aquaculture perspective, if the breeding cycle can be closed and the females provided with sufficient resources, it may be possible to both increase egg production and negate the egg losses likely to be currently experienced through the process of wild capture. Assuming the same extent of the potential losses reported above for trawling, then *M. challengeri* has the potential to produce a mean of 640 eggs per clutch. This is a significant increase in egg production but still relatively low for an animal of these dimensions. *Metanephrops challengeri* has a mean of 7.1 eggs g^{-1} bodyweight (this study), contrasting with *H. americanus* which is reported to have 29 eggs g^{-1} of adult female body weight (Pollock, 1997). In terms of egg dimensions, *M. challengeri* was recorded having eggs of 2.08 – 3.15 mm in length (Fenaughty, 1989), this study recorded egg lengths of 2.10 - 3.23 mm, while *M. mozambicus* has eggs of 1.95 – 2.83 mm (Berry, 1969) and *M. thomsoni* is reported to have eggs of 1.78 - 2.66 mm (Hamasaki & Matsuura, 1987). *Nephrops norvegicus* eggs are between 1.2 - 1.56 mm in diameter (Smith, 1987), although some researchers report eggs as large as 2 mm (Mori et al., 1998). The size of the eggs has been found to increase with the duration and extent of egg development (Mori et al., 1998). Generally larger eggs are also thought to be associated with smaller broods, better egg or larval survival and abbreviated free-living larval periods (Pollock, 1997).

Data collected from the commercial fishery suggest an annual spawning cycle in *M. challengeri* (Tuck, 2010). However, based on observations during this study, spawning events may only occur every two years at lower temperatures.

2.4.2 Larval development

It has been shown that *M. challengeri* has three larval development stages prior to moulting to a post-larva or megalopa, these are the pre-zoea stage, stage I zoea and stage II zoea. These

larval stages are reflected in *M. thomsoni* (Uchida & Dotsu, 1973) and *M. sagamiensis* (Iwata et al., 1992), but not in *M. japonicus* (Okamoto, 2008a) which lacks stage I and stage II zoea.

Of the larval stages in *M. challengeri,* only stage II zoea feed, the pre-zoea and stage I zoea are lecithotrophic. In *M. sagamiensis* all of the zoeal stages are reported to be lecithotrophic (Iwata et al., 1992). It is assumed that the pre-zoea stage, which is the only zoeal stage of *M. japonicus* is also lecithotrophic.

Upon metamorphosis to a post-larva (or megalopa), the swimming activity is taken over by the pleopods which are well endowed with natatory plumose setae. The first stage post-larva of *M. challengeri* can still be an active swimmer in the water column, spending most of their time in suspension which suggests it could still utilise water currents for dispersal. The second stage post-larvae become more sedentary, starting landscaping and showing pigmentation.

All members of the Nephropidae show abbreviated larval development (Goy, 2014). *Nephrops norvegicus* has three swimming zoeal stages and a pre-zoea stage, as does *H. americanus* and *H. gammarus,* although Charmantier and Aiken (1987) argue for an intermediate stage between stage III and the metamorphosed post-larvae of *Homarus* species. Williamson (1982) considers the pre-zoea to be a late embryonic stage and not an early zoeal stage. However, this stage continues to be described by most authors as a pre-zoea. In order to reach the first post-larval stage *N. norvegicus* requires 18 - 33 days (Powell & Eriksson, 2013), while *H. americanus* requires 9 – 33 days (Hughes & Matthiessen, 1962) and *H. gammarus* requires 11 - 55 days (Ennis, 1995). In contrast, *M. sagamiensis* has a zoeal duration period of 6 to 8 days. In this study *M. challengeri* has a mean zoeal period of only 9-11[3] days. Larval development has been shown to be temperature dependant in the Nephropidae (Ennis, 1995; Quinn & Rochette, 2015) so variations in larval durations in *Metanephrops* are likely to vary in part in relation to ambient temperatures.

All five of the *Metanephrops* species that have been partially or fully reared from eggs have a short-lived pre-zoea stage ranging from minutes to 22 h in duration (Berry, 1969; Iwata et al., 1992; Okamoto, 2008a, 2008b; Uchida & Dotsu, 1973; Wear, 1976). *Metanephrops thomsoni* (Uchida & Dotsu, 1973), *M. sagamiensis* (Iwata et al., 1992) and *M. challengeri* (this study) are shown to have a pre-zoea stage, a stage 1 zoea and a stage 2 zoea taking a minimum of hours, 4 and 9 days to reach post-larvae metamorphosis respectively. *Metanephrops japonicus*

[3] Post-publication research refined this to 8.3 days (see Chapter 4).

has been shown to have a pre-zoea that metamorphoses directly into a post-larva (Okamoto, 2008a). Although *M. andamanicus* (Berry, 1969) is suggested to only have a pre-zoea stage before metamorphosis, this suggestion is based on observations of the pre-zoea and its internal structure and therefore may be incorrect.

2.4.3 Life history and natural behaviour activity

A trend towards increasing egg size, incidence of lecithotrophic larvae and short planktonic larval stages, all of which are found in the known *Metanephrops* zoeal stages, has been associated with decreasing water temperature at higher latitudes and greater water depth (Thorson, 1950). This trend has been attributed to reduced planktonic food available in deep waters at higher latitudes, which is suggested to be an attribute of, and consequence of, the low productivity during ice ages (Poulin & Feral, 1996). In the case of *Metanephrops*, this may be correct as the current zoogeographic hypothesis is that the *Metanephrops* genus originated in the Antarctic region in the Cretaceous Period (Tshudy, 2013) and is thought to have diversified as it dispersed northward into new habitats (Chan et al., 2009; Tshudy, 2003; Tshudy et al., 2007).

It is possible that some members of the *Metanephrops* genus are evolving more of a precocial life history with more of the larval stages being included in the embryonic stages of the egg. The decreasing larval period shown in *M. japonicus* and lecithotrophic early stages are potential examples of this possible trend.

Nephrops and *Metanephrops* species are known to have more specific habitat requirements (Cobb & Wahle, 1994; Tully & Hillis, 1995; Wahle et al., 2012). All the *Metanephrops* species are reported to live on muddy substrate (Wahle et al., 2012) except *M. armatus* which is associated with rocky substrate (Chan & Yu, 1991). Genera such as *Homarus* can use a number of substrates (Cobb & Wahle, 1994) providing they have cover and therefore it is suggested that they can afford to have a longer pelagic life history with greater dispersal capacity. It is suggested that the first post-larval stage of *M. challengeri*, which can be benthic or pelagic for as long as 28 days, has the ability to settle to the seafloor or continue swimming to find suitable habitat during this period, greatly extending its dispersal potential. It follows that populations of this species may be relatively restricted in their distribution or gene flow within the population, as has been recently demonstrated (Verry, 2017).

Communal holding of mixed sex adults tended to result in some aggression among individuals but with little damage generally to the animals themselves unless an animal moulted in which case it was set upon by the others and eaten. Aggression was generally only observed between males, which may be involved in the establishment of a social hierarchy as seen in *N. norvegicus* (Sbragaglia et al., 2017). Katoh (2011) showed that the majority of mating in *N. norvegicus* occurred generally with softshell females. However, the presence of a moulted female may induce mating behaviour from males with hard-shell females. This may help to explain some of the similar social interactions that were observed in *M. challengeri*.

2.4.4 Implications for Aquaculture

Metanephrops challengeri, like many of the other members of the Nephropidae, has many biological characteristics which make it a suitable candidate for aquaculture, in addition to its high commercial value as seafood.

To culture this species successfully in the future it is important to be able to mate selected adults and produce high quality and abundant eggs. In this study it has been shown that mating and egg laying will take place in captivity. Spawning and egg rearing in captivity without intervention is also a benefit to aquaculture practices. While a single successful mating and spawning event was observed, there is uncertainty surrounding the requirement for female scampi to mate shortly after moulting and the duration of sexual receptiveness of the female. Except for the female that nurtured her clutch of eggs to hatch in this current study, it is unknown if all the mating that took place resulted in fertilisation as females may be able to store sperm for some time for subsequent egg extrusion and fertilisation. Other females (n = 10) which were in isolation produced eggs while in the facility, which suggests an ability by females to store sperm from prior mating. The female that spawned in the tank three days after mating was not a new moult so either the female does not necessarily need to be newly moulted to be fertilised or she may have carried sperm over from a previous mating. This has yet to be tested to establish the potential for egg production in scampi under aquaculture conditions.

In the aquaculture facility, the zoea were well advanced upon hatching, as a result of substantial maternal investment into large eggs (although egg volume can be influenced by temperature - see Chapter 3). So female conditioning prior to mating will be important for ensuring adequate egg development in the future. The advanced zoea has a short larval period which is a desirable attribute for culturing this species as it reduces larval rearing requirements and mortality

normally found in species with long larval rearing periods. The pre-zoea and stage I zoea are lecithotrophic, before becoming a reptant juvenile within 9 to 11 days[4] but taking up to 21 days for very slow developers. This variation may be an artefact of husbandry methods or perhaps a result of limited nutritional reserves in the egg leading to slow development. All the feeding zoea stages will consume inert food, and on becoming a juvenile readily consume artificial feed pellets. They are non-aggressive in the zoeal stages and so can be held in relatively high densities, especially when compared to *Homarus* species which have highly cannibalistic larvae (Romano and Zeng, 2017).

Diets are an important part of final aquaculture production. In *M. challengeri* the larval feeding stage rapidly reaches a benthic life stage which is a significant advantage, providing maternal investment in the ova has been sufficient. This places emphasis on understanding diet development to support the broodstock and feeding zoeal stages (Rosa et al., 2003; Jeffs & O'Rorke, 2020; Li et al., 2021; McConaugha, 2017; Shu-Chien et al., 2017).

There are some challenges in terms of the aquaculture potential of *M. challengeri*. This species produces a small clutch size and the eggs take an extended period to hatch (~225[5] days at 10.5 °C) during which time the female may discard or consume the eggs. The eggs are also prone to fungal infection if removed from the female thus requiring the facilities to maintain the female broodstock through the egg brooding period. All life stages of *M. challengeri* appear to require maintenance of high-quality seawater, which can only be derived from a reliable seawater control system. The gills of the zoea and post-larvae 1 are poorly formed thus requiring high levels of oxygen saturation.

High mortalities were initially encountered during the early stages of zoea development. However, this may be reduced through further improvements in larval rearing arrangements.

Improvements in culture techniques and parameters and greater understanding of larvae dietary requirements are likely to make significant improvements in future larviculture outcomes.

2.4.5 Conclusions

Metanephrops challengeri has a long embryo development period and a short larval period consisting of three larval stages. The improved understanding of the reproduction in this

[4] Post-publication research refined this figure (Chapter 4).
[5] Post-publication research refined this figure (Chapter 3).

species is useful for improving fisheries management and evaluating their aquaculture potential. Larval duration is short resulting in an advanced post-larva with minimal requirement for providing pelagic larval rearing conditions. This short larval period and ease of husbandry during this phase of development is conducive to aquaculture production either to support fisheries or grow-out production.

Chapter 3 : Patterns of embryo development in New Zealand scampi (*Metanephrops challengeri*)

3.1 Introduction

The New Zealand scampi, *Metanephrops challengeri* (Balss, 1914), is a burrowing lobster which is primarily distributed in waters from 200 to 600 m deep around much of New Zealand. It is endemic to New Zealand waters where temperatures range from 6 – 9 °C in areas around the Auckland Islands (SCI 6A) (Figure 3-1) (Morris et al., 2001; Sutton, 2003), 7 – 10 °C on the Chatham Rise (Hadfield et al., 2007) and 7 – 13.5 °C on the east coast of the North Island (Cryer et al., 2005).

It is an important commercial seafood species in New Zealand with a total allowable catch of 1272 t (Fisheries-New-Zealand, 2021) that is harvested by bottom-trawling with landings of around 800 t a year and valued at over US$20.4 million (MPI, 2018).

Of the 18 extant species of *Metanephrops* found around the world, only five have had their larval stages partially or fully described. Other than accounts of fecundity (Heasman & Jeffs, 2019; Robey & Groeneveld, 2014) there is limited published research that has focused on describing the egg and embryo development in any *Metanephrops* species, especially in relation to key environmental variables. For example, temperature is known to have a major influence on the rate of development in crustacean embryos (Charmantier & Mounet-Guillaume, 1992; Gendron & Ouellet, 2009; Green et al., 2014). For scampi, the females incubate the egg clutch for an extended duration until hatching, a period that varies considerably depending on temperature. This incubation period is important as it may influence the frequency of breeding cycles and relative fecundity of different populations, which is an important parameter in fisheries management, and for aquaculture, for which this species is under consideration.

The Perkins eye pigment method (Perkins, 1972) has been used effectively to estimate embryo developmental rate and total development time of clawed lobsters (*Homarus americanus* and *H. gammarus*) based on changes in the dimensional parameters of the orbital (eye) in the egg (Charmantier & Mounet-Guillaume, 1992; Gendron & Ouellet, 2009) over time and relative to temperature. The method has been adapted for use in the other lobster species, (i.e., spiny lobsters) given its usefulness in predicting hatch times for aquaculture production (Tong et al., 2000).

The aim of this study was to characterize the effects of temperature on the total development time and other metrics of the embryo advancement of *M. challengeri* eggs and embryos.

3.2 Methods

3.2.1 Collection and transport of egg bearing females

For measuring the egg volume of wild scampi, eggs were taken from samples of 150 egg bearing scampi that were collected on four separate occasions with a short duration trawl from a depth of between 250 and 400 m on the Chatham Rise, off the eastern coast of the South Island of New Zealand (SCI 3, Figure 1-3) and held as described below and in accordance with animal ethics approval (NMIT- AEC2014-CAW-02).

Upon recovery of the trawl, the scampi were immediately placed in a bespoke temperature-controlled seawater system on the deck of the trawl vessel. The holding system consisted of a 1000 l insulated food-grade high density polyethylene (HDPE) bin with an insulated lid (Stowers Containment Solutions Ltd, #6943). Trays made from HDPE (80 × 45 × 12 cm) with a 6 mm mesh were stacked five deep into the bin. Perforated HDPE baffles were inserted into the bin to reduce seawater movement within the bin. The water in the bin was aerated with air chilled to between 5 and 8 °C. Seawater was partially exchanged every 6 h for the duration of the transport phase. Upon arrival at the Port of Picton, within 24 h of capture, the entire holding system was loaded onto a vehicle and transported to the research aquarium facility (Nelson, New Zealand), a 2 h journey.

The egg-bearing scampi arriving at the research facility were transferred individually into tanks (40 × 15 × 20 cm, L × W × H) holding 12 l of water volume. The tanks were supplied with seawater from a recirculating aquarium system, which included mechanical and biological filtration, degassing, aeration, UV sterilisation and temperature control. They were maintained at a temperature of 10.5 °C (± 0.2 °C, S.E.), pH of 8.2 ± 0.05, salinity of 34 ± 0.1 PPT, oxygen at 100 % saturation and ammonia at trace concentrations only (Heasman et al., 2020). Scampi were held under 12:12 h cycle of dark/deep red light ($\lambda > 915$ nm) to avoid light-induced stress to which the scampi from depths greater than 200 m would be unaccustomed (Raymond & Widder, 2007; Warrant & Locket, 2004). Once the scampi began to accept food, which indicated acclimation to the holding conditions, the scampi were fed to satiation (i.e., the point where they actively rejected food) every 2 days on an alternating diet of squid, fish tissue (from

local fish monger) and Greenshell™ mussel tissue (from local mussel farms). Tanks were cleaned of food after 24 h and gently wiped down every week.

3.2.2 Temperature selection, experimental system and assessment methods

The temperatures used for holding the scampi and for the experiments in this study were derived from data obtained from 46 expeditions which took 1390 measurements from depths ranging from 192 to 644 m from four areas around New Zealand (SCI 1, 2 ,3, and 6, Figure 3-1, Table 3-1) spanning 20 years. (Source: New Zealand Ministry for Primary Industries FishServe Database). Additional temperature data was gathered by deploying logging thermistors attached to commercial and research fishing equipment associated with the current research (Table 3-1).

The scampi selected for the eye index staging were held in the above rearing system for 10 days to acclimate. Sixty egg-carrying scampi (mean mass 57.8 g ± 10.5 g, mean orbital carapace length 38.5 mm ± 3 mm) were randomly selected from the females that had eggs at an early stage of development for use in assessing the Perkins eye index for scampi (Perkins, 1972). Fifteen of the 60 egg-carrying scampi were then moved to each of five separate rearing units with each unit providing a single temperature treatment (i.e., 7, 9, 11, 13 and 15 °C) that were selected and centred on the basis of the range of water temperatures that *M. challengeri* are known to inhabit (Table 3-1). The four units were situated in an insulated temperature-controlled room (16 °C) with a 12:12 h cycle of dark/deep red light ($\lambda > 915$ nm). Within each treatment, scampi were housed independently in a HDPE chamber (40 × 15 × 20 cm, L × W × H) which held 12 l of water. Each set of 15 chambers in a treatment were supported with their own water recycling and chilling system, which maintained the same water quality parameters as outlined in the receiving system. To acclimate each group of scampi to their treatment temperature each seawater system started at 10.5 °C and after a 5 day initial acclimation period, the systems were either increased or decreased in temperature at a rate not exceeding 1 °C per day until reaching their target temperature. The target treatment temperature in each system did not vary by more than 0.2 °C over the remaining duration of the experiment.

Table 3-1 The mean (± S.D), minimum and maximum seawater temperatures recorded in New Zealand scampi habitat i.e., SCI 1, 2, 3 & 6 (Figure 3-1). (Source: New Zealand Ministry for Primary Industries FishServe Database, (1998 to 2018†) and Cawthron data (2016)‡.

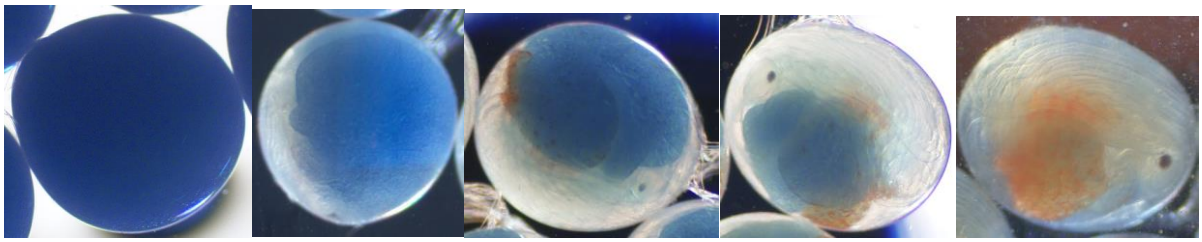
Location & Latitude	Period of sample collection	n		Depth (m) (S.D.)			Temperature at depth (°C) (S.D.)		
		Sampling expeditions over 20 years	Total samples	Mean	Min	Max	Mean	Min	Max
SCI 1† **Lat ~35º S**	Sept-Apr	14	792	387 (80)	195	644	10.3 (1.1)	7.0	13.5
SCI2† **Lat ~39º S**	Jul-May	19	522	359 (80)	192	594	9.8 (1.2)	7.0	13.5
SCI3† **Lat ~ 45º S**	Sept -Oct	8	221	397 (50)	273	536	8.7 (0.6)	7.2	11.9
SCI3‡ **Lat ~ 45º S**	October	12 transects (over 10 days)	10664	367 (9.8)	345	378	9.8 (0.1)	9.17	10.50
SCI6† **Lat ~ 50º S**	Feb-Mar	5	155	446 (46)	278	541	7.6 (0.3)	6.7	9.0

Clutches of eggs on all experimental scampi were checked weekly to determine if the eyespot was developing. Once confirmed, eggs were sampled every 7 days and the eye parameters were measured, following the methods of Perkins (1972), by removing a maximum of five eggs at each sampling event to ensure sufficient eggs were carried by the female until peak hatching given the limited size of clutches in scampi (Heasman & Jeffs, 2019) (Refer to Chapter 2). To sample the eggs, a female scampi was gently taken from her chamber and held in such a way to avoid tail flicking, then turned onto her back and the eggs checked. Eggs were removed by gently holding sterilised forceps astride the base of the pleopod and slowly lifting it until the desired number of eggs were randomly dislodged from the clutch. The dislodged eggs were then placed in a petri dish with 2 µm filtered seawater and immediately placed under a dissecting microscope, and the maximum length and maximum width of the eye of the developing embryo was measured using a calibrated reticule.

3.2.3 Volume of wild reared eggs relative to stage development

Live egg-bearing female scampi collected from the Chatham Rise in area SCI3 (Figure 3-1) were transported to the aquarium facility using the same methods as described previously. The eggs were removed (4 to 10 eggs per sample) and measured as outlined previously and photographed. From the photographs the eggs were then categorised into five stages based on their developmental progress: Stage 1 – eggs which had minimal or no visible embryo; Stage 2 - the embryo present and ranging from minimal visible embryo up to the embryo taking up

approximately 30% of the egg volume; Stage 3 – the embryo occupied from 30 to 50% of the egg volume with a very early eye spot; Stage 4 - the embryo occupied from approximately 50 to 70 % of the egg volume and the hepatopancreas was showing; and Stage 5 - was an egg with an advanced embryo (>70% of egg volume) and close to hatching. (Fig 3.1)



Stage 1 Stage 2 Stage 3 Stage 4 Stage 5

Figure 3.1 The various stages of the developing embryo in the egg

3.2.4 Egg volume measurement

Egg volume was estimated from the following formula for an ellipsoid and using the microscope measures from the sampled eggs.

Egg volume = $4/3*\pi*wr^2*lr$

Where:

wr = width radius

lr =length radius

3.2.5 Perkins eye index

Following Perkins (1972), the eye index was estimated by measuring the maximum length and width of the eye. These two measures were summed and then divided by 2 providing the index.

3.2.6 Hatch time estimate

Observations indicated that scampi eggs from the clutch of one female will hatch over a mean period of 4 days (range 0 to 10 days with rare durations of up to 20 days). For the purpose of this experiment the first eggs observed to hatch were taken as the hatch time.

3.3 Statistical analyses

The effect of temperature on the developmental rates of the egg volumes and eye sizes, was analysed using general linear models (GLMs) with temperature (as a factor) and week (continuous) as the independent variables. Gamma distributions were used in the GLMs as both the egg volumes and eye sizes had non-normal distributions. Post-hoc Tukey analysis was then used to assess any significant differences in the relationship between the developmental variables and time between the different temperatures. The maxima of the different variables were assessed using non-parametric Kruskal-Wallis tests, due to the unbalanced nature of the data. Post-hoc Dunnett tests were then used to conduct pairwise comparisons of these maxima among the different temperatures. All statistical analyses were done in R 4.1.3 (RCoreTeam, 2022), using the DHARMa package (Hartig, 2022) for diagnostic analysis of the gamma family GLMs.

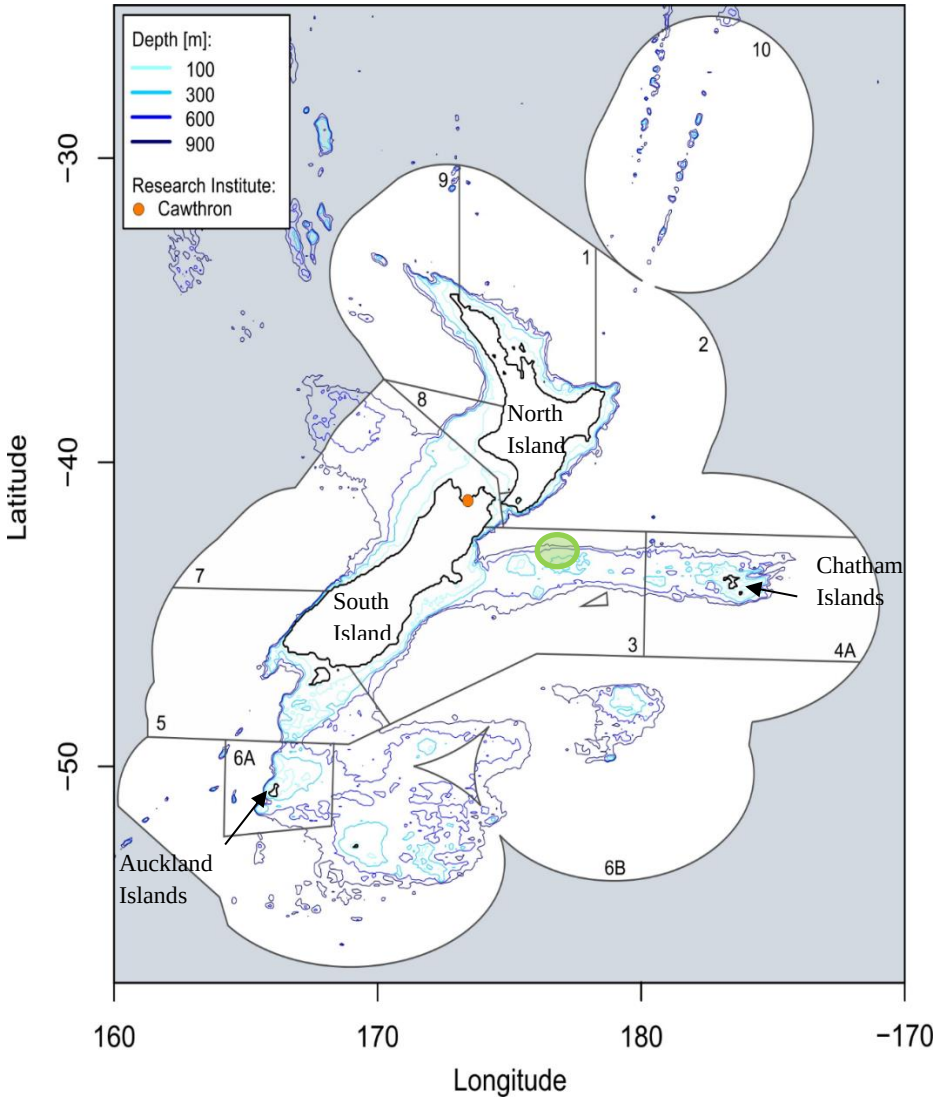


Figure 3.2 Map of New Zealand scampi commercial fisheries management areas showing the four areas where measurements of the seawater temperature in scampi natural habitat were collected, i.e., 1 = SCI 1 in the north, 2 = SCI 2 in the north east, 3 = SCI 3 central & 6A = SCI 6A south west. Live scampi used for this study were captured in 3 (SCI3) and transferred to aquarium facilities at Cawthron Institute's Aquaculture Park (orange dot). The location on the Chatham Rise from which the scampi were collected for this study is shown as a green circle.

3.4 Results

3.4.1 Embryo egg development at controlled temperature

When introduced to the facility, the eggs on the females that were used for the Perkins eye measurement and egg volume at controlled temperatures, were all at the very early stage (stage 1 as described above). Once the scampi had acclimated to the captive conditions, they were maintained with minimal interference and handling and had a low mortality rate of < 3% in the system over the duration of the experiment except for the scampi at 15 °C. The scampi held at

15 °C declined in health over the first 5 to 10 days, becoming lethargic and ceasing to feed and clean. Therefore, they were euthanized to meet animal welfare requirements. For those scampi being held at the remaining controlled temperatures, the mortality at each temperature, out of 15 animals, was 20%, 6.7%, 13.3% and 6.7% for 7, 9, 11 and 13 °C, respectively over the full egg development period, which varied in extent in relation to the temperature (Table 3-2).

Egg loss from the females (either through being discarded by the females or consumed by the females) was observed to increase markedly in the first 7 weeks, particularly at 13 °C (Table 3-2). This trend continued, although to a far lesser extent after week 7. Many of the scampi females had lost all their eggs or had them sampled by the time the eggs started hatching.

Table 3-2 Key measures of scampi egg development parameters for clutches of eggs raised at experimental temperatures 7, 9, 11 and 13 °C. The regression equation is based on Perkins development index (time in weeks and temperature) and the calculated extrapolated development time from extrusion to hatch is based on methods by Charmantier and Mounet-Guillaume (1992).

Temperature (°C)	**7**	**9**	**11**	**13**
Total eggs counted	845	825	605	440
Egg samples (n)	169	165	121	88
Scampi mortalities by week 7	3	1	2	1
No. of females that discarded/consumed all eggs before the end of week 7	0	1	2	8
Perkins eye index µm (S.D.)	375.5 (76)	423.2 (76)	455.2 (70)	423.2 (51)
Time taken from first eye pigment to first hatch (weeks)	19	19	14	12
Regression (time/eye size)	$y = 11.4x + 285$ $R^2 = 0.50$	$y = 14.9x + 320$ $R^2 = 0.70$	$y = 16.1x + 367$ $R^2 = 0.58$	$y = 15.5x + 357$ $R^2 = 0.59$
Estimated time to hatch from extrusion in weeks (days).	45 (318)	39 (272)	35 (244)	32 (226)

3.4.2 Embryo eye development

There was a higher attrition rate of eggs from clutches with higher treatment incubation temperatures resulting in decreasing numbers of eggs as the experiment progressed, and ultimately fewer eggs being carried to hatch (Table 3-2). The mean final embryo eye size (i.e., immediately pre-hatch) increased with increasing temperature from 7 to 11 °C and then declined at 13 °C when compared with 11 °C (Figure 3-3). The linear trend lines (Figure 3.4) for 7, 9, 11, and 13 °C have r^2 values of 0.50, 0.71, 0.59, 0.60, respectively.

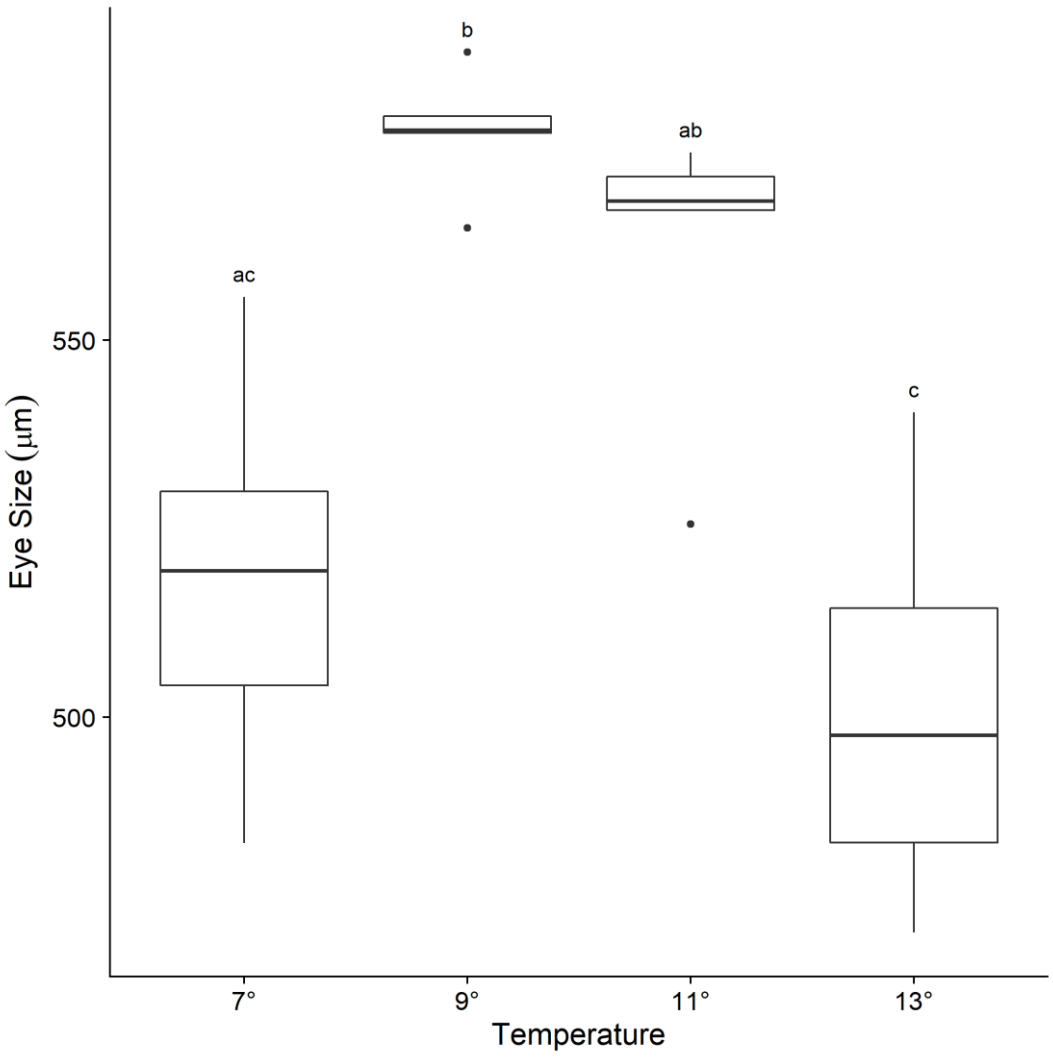


Figure 3.3 A boxplot of the embryo eye size data in the final week prior to hatch for clutches of scampi eggs raised at experimental temperatures 7, 9, 11 and 13 °C. Different letters indicate significant differences in eye size in the final week according to Dunnett tests (P <0.05).

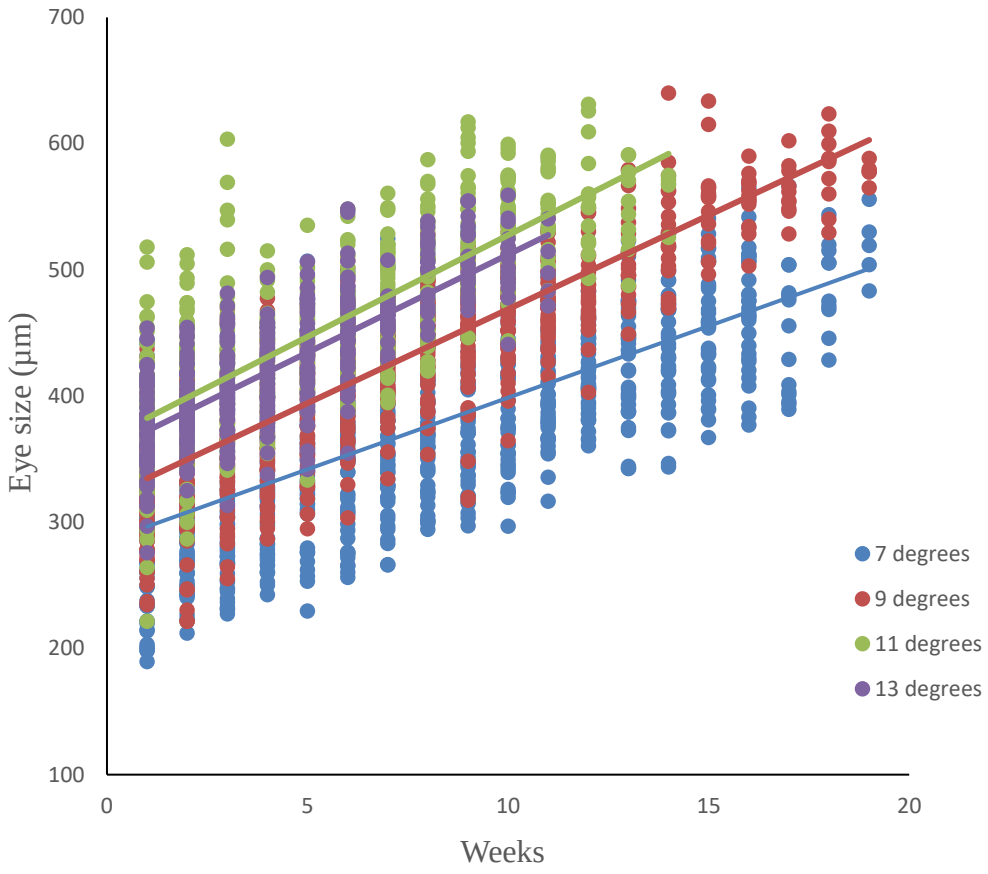


Figure 3.4 The change in eye size for scampi embryos, from first visible eye pigment to hatch, raised at experimental temperatures 7, 9, 11 and 13 °C. The plotted lines show the fitted trendline for each temperature.

The rate of development of scampi embryos at 7 °C as measured by eye size was significantly slower than at all other temperatures. The rate of embryo developmental at 9 °C was significantly slower than at both 11 and 13 °C (Figure 3-4).

The duration of development from the onset of the eye pigment to hatch was equal at 7 and 9 °C (19 weeks each) and then reduces at 11 and 13 °C to 14 weeks and 12 weeks, respectively (Table 3-3, Figure 3-5).

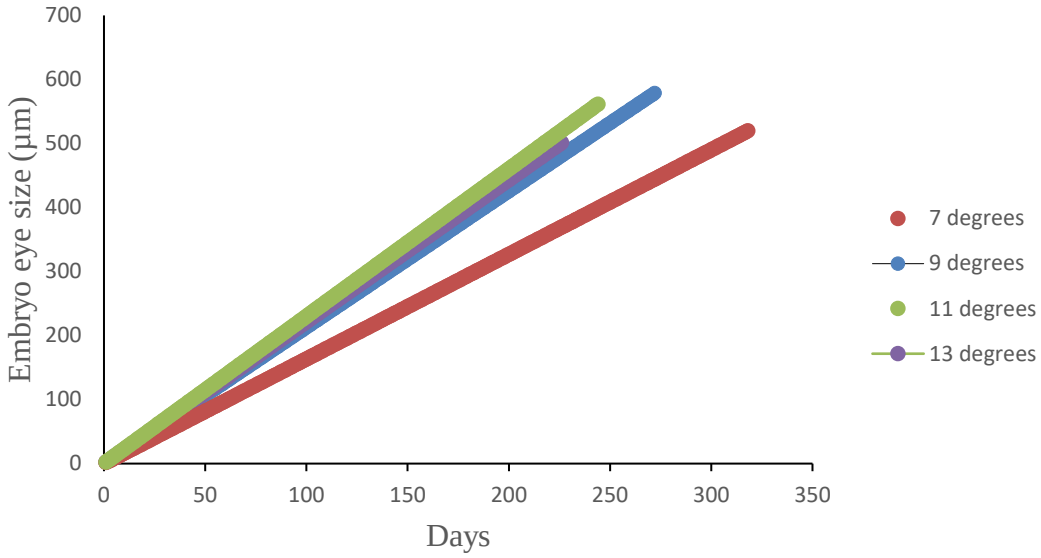


Figure 3.5 The size of the embryo eye of scampi reared at four different experimental temperatures and extrapolated from egg extrusion to time of hatch based on the method developed of Charmantier and Mounet-Guillaume (1992).

The embryo development from extrusion to hatch of *M. challengeri* is directly related to temperature according to the equation $y = 131.07x^{-055}$ $r^2 = 0.99$ (weeks) which is based on the general reptantian crustacean egg incubation equation derived from eight species (Figure 3-6) (Green et al., 2014).

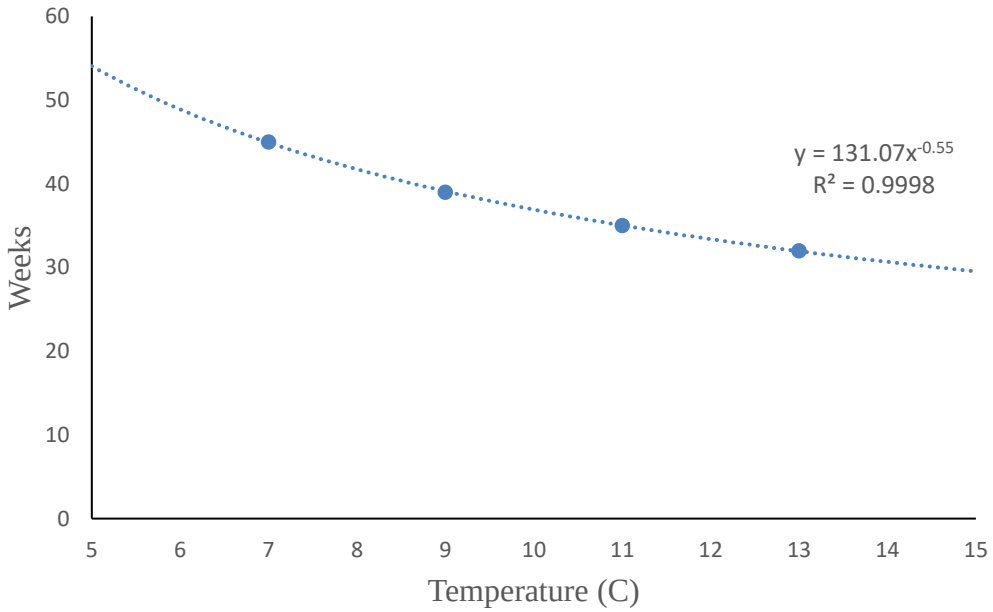


Figure 3.6 The estimated mean time taken for scampi embryos to develop from egg extrusion to hatch based on experimental rearing embryos at four experimental temperatures in this study.

3.4.3 The volume of the eggs raised in controlled temperatures

There is a high variation of egg volume within the same rearing temperature, and these variations appear to be mostly due to natural variation in egg size found in this species (Figure 3-6). Overall, the increase in the volume of *M. challengeri* eggs during development was greater with increasing experimental rearing temperatures (Figure 3-7). There is also an increase in volume of the eggs immediately prior to hatch regardless of rearing temperature (Figure 3-6). There was a significant difference in the increase in the volume of eggs over the measured period between all temperatures except 11 and 13 °C (Figure 3-7). The volume of eggs in the final week of development was not different for eggs at 9 and 11 °C, 11 and 13 °C and 7 and 13 °C (Figure 3-8). However, the final egg volume was different between 7 and 9 °C, between 7 and 11 °C and between 9 and 13 °C (Figure 3-8).

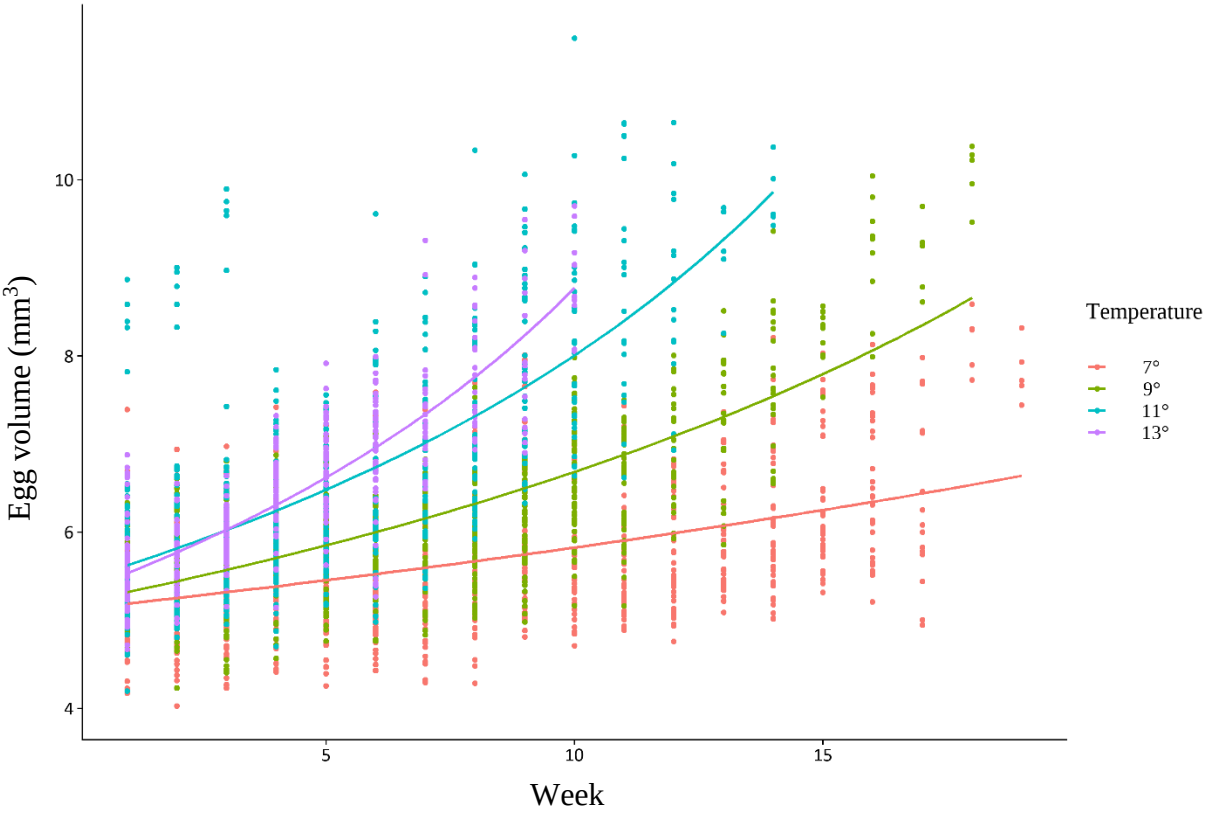


Figure 3.7 The volume of scampi eggs measured at weekly intervals, from first eye pigment development to hatch, when reared experimentally at different temperatures 7, 9, 11 and 13 °C. The plotted curves show the fitted trendline for each temperature. Orange = 7 °C, green = 9 °C, blue = 11 °C and purple = 13 °C

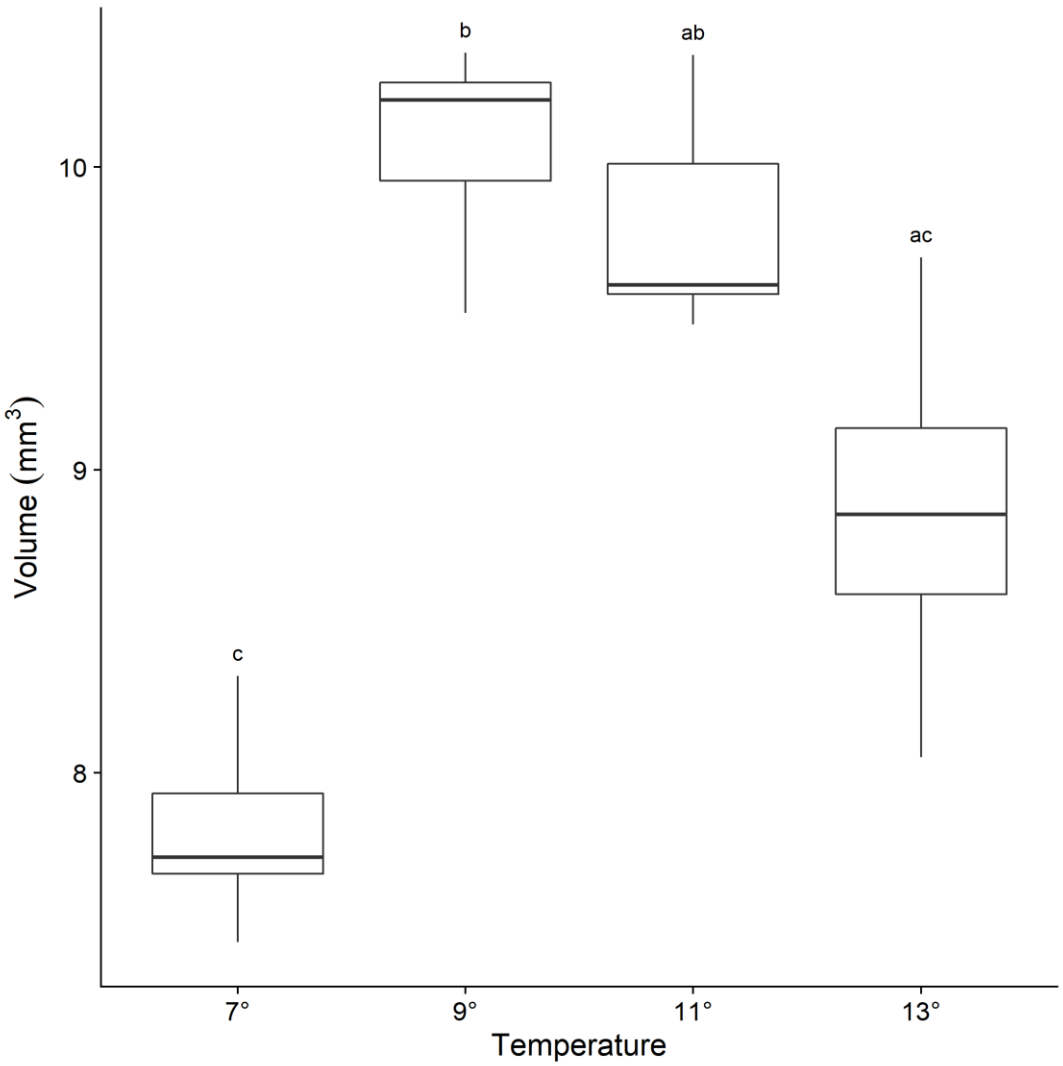


Figure 3.8 A box plot comparing scampi egg volume in the last week of development (pre-hatch) for eggs raised at four different experimental temperatures, 7, 9, 11 and 13 °C. Different letters indicate significant differences in eye size in the final week according to Dunnett tests (P <0.05).

3.4.4 Length and Volume of eggs collected from wild females (uncontrolled temperatures)

The mean length and volume of eggs sampled from wild scampi was found to increase with state of development (Table 3-3).

Table 3-3 The mean length (mm) and volume (mm^3) of wild scampi eggs at the various stages of embryo development.

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
n	105	42	70	39	183
Mean egg length mm (S.D.)	2.29 (0.10)	2.39 (0.11)	2.49 (0.13)	2.60 (0.08)	2.77 (0.16)
Mean egg volume mm^3 (S.D.)	5.30 (0.60)	5.87 (0.51)	6.54 (0.98)	7.32 (0.54)	8.41 (1.09)

3.5 Discussion

Perkins (1972) identified that the size of the eye of the embryo of the American lobster (*Homarus americanus*), increases uniformly from the onset of pigmentation to hatching, and that the rate of development increases in relation to temperature. Furthermore, the size of the eye and the volume of the eggs were found to increase with increasing temperature, while the time taken to hatch was reduced. Other studies on similar crustacean species have shown that the development of the embryo has a close relationship with temperature, e.g., *H. gammarus*, *Cancer setosus, Macrobrachium americanum*, *N. norvegicus* (Branford, 1978; Charmantier & Mounet-Guillaume, 1992; Fischer, 2009; García-Guerrero, 2010; Miller et al., 2016; Pisani & Greco, 2021; Smith, 1987; Yamamoto et al., 2021).

Hatch times from the current study were recorded from relatively small sample sizes of females brooding clutches, despite starting with 15 females for each of the four experimental temperatures. The small number of records of hatch time were the result of low numbers of females carrying eggs through to hatch due to egg loss through sampling, and from eggs being discarded or being eaten by the females particularly at the higher temperatures. This is likely to be a consequence of a stress response of the females from the weekly handling for egg sampling. It was observed that in other non-handled females held in the facility at 10 °C, the rate of eggs reaching hatch was routinely over 50% and as high as 76% (data not shown).

Eggs from the clutch of any one female were observed to hatch over a period of 4 to 20 days although the majority of eggs hatched within 4 to 10 days of the first egg hatching. The first hatches of eggs occurred up to a week apart for clutches of eggs belonging to different females that had started the experiment at a similar early egg development stage and were held at the same temperature in the current study. It is uncertain if this is an artifact of the culture activity or natural variation in development times among individuals. Furthermore, since the precise egg extrusion date was unknown the time to hatch may have been less certain.

The embryo eye development in scampi follows the Perkins formula in that the mean eye size of the embryos increased with increasing rearing temperature ranging from 7 – 13 °C. This suggests that the larvae are correspondingly increasing in size with temperature (Clarke, 1992; Herring, 1974). This is supported by the egg volume data which increased with increasing temperature except for 11 and 13 °C which were not significantly different from one another.

Changes in the scampi embryo eye size at different temperatures provided estimates of periods from extrusion to hatch in scampi (as per Charmantier & Mounet-Guillaume (1992)). The estimated mean time periods taken for the embryos to complete incubation follows the equation $y = 131.07x-0.55$ $r^2 = 0.9998$ (Figure 3-6) with a range of 318 days (45 weeks) at 7 °C to 226 days (32 weeks) at 13 °C. Egg incubation periods have been shown to decrease with increasing temperature across a range of reptantian crustacean species, which has been described by the equation $y = 0.5906x^2 - 29.725x + 429.09$, $r^2 = 0.862$ (Green et al., (2014) (Figure 3-8). Egg incubation in *M. challengeri* follows a similar trend to these other crustacean species over the experimental temperature range in this study (7 to 13 °C). However, egg development times for scampi are longer at all temperatures (Figure 3-9). A trend of faster embryo development with increasing temperature has also been observed in other crustacean species, such as *H. gammarus*, *H. americanus*, *Cercopagis pengoi*, and *Cancer setosus* (Charmantier & Mounet-Guillaume, 1992; Fischer, 2009; Gendron & Ouellet, 2009; Sopanen, 2008; Steele & Steele, 1975), but it appears that if the temperature approaches the upper limits for the species there can be a cost in terms of metabolic inefficiency and no further corresponding increases in the rate of embryo development (Heming, 1982; Pandian, 1994). In this study, the number of eggs being successfully carried by the females to hatching decreased with increasing temperature and was most reduced at 13 °C, suggesting that *M. challengeri* may be reaching an upper thermal limit (Table 3-2). This is supported by an additional study that showed that the metabolism of adult *M. challengeri* appears to plateau at 13 °C and a temperature of 15 °C was also found to be lethal in this study (see Chapter 5). Thus, the temperature tolerance range for *M. challengeri* appears to be relatively narrow, most likely corresponding to the stable thermal deep-water environment in which this species resides. The narrow range and high sensitivity to temperatures at the margins of this range would make this species less resilient to fluctuating aquaculture conditions and is likely to make this species susceptible to future climate change at cooler latitudes.

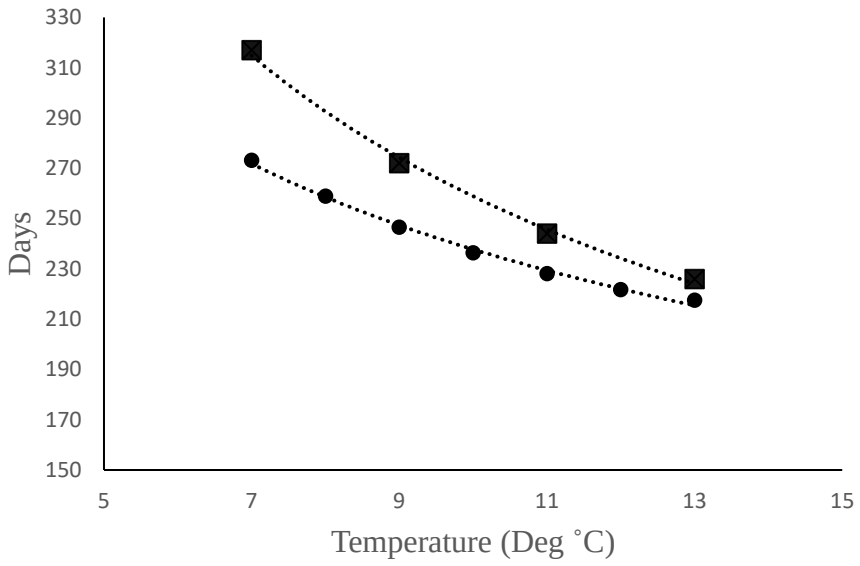


Figure 3.9 The embryo development time taken (in days) in relation to temperature for *M. challengeri* (■) and the general reptantian crustacean egg incubation equation based on eight species (●) according to Green et al. (2014).

The length and volume of the scampi eggs increase with development of the embryo as has been observed in other species of decapods (Bakır, 2009; Smith, 1987). The rate of egg volume increase over the course of development was lowest at 7 °C and increased with rising temperature up to 13 °C. The wild egg mean volumes at hatch were seen to fit with the range found in the experimental study (Table 3-3).

The increasing egg length and volume with rising temperature suggests an increasing moisture content of the eggs, particularly immediately prior to hatching as found in other crustacea eggs (Figueiredo & Narciso, 2008; Pandian, 1970a, 1970b; Powell & Eriksson, 2013)(Figure 3-7). Charmantier and Aiken (1987) suggests that there are three patterns of ontogeny of osmoregulation in crustacean embryos: pattern 1, osmoregulation varies little (adults are generally marine and stenohaline osmoconformers); pattern 2, are generally euryhaline and freshwater, and pattern 3, larvae osmoconform (adults are mesohaline or euryhaline). The results from the current study suggest New Zealand scampi follow pattern 1. The egg membranes of *H. americanus, H. gammarus* have been shown to increase permeability to water and metals just prior to hatching (Pandian, 1970a, 1970b). Hendrick and Papiol (2019), working on the squat lobster, *Galacantha diomedeae*, which inhabits deep, cold water ocean areas, found that there was a small increase in egg size with embryo development which indicates that even at great depths eggs may still show increased osmolarity assisting or causing the egg to hatch.

The use of the Perkins eye index and volume assessments at different temperatures has provided useful information on embryo development in scampi. Rearing temperature can be used to manipulate the development times of scampi embryos, which is a useful attribute in aquaculture for adjusting hatch times to suit the availability of hatchery facilities. Furthermore, elevated rearing temperatures would help to reduce development time in this species, reducing the incubation time by nearly a third (i.e., 32 versus 45 weeks for 13 and 7 °C, respectively). There is some indication that the embryos may hatch larvae of larger size when incubated at higher temperatures, but they may ultimately be of poorer quality because of faster metabolic consumption of egg reserves. However, egg losses increased with increasing incubation temperature, which would be a significant drawback to using elevated temperatures to artificially truncate incubation times, especially as the clutch sizes of scampi are small (Chapter 2).

The data provides a means of establishing an egg rearing temperature which can balance the reduction in the time taken from egg extrusion to hatch versus the extent of egg mortality experienced by using increasing incubation temperatures. The temperature suggested to provide this balance for optimal aquaculture production would be between 10 and 11 °C, vis 10.5 °C. This would suggest a time to hatch of 36 weeks with low mortality and a large volume and eye size suggesting a larger, more robust larvae.

Chapter 4 : The larval development of the New Zealand deepwater scampi, *Metanephrops challengeri* (Balss, 1914) (Decapoda: Nephropidae).

4.1 Introduction

Relatively little is known about the reproductive and larval biology of the New Zealand scampi, *Metanephrops challengeri*, or for most of its congeners. Of the 18 extant *Metanephrops* species (Chan, Ho, Li & Chu, 2009) only five, *M. japonicus* (Tapparone Canefri, 1873), *M. thomsoni* (Bate, 1888), *M. andamanicus* (Wood-Mason, 1892), *M. sagamiensis* Parisi, 1917), *and M. challengeri* (Balss, 1914), have had their larval development described, in part or in full, based on larval rearing in captivity (Berry, 1969; Iwata et al., 1992; Okamoto, 2008; Uchida & Dotsu, 1973). The eggs of wild captured adult females were hatched in laboratories from late-stage embryos of these five species. Some assumptions of the larval stages of these five species have been made by researchers as not all the larvae of the various species were reared successfully through to a first instar juvenile. The larval durations of the *M. japonicus, M. thomsoni, M. sagamiensis, and M. challengeri* were found to have, or thought to have an abbreviated pelagic larval development which lasted 4 – 9 days (Anger, 2001). *Metanephrops andamanicus* larvae were not raised through all larval stages and assumptions have been made regarding the larval development. It is assumed that since the biology of *M. andamanicus* is similar to the other four species, then it also has an abbreviated larval development period. Larval development has been shown to be temperature dependant in the Nephropidae (Ennis, 1995; Quinn & Rochette, 2015) so variations in larval durations are likely to occur in relation to ambient temperatures.

One previous attempt has been made to rear *M. challengeri* larvae in captivity by hatching eggs and only achieving raising them until they reached a first, free-swimming zoeal stage (Wear, 1976).

Given the depths and offshore distances of *M. challengeri* habitats, the collection of wild larvae for determination of larval development is impractical. Therefore, the aim of this study was to rear and describe the complete larval development of *M. challengeri* through to the settled benthic juvenile (post-larvae) by hatching and culturing eggs from clutches on captured wild female scampi.

4.2 Material and Methods

4.2.1 Nomenclature

The nomenclature for the various developmental stages of members of the Family Nephropidae, from embryo to early juvenile is contentious (Anger, 2001; Farmer, 1974; Goy, 2014; Okamoto, 2008; Powell & Eriksson, 2013; Rotllant et al., 2001). The identification of some developmental stages are further confused by the use of behavioural criteria, requiring observations of live animals to ascertain that zoea utilise thoracic appendages to swim while the megalopa use abdominal appendages to swim (Felder et al., 2017; Gurney, 1942).

To avoid confusion the larval terminology of Powell and Eriksson (2013), is adopted for this study, i.e., in order of occurrence in scampi development: egg; pre-zoea; stage I zoea; stage II zoea; and post-larva. For referring to all the zoea stages collectively the term “larvae” is used.

Some studies consider the pre-zoea to be essentially an extension of the embryonic phase. For the purpose of the current study it is accepted that this is appropriate in the case of *M. challengeri* as this stage is described and discussed as a pre-zoea in the *Metanephrops* literature.

4.2.2 Measurements

The following morphometric measurements of larvae were taken using a digital micrometer on a binocular microscope:

1. Total length (TL) of the whole larva is measured from tip of rostrum to rearmost margin of telson.
2. Total length of appendages includes the basal endite to the terminal end of the appendage excluding exopods.
3. The Orbital Carapace Length (OCL) is the distance from the posterior margin of the orbit to the dorso-mesial (posterior) margin of carapace.

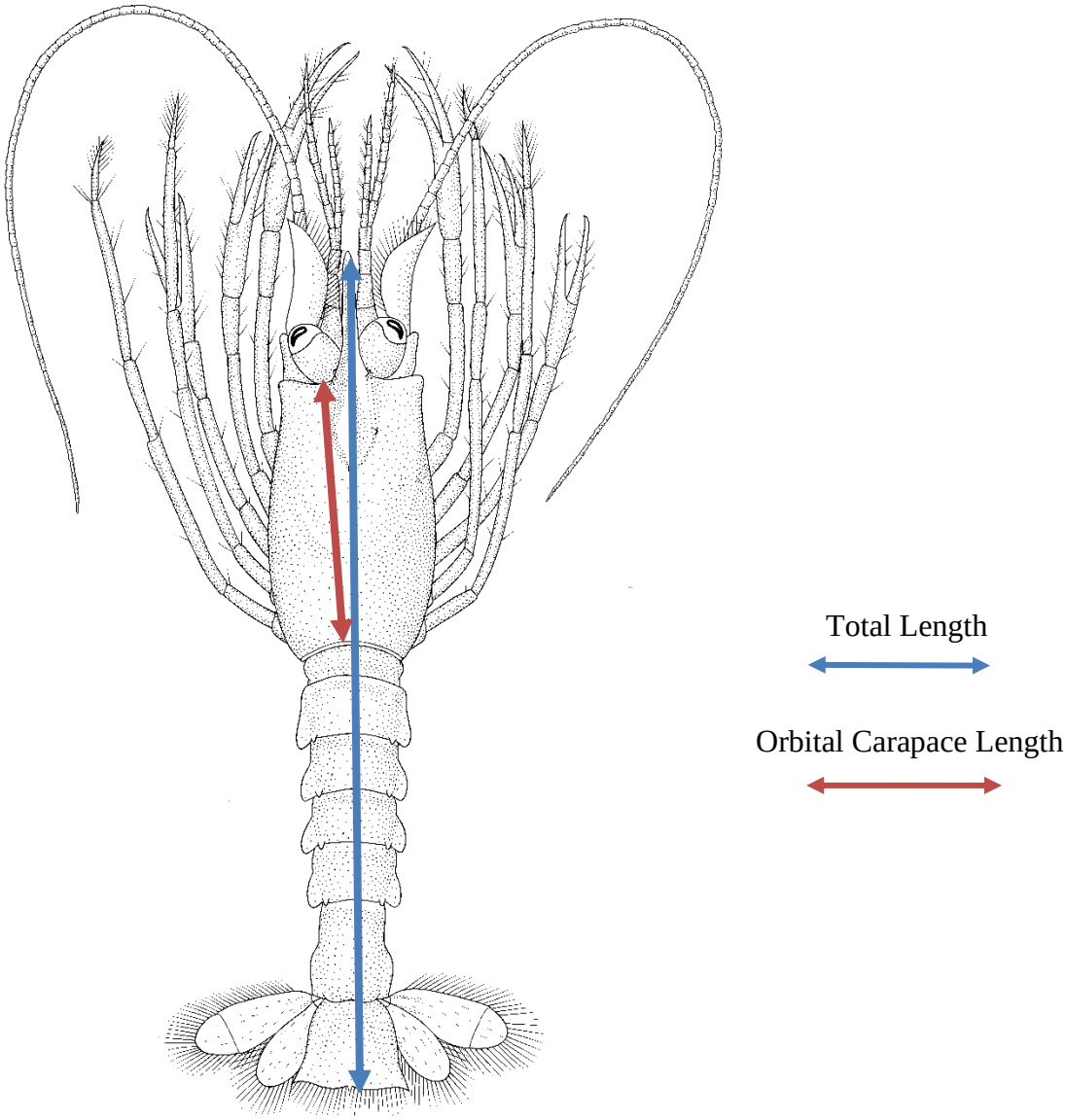


Figure 4.1 Figure showing the measurement of the orbital carapace length (OCL) and the total length in scampi developmental stages.

4.2.3 Collection of ovigerous females

Live ovigerous adult female scampi (OCL = 35 – 52 mm) were collected from 2014 to 2016 using bottom trawling at a depth of around 350 m on the Chatham Rise, some 280 km off the east coast of the South Island of New Zealand (within 100 km of 42.924394° S, 177.260249° E). During each fishing trip, up to 75 lively, ovigerous females were taken from the trawl net and placed in lidded plastic holding trays (80 × 45 × 12 cm), with all surfaces perforated including the lid, to allow water flow. The trays were stacked inside a 1,000 l insulated

transport bin containing seawater which had an insulated, light-tight lid. The seawater in the insulated bin was circulated and chilled to the ambient temperature from which the adult scampi were captured (i.e., 8 - 9 °C) and aeration used to maintain dissolved oxygen. A third of the seawater was replaced every 12 h during the subsequent 48 h vessel trip back to port, where the insulated container was lifted onto a truck and driven to the Cawthron Aquaculture Park (CAP) near Nelson, New Zealand.

4.2.4 Holding ovigerous females

On arrival at the research facility, the scampi were transferred into a purpose-built holding system which provided high quality recirculated seawater at a constant 10.5 °C. Potentially damaging ammonia, nitrite and nitrate compounds in the water were kept at trace levels and carbonate levels were maintained by dosing with sodium carbonate. Dissolved oxygen was always maintained above 98% through continuous water exchange and aeration. The animals were fed a mix of pellets (Prochete™) and fresh fish tissue. Dim red (915 nm) LED lighting was provided at the research facility on a 12 h on, 12 h off regime to allow staff to work in the facility. It is believed that this light would not interfere with the scampi, as decapod crustaceans are thought to be generally insensitive to red light above 915 nm (Meyer-Rochow, 1994).

The female scampi were housed individually in plastic trays (40 × 15 × 20 cm) to avoid any stress or damage to broods of eggs that might be caused by agnostic interactions. Clean seawater was introduced from one end of the scampi holding tank (at 0.6 l m^{-1} sec) and exited through holes in the floor of the tank to create a down-current designed to remove the larvae from the chamber. This was done to avoid females consuming their hatched larvae and to assist in the collection of hatching larvae. The exiting water from the trays flowed slowly through a screened (1 mm mesh) larval catching sump where they could be recovered and transferred to larval rearing units.

4.2.5 Larval rearing

Larvae were cultured in modified kreisel tanks (Serfling et al., 1974). Initial observations found the scampi larvae were susceptible to shear forces resulting from water injected into prototype kreisel tank upwelling units that were intended to encourage suspension of the larvae in the water column. The acrylic kreisel tanks were thus modified to reduce the shear forces of the introduced water. The tanks were supplied with UV sterilised, 10 µm filtered

seawater at 10.5 °C. The water was introduced at a rate of 0.3 - 0.5 l min^{-1} across the width of each tank and was drained from the centre of each tank encouraging a flow of water from the kreisel tank walls to the centre of the tank. This arrangement created an even, circulating flow of water that maintained larvae in suspension. Larvae were susceptible to fungal and bacterial infection, and therefore the water in the culture system was subjected to a strict ozonation and U/V treatment regimen.

4.2.6 Post-larval holding

Post-larvae were initially held in tanks with a sandy bottom (sand grains from 250 - 750 µm). Later the post-larvae were held in Aquahive™ trays and then in custom built rearing chambers. Post-larvae were fed prepared pellets (ProChaete™).

4.2.7 Larval feeding

Pre-zoea and stage I zoea are lecithotrophic. Stage II zoea were fed live or frozen cultured brine shrimp stage II nauplii (*Artemia salina)* (from Brine Shrimp Direct, USA) enriched with lipids (Selco®) and rotifers (*Brachionus plicatus*). The addition of cultured microalgae into the water of the stage II larvae increased the appetite and survival of the larvae significantly.

4.2.8 Larvae preservation and analysis

Samples of several scampi zoea at different stages of development were euthanized and preserved in 10% formaldehyde in buffered seawater. The various appendages were then dissected out under a dissecting microscope and photographed for morphological examination and measurement.

4.3 Results

4.3.1 Hatching and larval production

Larvae generally hatched at night. The pre-zoea stage of healthy larvae was short-lived, moulting to a stage I zoea within minutes to hours. The pre-zoea did not swim as they had no setae on the exopods of the pereiopods (a defining character of the pre-zoea stage) (Fig. 4-2). The stage I zoea, which bear natatory setae on the exopods of the pereiopods, were predominantly pelagic, slowly swimming forward and upwards or maintaining position in the

water column by the continuous beating of the pereiopodal exopods. It took a mean of 5.8 days (SD ± 3.2, n =10) for the stage I zoea to moult to stage II zoea. The stage II zoea were primarily pelagic, only descending to the floor of kreisel tanks periodically.
The moult to the first post-larval stage took place at 8.3 days (SD ± 1.0, n = 10) after hatching.

4.4 Morphological descriptions of larval and post-larval stages

4.4.1 Pre-zoea stage (Fig 4-2)

The pre-zoea is the cuticle-covered zoea larva as it hatches from the egg, in many decapod species. In *M. challengeri* when the pre-zoea hatches, it is covered by extremely thin, transparent cuticle that lacks any vestige of functional appendages. In this state the larva does not appear to undertake any activity other than movements to shed the pre-zoeal cuticle. The cuticle is shed within minutes to hours and the larva then assumes the active form of the first zoea. As a pre-zoea its whole body is contained within the pre-zoeal cuticle, with the body and appendages variously folded and compressed. The description of the pre-zoea is therefore brief but includes observations on its shape and appendages that can be compared to the first zoea. The description of the first zoea, which displays the morphology of the larva in its functional form and proportions, is more detailed.

In the rearing experiments, the pre-zoea of scampi were 11.1 mm (SE ± 0.7, n = 10) TL (Fig. 4-2). They were transparent with an obvious orange coloured hepatopancreas visible within the cephalothorax and residual blue coloured yolk visible in two anteriolateral lobes forward of, and on either side, of the hepatopancreas, when viewed dorsally. The rostrum of the pre-zoea stage was 75 % of or equal in length to the carapace; with one or more pairs of subterminal asymmetrical spines. The orbital carapace length (OCL) of the pre-zoea was 2.5 mm. The carapace had a pitted surface with minimal relief. There is a forward projecting spine of 610 µm length laterally and slightly posterior to the orbital margin. The eye is oval with visible pigment and measures 530 × 422 µm in size. The first antennal scale is 1.3 mm in length with 14 setae increasing in size (53 – 250 µm long) in a row on the inner margin of the peduncle. There are five pairs of plumose setae (260 µm) on the inner surface of flagella. The second antennal scale is 2.8 mm in length. The exopod is 1.7 mm length. It is a dorsoventrally flattened antennal scale with 23-25 plumose setae (100 – 343 µm) (absent or reduced on rare occasions)

on distal two thirds of the inner margin. Each seta was covered with numerous fine hairs (up to 40 µm) on the dorsoventral plane.

The first maxilla has 9 to 11 teeth on the endite. The second maxilla is flattened with numerous plumose setae on the outer edge of all the lobes, and each seta has abundant fine hairs on the dorsoventral plane. The first maxilliped exopod is up to 1.6 mm in length with four plumose setae (200 µm to 1.1 mm). Each seta has numerous fine hairs (up to 35 µm in length) on the dorsoventral plane. The second maxilliped endopod is segmented with small setae at the terminal end. The exopod is up to 1.7 mm in length with four setae (ranging from 500 µm to 1.3 mm in length). Each seta has numerous fine hairs on the dorsoventral plane. The third maxilliped endopod is segmented with small setae at the terminal end. The exopod is 1.7 mm in length with four setae (600 µm to 1.2 mm). Each seta has numerous fine hairs on the dorsoventral plane.

The first pereiopod chelate is up to 2.7 mm TL with the associated exopod being plain with no setae. The second pereiopod chelate is up to 2.7 mm TL with the associated exopod being plain with no setae. The third pereiopod chelate is up to 3 mm TL with the associated exopod plain with no setae. The fourth pereiopod is non-chelate, up to 3.2 mm TL with the associated exopod plain with no setae. Fifth pereiopod is non-chelate, up to 3.3 mm TL with the associated exopod plain with no setae.

The pleon is transparent and 7 mm in length and the somites of the pleon are distinct. There is a single mid dorsal spine on the posterior margin or tergite. There are two lateral spines on each side of the posterior margin of the 2nd to 5th pleura. The 6th somite is elongate with no spines. While the 6th somite and telson combined is described as “the telson” by (Wear, 1976), other morphological descriptions of decapods (Berry, 1969) describe a 6th somite and telson separately. In this instance the latter terminology is used in this current study.

The pleopods are biramous, and plain without setae. The anterior pleopod is 570 µm in length and the posterior pleopod is 600 µm in length.

The telson is triangular with a prominent single central spine on the rear-most margin with three to five spines extending posterolaterally from the terminal lateral corners. The dorsoventral plain has 16 to 18 intermediate spines (150 – 190 µm) located between the corners and the central spine. Fine hairs are randomly distributed over the dorsal surface of the telson. Developing uropod endopodites and exopodites are visible within the telson.

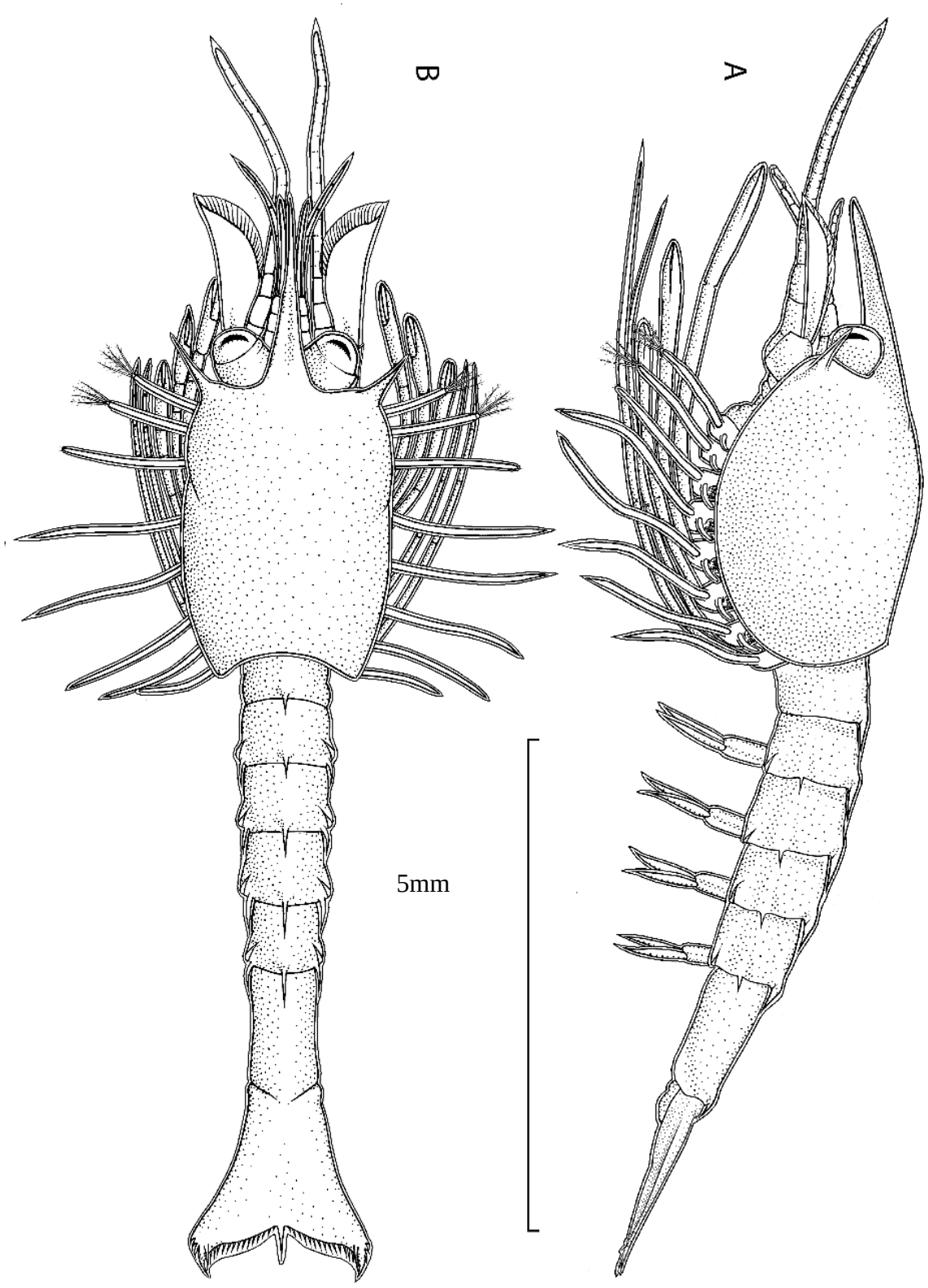


Figure 4.2 Pre-zoea of *M. challengeri*, whole animal, a lateral view, b dorsal view (Drawing: R. Webber).

4.4.2 Stage I Zoea (Figs 4-3 – 4-5)

The stage 1 zoea of *M. challengeri* (Figs. 4.3 & 4.4) is 11.3 mm (S.E ± 0.80, n = 10) TL and transparent with an obvious orange hepatopancreas visible within the cephalothorax. There are two residual deposits of blue yolk visible anterolaterally to the hepatopancreas when viewed dorsally. The OCL was 2.6 mm (S.E. ± 0.14, n = 10) and the length of the rostrum was 2.3 mm. The dorsal surface of the rostrum forms a V-shaped trough that runs posteriorly from the rostrum tip to between the eyes. The trough thus lies between two ridges that converge at the rostrum tip, with about 12-15 small spines irregularly distributed along each of the two ridges. The eyes are oval, and approximately 550 × 450 µm and dark pigment is visible within each eye. Carapace with an anterodorsally projecting 550 µm long spine positioned lateral and posterior to the orbital margin. The carapace is otherwise unarmed.

The first antenna (antennule) (Figs 4-3; 4-5a) is up to 2.6 mm long with setae increasing in length from 60 – 300 µm in a row on the inner margin of the peduncle and five pairs of plumose setae each up to 260 µm on inner surface of the flagellum with fine hairs on the setae surfaces (Fig. 4-5a). The second antenna is up to 5.2 mm in length (Figs 4-3; 4-5b) and its exopod (antennal scale) is 1.7 mm long and dorsoventrally flattened with 23 to 29 plumose setae on the distal 2/3rds of its inner margin, each seta having numerous fine hairs (up to 40 µm long) in the dorsoventral plane (Fig 4-5b).

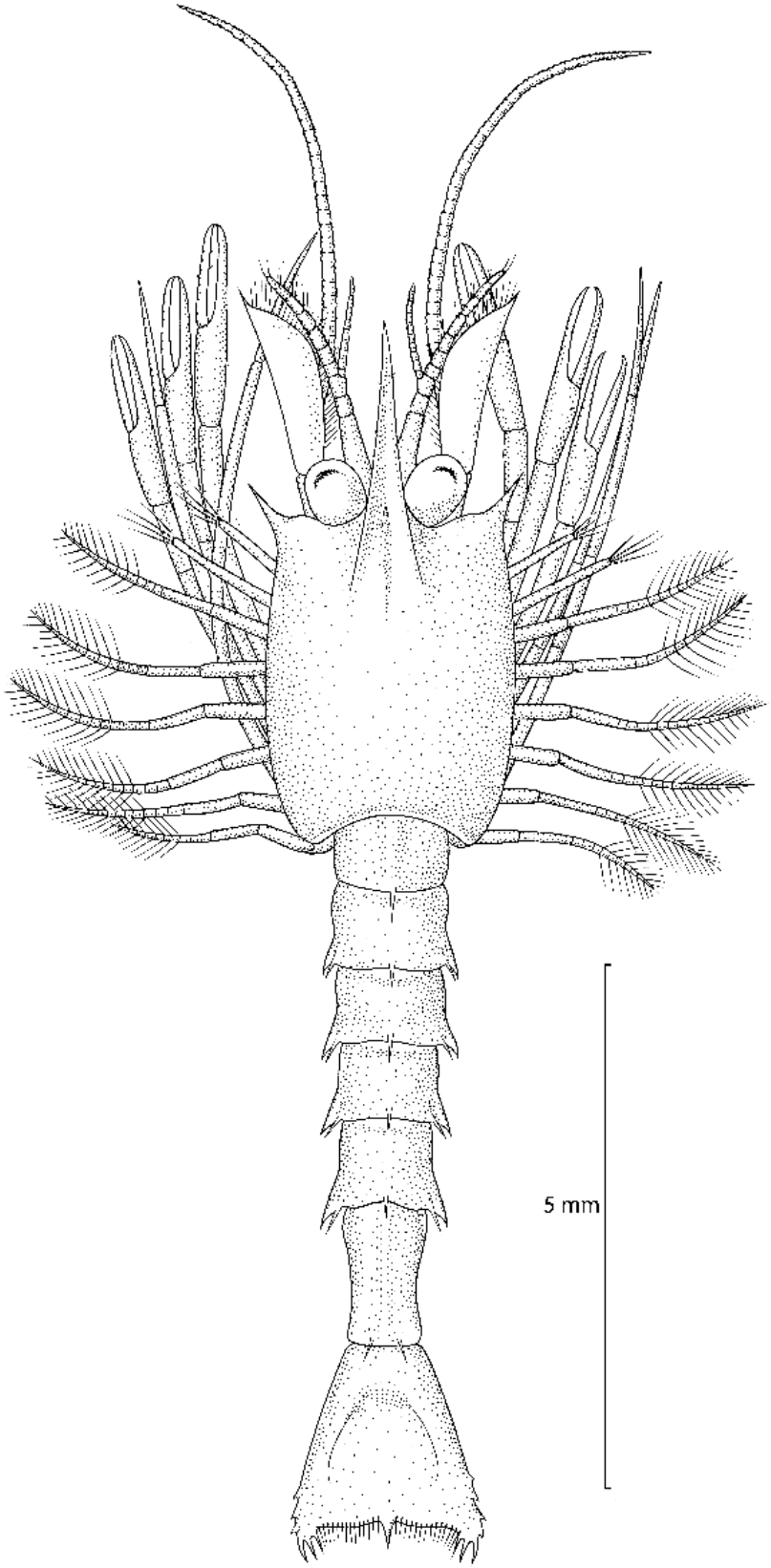


Figure 4.3 Stage 1 zoea of *M. challengeri*, whole animal, dorsal view (Drawing: R. Webber).

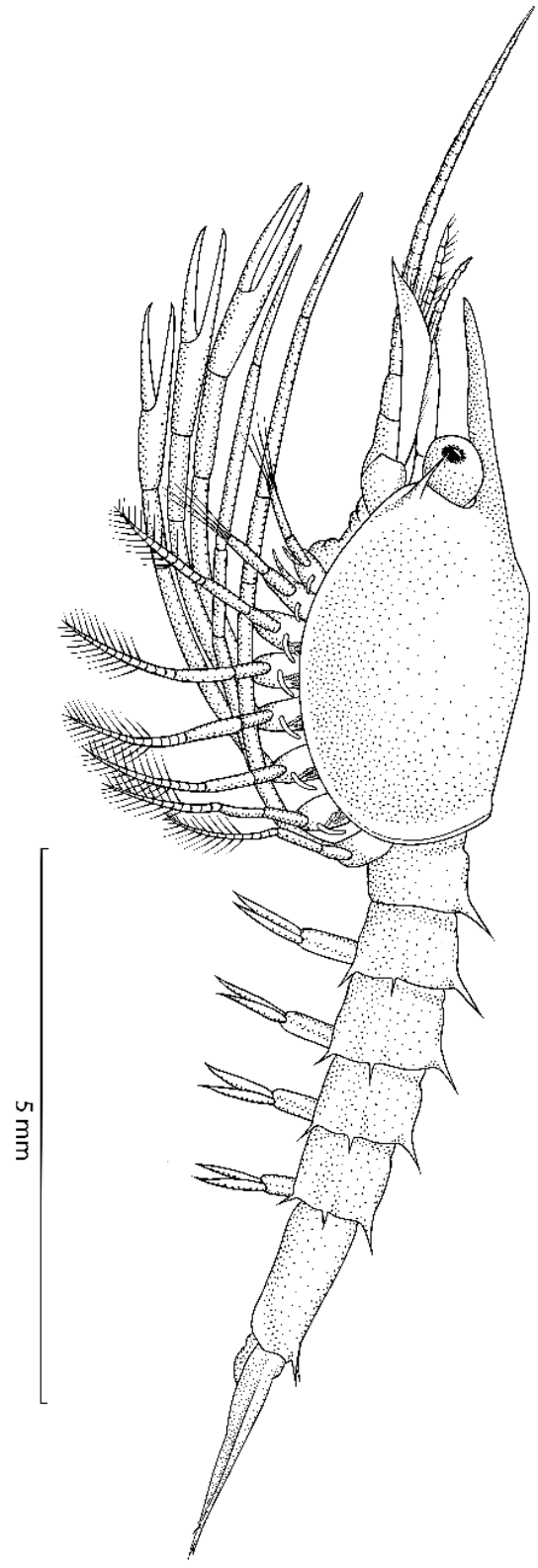


Figure 4.4 Stage 1 zoea of *M. challengeri* whole animal, lateral view (Drawing: R. Webber).

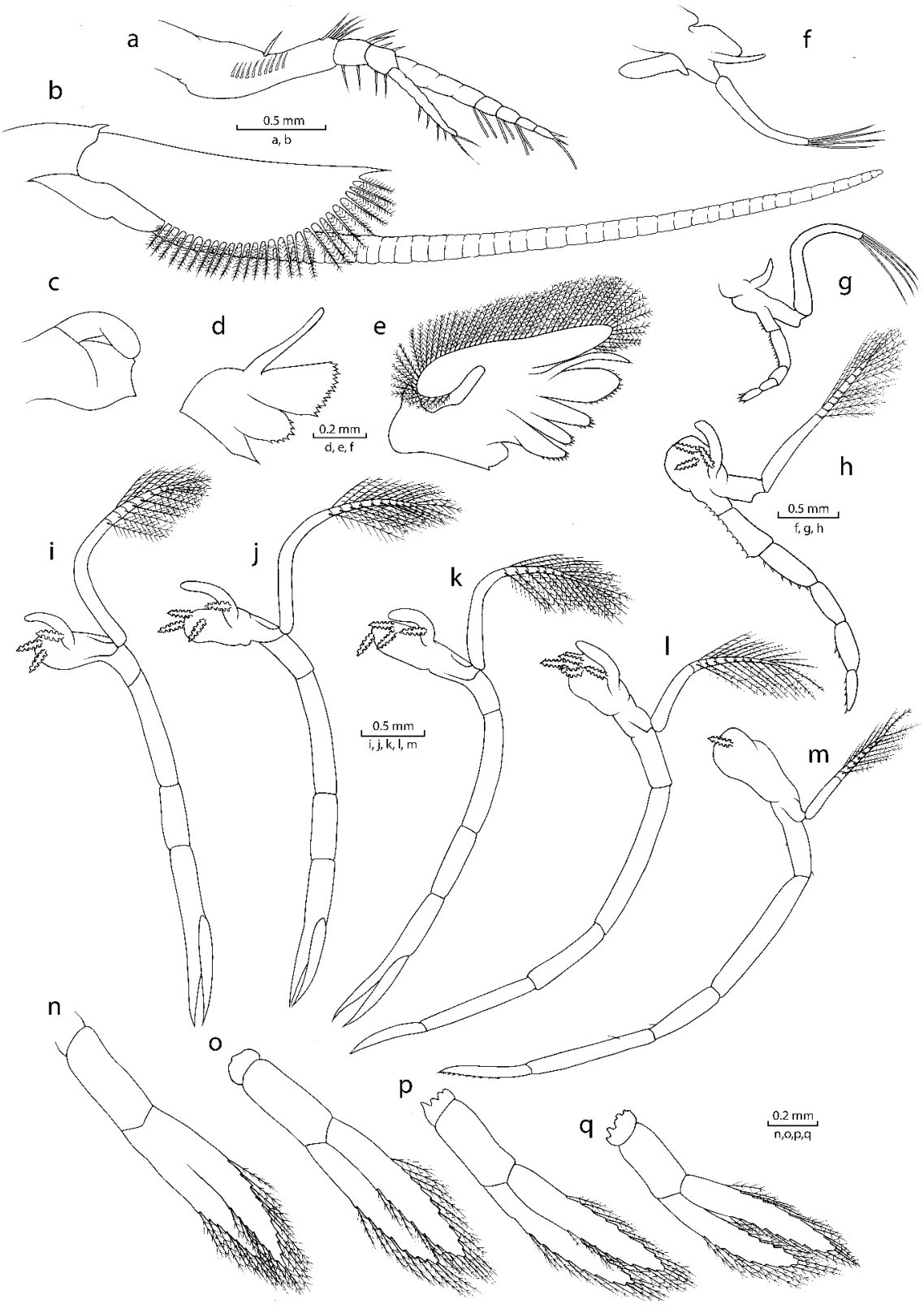


Figure 4.5 Appendages of stage 1 zoea of *M. challengeri*; a) antennule, b) antenna, c) mandible, d) maxilla 1, e) maxilla 2, f) maxilliped 1, g) maxilliped 2, h) maxilliped 3, i) pereiopod 1, j) pereiopod 2, k) pereiopod 3, l) pereiopod 4, m) pereiopod 5, n) pleopod 1, o) pleopod 2, p) pleopod 3, q) pleopod 4. (Drawing: R. Webber).

The mandible (Fig. 4-5c) has an unarmed endopod, incompletely jointed at its base but otherwise unjointed, with the molar process square-shaped with two corner-like projections but otherwise unarmed. The first maxilla (Fig. 4-5d) has approximately seven small, strong teeth on the coxal endite, 11 to 16 similar teeth on the basal endite. The endopod is unsegmented and unarmed and the second maxilla (Fig. 4-5e) is flattened with numerous small spines around the tips of the coxal and basal endite lobes, with a narrow endopod that is unarmed and a scaphognathite fringed by a close-set row of many plumose setae.

The exopod of the first maxilliped (Fig 4-5f) is armed with four long, unarmed setae at its tip and the endopod is small and simple with no setae. The exopod of the second maxilliped (Fig. 4-5g) bears four long, unarmed setae at its tip, while the endopod has four to five-segments with the mesial margin of the proximal segment bearing a short row of spines. The spine-row continues along the mesial margin of the following segment while the last three segments bear a few scattered small setae and several tiny, spine-like setae are present around the tip of the terminal segment. The third maxilliped (Fig. 4-5h) is up to 1.4 mm in length, consisting of a coxa/ basis, and an epipod, exopod and endopod with three gill buds present, and two arthrobranchs on the coxa/ basis and a podobranch on the epipod. The exopod has four plumose 'natant' setae at its tip, similar to, but somewhat smaller than, those of the pereiopods posterior to it. The endopod has five-segments with a coxa/ basis with a short mesial row of spines and a row of similar close-set spines continuing along the mesial margin of the next segment (ischium). The merus has a row of more widely spaced small spines mesially. The distal three segments (carpus, propodus, dactylus) are without spine rows but scattered small spines are present, particularly on the dactylus.

The five pereiopods (Fig. 4-5i-m) each consist of a coxa/ basis bearing an epipod an exopod and an endopod. All five pereiopods (Fig. 4-5i-m) also bear gill buds as follows: P1 (Fig. 4-5i) 2 arthrobranchs, 1 podobranch; P2 - P4 (Figs 4-5j – 4-5l) 1 pleurobranch, 2 arthrobranchs, 1 podobranch; P5 (Fig. 4-5m) 1 pleurobranch only.

The exopods of all five pereiopods are prominent, each bearing plumose natatory setae distally, with the numerous fine setules on each natatory seta being up to 35 µm in length and attached along the dorsoventral plane. The endopod of pereiopod 1 is chelate, and up to 3.7 mm TL and the exopod is 2.2 mm TL and bears 17 - 19 setae, each up to 550 µm long. The second pereiopod is also chelate and up to 3.7 mm TL, with an exopod of 2.3 mm TL, which hold 17 to 19 setae of up to 580 µm in length. The third pereiopod is chelate and up to 3.8 mm TL, with

an exopod of up to 2.1 mm TL, giving rise to 17 to 19 setae of up to 630 µm in length. The fourth pereiopod is not chelate and is up to 4.6 mm TL, with an exopod of 1.6 mm TL and giving rise to 13 to 16 setae up to 520 µm long. The fifth pereiopod is not chelate and is the longest at up to 4.7 mm TL, with an exopod up to 1.2 mm long, bearing 9 to 15 plumose setae of 350 µm in length.

The pleon (Fig. 4-4) is transparent and up to 7 mm in length. The somites of the pleon are distinct, with a single mid dorsal spine on the posterior margin or tergites 1-5, and two lateral spines on each side of the posterior margin of the 2nd to 5th pleura. The 6th somite is elongate with two dorsal spines.

The pleopods are biramous, with 18 setae of the next stage projecting from the biramous appendage (Fig. 4-5n–q). The anterior pleopod is 1.2 mm in length, while the hindmost pleopod is 980 µm long.

The telson (Fig. 4-3) is triangular with a prominent single central spine on the posterior margin and with up to seven spines extending posterolaterally from its lateral corners. A total of 16 to 18 intermediate setae of up to 260 µm in length are present on the posterior margin between the lateral corners and central spine, with these setae bearing numerous fine setules on the dorsoventral plane. Small setae are randomly distributed over the dorsal surface of the telson. Developing endopodites and exopodites are visible within the telson.

4.4.3 Stage II Zoea (Figs 4-6 – 4-8)

The description of the stage II zoea focuses mainly on changes that have occurred with the moult from Stage I to Stage II. These are not metamorphic changes, but they mainly involve increases in size, the addition of setae and spines and additional jointing in some appendages.

Zoea II (Fig. 4-6 & 4-7) are 11.76 mm (S.E ± 0.51, n = 10) TL and are transparent with an obvious orange hepatopancreas visible within the cephalothorax. The OCL is 2.92 mm (S.E ± 0.10, n = 10). The rostrum is up to 2.7 mm in length and is troughed dorsally and has up to 18 randomly spaced small spines along the dorsolateral crests. The eyes are 580 × 520 µm in size. The long spine positioned lateral and posterior to the orbital margin is 550 µm long. The first antenna is up to 2.6 mm in length with setae increasing in size from 60 – 300 µm in a row on the inner margin of the peduncle and five pairs of plumose setae each up to 350 µm in length on the inner surface of the minor ramus setae (Fig. 4-8a). The second antenna is up to 6.8 mm

in length (Fig. 4-8a). The antennal scale is 1.8 mm long with 26 to 31 plumose setae on the distal 2/3rds of the inner margin, with its fine setules up to 40 µm long.

The mandibular palp (Fig. 4-8b) has a single segment at its tip and its anteriolateral margin bears about 10 small spines. The first maxilla (Fig. 4-8c) has 17 to 21 teeth on the basal endite. The second maxilla (Fig. 4-8d) is similar to that of stage 1 but larger with a small seta at the tip of its endopod and many more plumose setae fringing the scaphognathite. The first maxilliped endopod (Fig. 4-8e) is unsegmented with a few tiny spines at its base, while the exopod is up to 1.2 mm long with the four naked setae at its tip that are up to 400 µm in length. The second maxilliped (Fig. 4-8f) has an endopod of 4-5 segments with up to 1 mm high, mesial serrations (spines) on the first two segments, and with small setae at its tip, and an exopod of up to 1.2 mm long with four naked natatory setae at its tip up to 400 µm in length. The third maxilliped endopod (Fig. 4-8g) is up to 2.6 mm in length, segmented with serrations (rows of small spines) on the mesial surface of the 1st, 2nd, 4th and 5th segments, and small setae on the dactyl while the exopod is 2.3 mm long with four plumose natatory setae of up to 450 µm long distally.

Pereiopods 1 to 5 (Fig. 4-8h–l) are of similar form to those described in stage 1. The first pereiopod is up to 4.3 mm TL, while the exopod is up to 1.8 mm TL, and has 17 to 19 plumose natatory setae, each up to 600 µm long. The second pereiopod up to 3.9 mm TL, while the exopod is up to 1.9 mm TL, and bears 17 to 19 setae, each up to 580 µm long. The third pereiopod is up to 3.9 mm TL, with an exopod of up to 2.0 mm long and its 17 to 19 natatory setae are up to 600 µm in length. The fourth (non-chelate) pereiopod is up to 5.1 mm TL, while the exopod is up to 1.3 mm TL, and it bears 13 to 16 setae, each up to 520 µm in length. The fifth pereiopod is up to 4.8mm TL, while the exopod is up to 1.2 mm long and bearing11 to 15 setae each up to 450 µm in length.

The pleon is transparent and up to 5.5 mm in length (Figs. 4-6 & 4-7). The somites of the pleon are distinct and similarly armed to those of zoea 1. The 6th somite is elongate with a single lateral spine in addition to the paired dorsal spines as seen in Stage 1.

The pleopods (Fig. 4-8m-p) are biramous, with up to 18 setae on the distal half of each ramus that reach 130 µm long. The foremost pleopod is the longest and up to 1.6 mm long, while the hindmost pleopod is up to 1.2mm in length.

The telson (Fig. 4-6) is triangular and similar to that described for Stage 1. The intermediate plumose setae between the mid-posterior spine and the telson lateral corners have increased in length to 350 µm but the main development is that of the endopod and exopod of the developing uropods are present and about half the length of the telson are fringed by long, plumose setae.

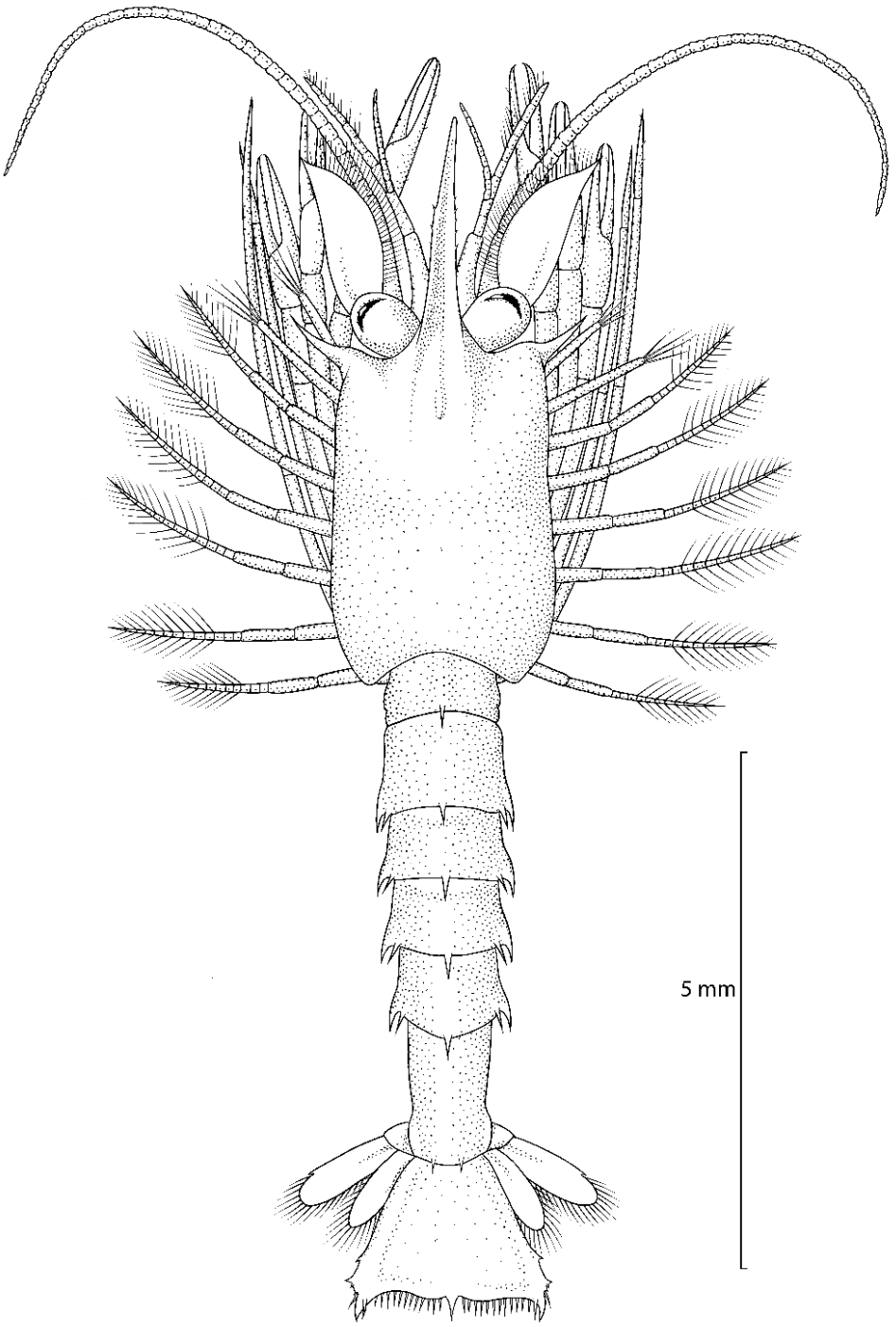


Figure 4.6 Stage 2 zoea of *M. challengeri*, whole animal, dorsal view (Drawing R. Webber).

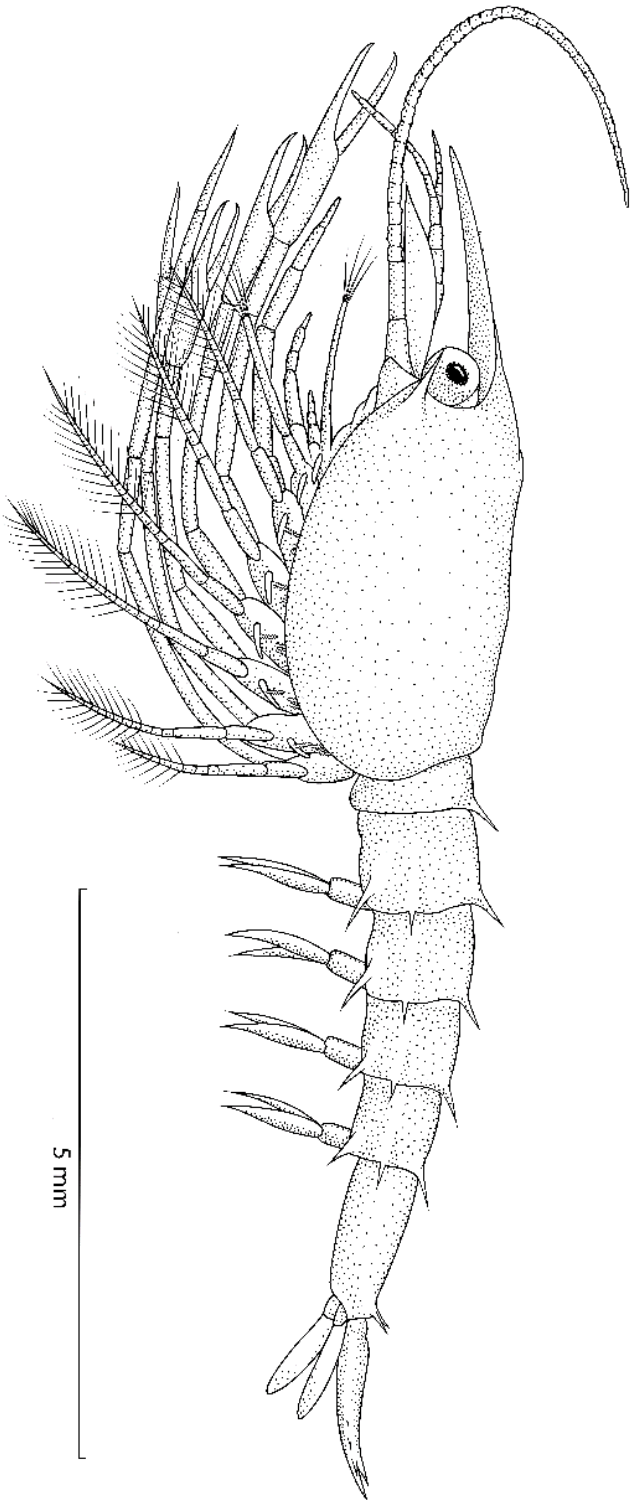


Figure 4.7 Stage 2 zoea of *M. challengeri*, whole animal, lateral view (Drawing: R. Webber).

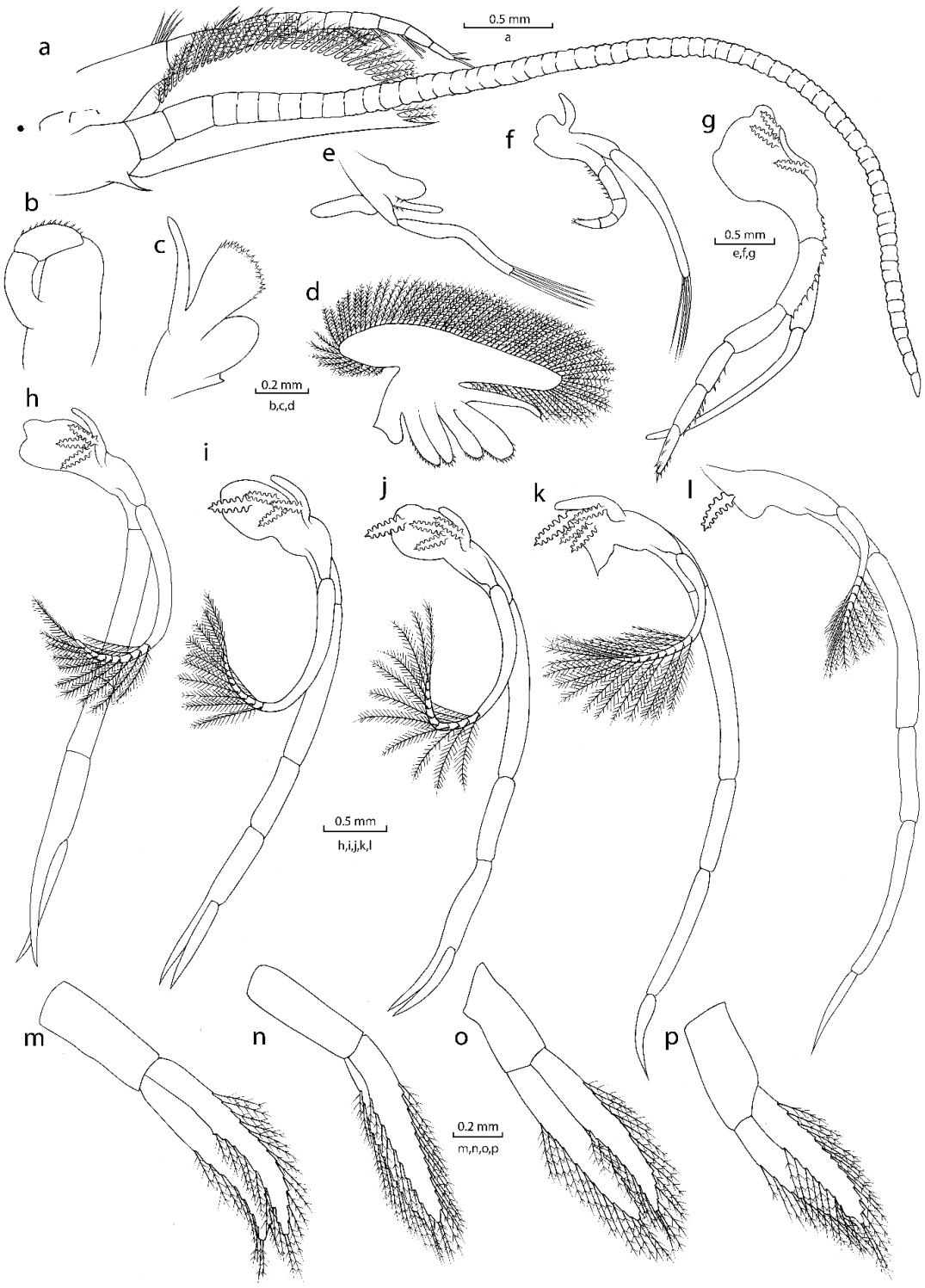


Figure 4.8 Appendages of stage 2 zoea of *M. challengeri*; a) antennule and antenna, b) mandible, c) maxilla 1, d) maxilla 2, e) maxilliped 1, f) maxilliped 2, g) maxilliped 3, h) pereiopod 1, i) pereiopod 2, j) pereiopod 3, k) pereiopod 4, l) pereiopod 5, m) pleopod 1, n) pleopod 2, o) pleopod 3, p) pleopod 4. (Drawing: R. Webber).

4.4.4 Post-larva (Figs 4.9 – 4.12)

The post-larva is 11.8 mm (SE ± 0.53, n = 10) TL (Figs. 4-9 & 4-10). It is transparent with an obvious orange hepatopancreas visible within the cephalothorax. No residual yolk remains at this stage. The OCL is 3.13 mm (SE ± 0.14, n = 8). The rostrum is equal in length to the carapace (dorsal) and is concave in shape along the dorsal axis. There are one or more pairs of terminal asymmetrical spines and random lateral and dorsal setae (80 to 210 µm long) along the edges of the rostrum. The eye is oval, with visible pigment and is 680 × 544 µm in size.

The first antenna (Figs 4-9 & 4-11a) is 2.12 mm in length with 14 setae increasing in size (53 to 250 µm) in a row on the inner margin of the peduncle (Fig. 4-11a). The flagellum is 1.75 mm in length with six pairs of plumose setae (160 µm long) on its the inner surface.

The second antenna (Figs 4-9 & 4-11a) is 16.8 mm in length and segmented. The antennal scale is 1.95 mm in length and dorsoventrally flattened with 32-35 evenly spaced plumose setae (135 to 567 µm long) on the distal 3/4 of its inner margin. The setules on each seta are up to 40 µm in length and inserted on the dorsoventral plane of each set.

The mandible (Fig 4-11b) has a fully articulated palp of three segments. The terminal segment is armed with dense tufts of strong setae, while the middle segment bears two longitudinal rows of setae and the basal segment is unarmed. The molar process has a hardened and slightly concave distal edge and retains the corner-like processes of the zoeal stages. The molar process is also now divided from the basis by a single joint.

The first maxilla coxal and basal endites (Fig. 4-11c) have numerous spine-like setae (40 to 60 µm in length) along and around their tips. The exopod is jointed at its base and mid-point and bears four long setae at its tip. The second maxilla (Fig. 4-11d) is flattened with the tips of the coxal and basal endites bearing numerous setae around their tips while the slender endopod has a few long setae near its base and one or more terminal setae. The scaphognathite margin is fringed by many close-set plumose setae.

The coxal endite of the first maxilliped (Fig. 4-11e) is small with scattered seta; the basal endite is flattened and oval in shape with numerous marginal and sub-marginal setae. The endopod is not segmented but bears a scattering of setae along its margins; the exopod bears no more than a tiny seta at its tip and is unsegmented and unarmed.

The endopod of the second maxilliped (Fig. 11f) is up to 1.4 mm long and is segmented into at least four segments bearing setae up to 240 µm in length along their mesial margins with the terminal segment covered in small setae; the exopod is reduced to an unarmed and unsegmented appendage.

The third maxilliped endopod (Fig. 4-11g) is five segmented and up to 3.3 mm in length with abundant setae (130 to 500 µm long) on the mesial surface of each segment. A mesial longitudinal row of approximately 13 robust spines is present mesially on the ischium, increasing in size distally. Three strong distally pointing spines also occur mesially on the merus. The carpus propodus and dactylus have rows of setae mesially and the dactylus is also armed with two or more long plumose setae at its tip. The exopod is articulated at its base but otherwise unsegmented and unarmed.

The five pereiopods (Fig. 4-11h-l) each consist of a coxa/ basis bearing gills, an epipod, endopod and vestigial exopod. Apart from a general increase in size, the main change from the condition of these appendages in the Stage II zoea to the post-larva is that the natatory exopods have become vestigial and they are now unarmed.

The first pereiopod (Fig 4-11h) is chelate and up to 5.6 mm TL and the cheliped fingers are 1.65 mm in length. There are numerous setae of up to 520 µm in length on all segments, which are most abundant on the chela, while the exopod is approximately 570 µm in length. The second pereiopod (Fig. 4-11i) is chelate and is up to 5.5 mm TL, and the cheliped fingers are 1.0 mm in length. There are numerous setae of up to 520 µm in length on all segments and they are most abundant on the chela. The exopod is approximately 740 µm in length. The third pereiopod (Fig. 4-11j) is chelate and is up to 5.9 mm TL and the chela is 1.3 mm in length, there are numerous setae on the chela and fewer on the remaining segments. The exopod is approximately 600 µm in length. The fourth pereiopod (Fig. 4-11k) lacks a chela and is up to 7.9 mm TL. It bears numerous setae mainly on the dactyl. The exopod is approximately 400 µm long. The fifth pereiopod is also without a chela (Fig. 4-11l) and is up to 8.5 mm TL, with setae mainly on the propodus and dactyl. The exopod is naked and 400 µm long. The gills are simple and 400 to 650 µm in length.

The gills on all five pereiopods (Fig. 4-11h-l) are the same in number and type as in the Stage I zoea but larger with P1 (Fig. 4-11h) consisting of 2 arthrobranchs and 1 podobranch (on the epibranch), P2 - P4 (Figs 4-11i – 4-11k) consisting of 1 pleurobranch, 2 arthrobranchs and 1 podobranch and P5 (Fig. 4-11l) consists of 1 pleurobranch only.

The pleon is transparent and 5 mm long with distinct somites (Figs. 4-9 & 4-10). There is a single mid-dorsal spine on the posterior margin of the tergite. There are also two lateral spines on each side of the posterior margin of the 2nd to 5th pleura. The 6th somite is elongate with no spines and is contiguous with the telson, a combination that was described as “the telson” by (Wear, 1976). In contrast, other morphological descriptions of decapods (Berry, 1969) describe the sixth somite and telson as distinct entities. In this current study the latter approach to morphological description is followed. Regardless, the telson in the post-larvae of scampi is rectangular with 24 to 28 setae and abundant fine hairs on the dorsoventral plane. The uropods are equal in length to the telson with up to 80 setae which have abundant fine hairs on a lateral plane.

The pleopods are biramous with up to 18 setae that are up to 700 µm in length (Fig. 4-12a - b). The setae have abundant fine hairs (55 µm) on the dorsoventral plane. The anterior pleopod is 1.9 mm and the most posterior pleopod is 1.7 mm long.

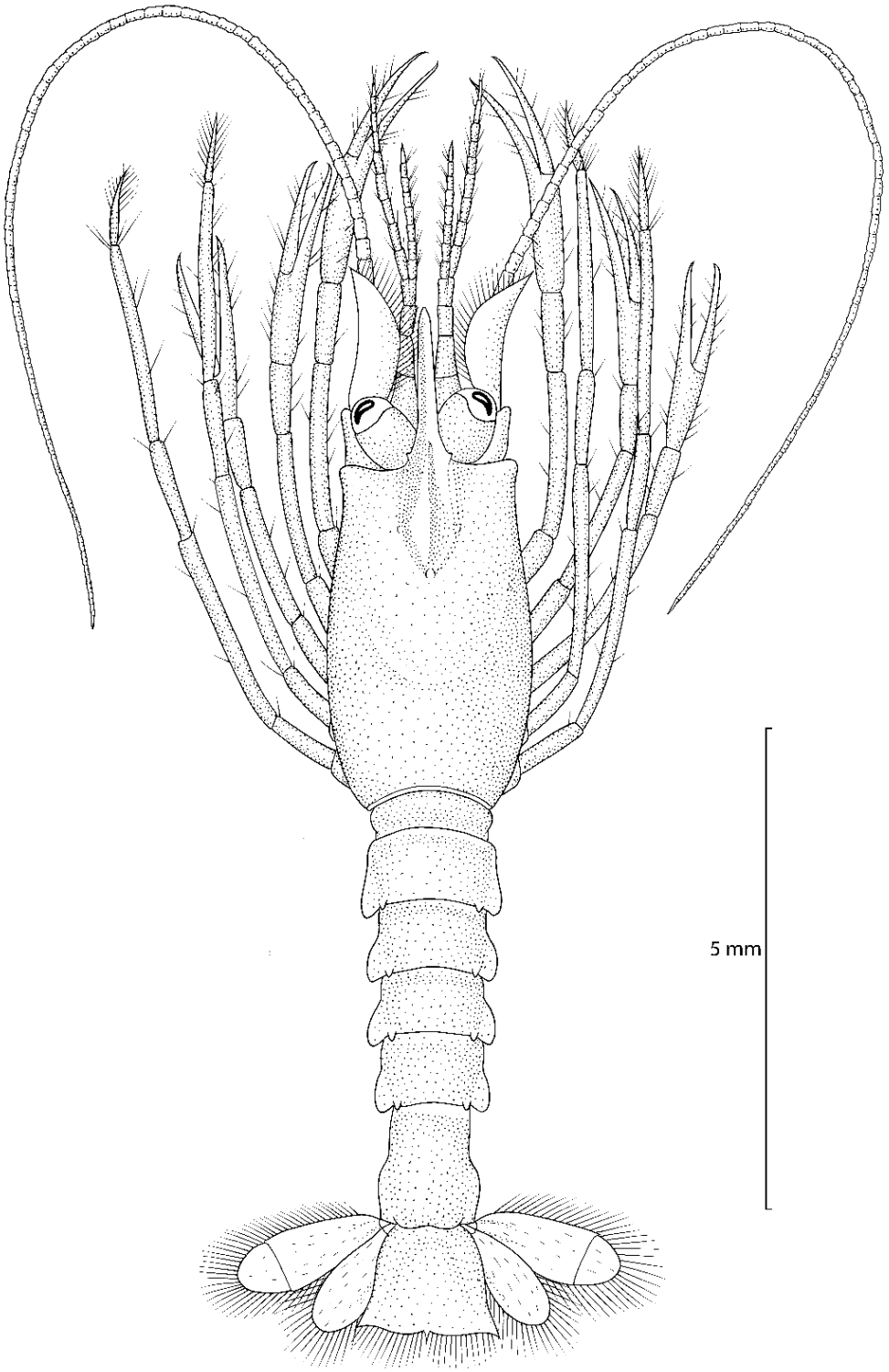


Figure 4.9 Post-larva of *M. challengeri* whole animal, dorsal view (Drawing: R. Webber).

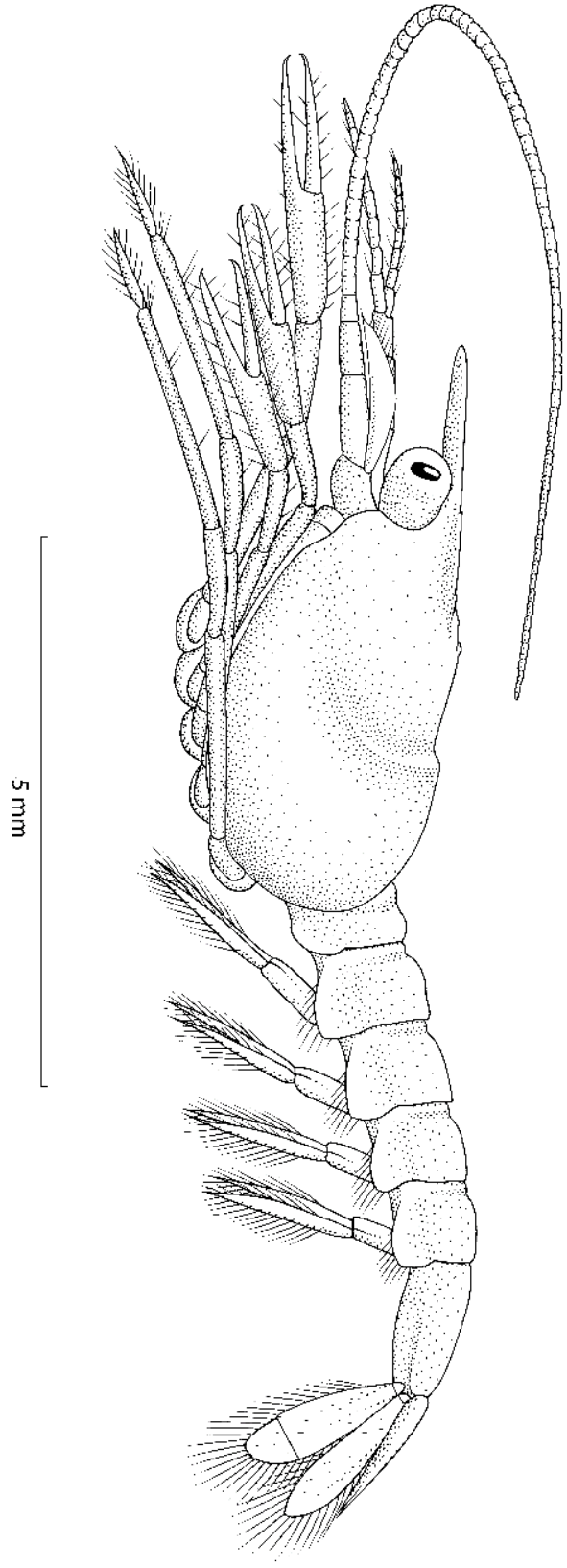


Figure 4.10 Post-larva of *M. challengeri* whole animal, lateral view (Drawing. R. Webber).

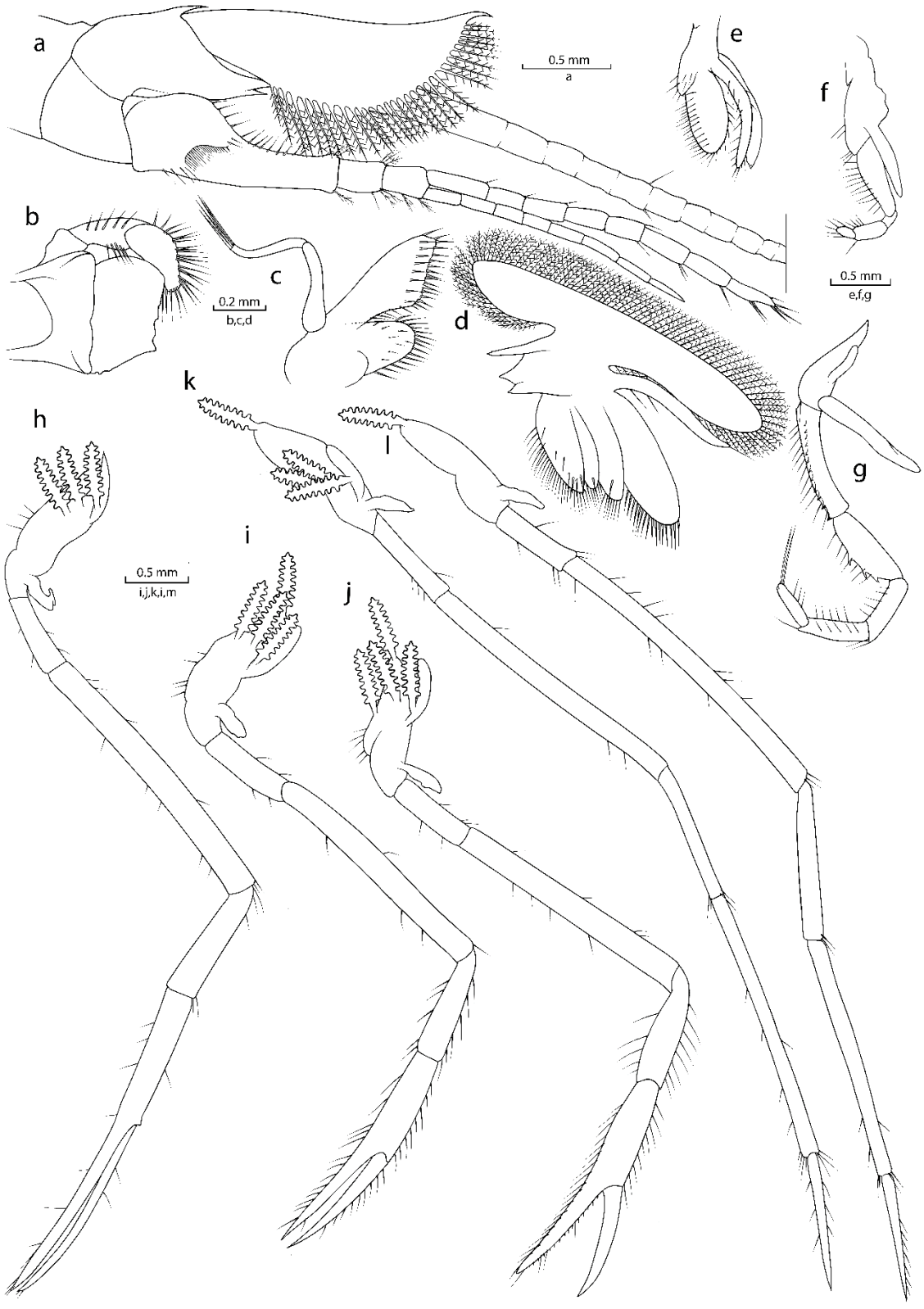


Figure 4.11 Appendages of post-larva of *M. challengeri*, a) antennule and antenna, b) mandible, c) maxilla 1, d) maxilla 2, e) maxilliped 1, f) maxilliped 2, g) maxilliped 3, h) pereiopod 1, i) pereiopod 2, j) pereiopod 3, k) pereiopod 4, l) pereiopod 5, m) pleopod 1, n) pleopod 2, o) pleopod 3, p) pleopod 4. (Drawing. R. Webber).

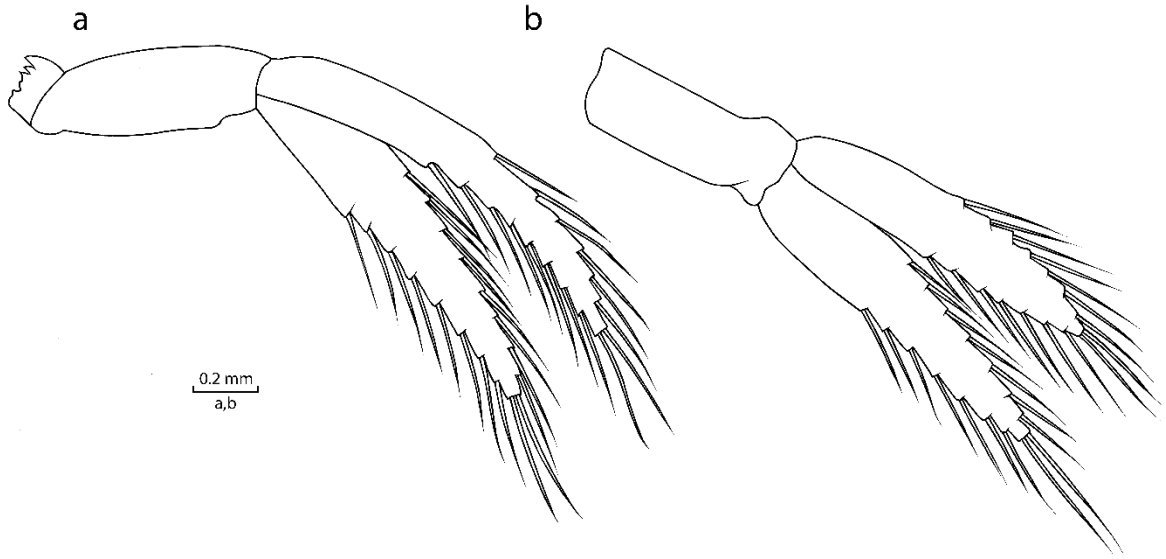


Figure 4.12 Pleopods of the post-larva of *M. challengeri*; a) pleopod 1, b) pleopod 4 (Drawing: R. Webber).

4.4.5 First Instar Juvenile

The first instar juvenile was not described as it is essentially a miniature of the adult. All vestigial elements that the post-larvae carried through the moult have disappeared and it is truly a benthic reptant lobster.

4.5 Discussion

Of all the 18 extant *Metanephrops* species only four have had larvae hatched from eggs and reared to post-larvae; *M. thomsoni* (Uchida & Dotsu, 1973); *M. japonicus* (Okamoto, 2008); *M. sagamiensis* (Iwata et al., 1992) and *M. challengeri* (this study). One species, *M. andamanicus* (Berry, 1969) has not had all the larval stages described but researchers have made some extrapolations, some of which are incorrect, of the likely intervening larval stages.

Of the larval stages of the *Metanephrops* species described, the larval stages of *M. sagamiensis* and *M. thomsoni* appear to be very similar to *M. challengeri* morphologically and have the same number of larval life stages. The moult periods for the larval stages of *M. thomsoni* are also very similar to *M. challengeri*. The early larval stages of *M. thomsoni* also appear to be lecithotrophic as they are in *M. challengeri*.

It has been shown that *M. challengeri* has a pre-zoea and two larval development stages prior to moulting to a post-larva or megalopa, i.e., the pre-zoea stage, stage I zoea and stage II zoea. *Metanephrops japonicus* has been shown to have a pre-zoea that metamorphoses directly into

a post-larva (Okamoto, 2008), while *M. thomsoni* (Uchida & Dotsu, 1973) and *M. sagamiensis* (Iwata et al., 1992) have both been confirmed to have a pre-zoea and two zoeal stages. *Metanephrops andamanicus* (Berry, 1969) has been suggested to only have a pre-zoea which metamorphoses directly to a post-larva but this has not been confirmed with larval rearing. *Metanephrops challengeri* (Wear, 1976) was previously assumed to have a pre-zoea and single stage I zoea before metamorphosing to a post-larva, however, the current study shows that there is an additional stage II zoea before metamorphosing to a post-larva.

It has been common to describe the anticipated larval stages based on observations of the developing stage made through the transparent cuticle of the preceding stage, e.g., Wear (1976) for *M. challengeri* and Berry (1969) for *M. andamanicus*. Hamasaki and Matsuura (1987) questioned the observations of the larval descriptions of *M. thomsoni* generated by Uchida and Dotsu (1973) because they were based on observations of the preceding last stage of unhatched embryos. However, in a recently translated version of Uchida and Dotsu (1973) it was shown that all the larval stages were actually seen and described. Berry (1969) thought that the pre-zoea of *M. andamanicus* settles almost immediately to post-larval stage even though it had not been observed. His conclusion was based on the lack of setae on the exopodites of the zoea making it less well-suited to a natatory existence and the naked pleopods on the first zoeal stage of *M. andamanicus*. Based on observations of *M. challengeri* it is likely that Berry (1969) had observed the pre-zoea stage, which has no setae on the exopodites of the pereiopods and therefore based on the observation of *M. challengeri* larvae, *M. andamanicus* may well have more zoeal stages.

Table 4-1 The pattern of larval development in five *Metanephrops* species which have been reared through full developmental stages.

	Developmental Stage				
Species	Pre -Zoea	Zoea I	Zoea II	Zoea III	Post Larvae
M. thomsoni *(1)*	O	O	O	A	O
M. andamanicus *(2)*	O	PA (SA)	PA (SA)	PA	P
M. japonicus *(3)*	O	PA	PA	PA	O
M. sagamiensis *(4)*	O	O	O	A	O
M. challengeri *(5)*	O	O	O	A	O

O = observed, P = proposed by paper author, SA = suggested alternative by this study, A = absent, PA = proposed absent by paper author

(1) = (Uchida & Dotsu, 1973), (2) = (Berry, 1969), (3) = (Okamoto, 2008), (4) = (Iwata et al., 1992), (5) = (Wear, 1976) and this study.

There appears to be some morphological variation within the same stages of the larvae of New Zealand scampi. For example, the most common state of the second antenna exopod in the pre-zoea is a broad dorsoventrally flattened appendage which is highly setose. However, in two individuals from separate cohorts the antennal scale lacked setae, having an unarmed margin. The setae on the exopods of the pre-zoea 2nd and 3rd maxilliped, which are normally exposed have also been observed to be sheathed and unexposed. The normal state of the stage I zoea 1st maxilliped is shown to have a short, plain exopod, however, on rare occasions there appears to be a setose exopod with four setae on the 1st maxilliped of this stage. This is unlikely to be a geographic variation within the species as the same life stages from the same cohort did not show these variations. It is unknown if the rearing of eggs in an artificial system will influence the morphology of the larval stages. For the purpose of this study, it was assumed not to be the case, but the possibility cannot be eliminated. There are no records of similar variations within other *Metanephrops* species bearing in mind that not many of the *Metanephrops* species have been described or observed as closely and in such large numbers as the current study.

Of the larval stages of *M. challengeri*, only stage II zoea feed while the pre-zoea and stage I zoea are lecithotrophic (Heasman & Jeffs, 2019). In *M. sagamiensis* and *M. thomsoni* all of the zoeal stages are lecithotrophic (Iwata et al., 1992; Uchida and Dotsu, 1973). It is assumed that the pre-zoea stage, which is the only zoeal stage of *M. japonicus*, is also lecithotrophic. The pre-zoea is a very short duration stage in this genus and the energy requirements to feed,

metabolise the food and moult, would probably exceed the energy uptake for the food, jeopardising the transition to the following stages. There is limited explanation for the lecithotrophy in the stage I zoea based on observations alone. Although the 2nd maxilla of the stage I zoea has fine setae which appear to be suitable for filtration, it does not appear to do so as no bolus has been seen in the alimentary canal which is visible through the translucent abdomen of the zoea. The mandibles (which are unarmed), maxilla, maxilliped 1 and maxilliped 2 in the pre-zoea and stage I zoea also lack the robust nature of the serrations seen on these appendages in the stage II zoea. It is also possible the chelae of the stage I zoea have limited strength to manipulate food items. Perhaps the stage I zoea can utilise soluble organic compounds in the culture water. Provasoli et al., (1959) presented evidence that *Artemia* nauplii could grow on soluble organics provided there was a stimulus to make them imbibe the water. There may be some relevance in this observation with regard to scampi stage I zoea as this study found that the addition of cultivated microalgae to the rearing water during larval rearing increased survival of the zoea I and zoea II larvae and produced more active post-larvae.

It is uncertain if stage II zoea filter feed, but they have been seen to eat live and frozen *Artemia*, suspended feed pellets (Prochete™) and even cannibalise larvae that have not successfully transitioned in a moult and died. Feeding was observed to be by contact with the food item rather than a directed hunt and lunge action. Stage II zoea, like the stage I zoea, show positive phototaxis during culture even though the light was at >915 nm. It is suggested that there may have been lower light frequency leakage from the LED red lights which provided this source. It could also be a negative geotaxis behaviour. The stage II zoea moulted in the water column to a post-larvae.

Some vestigial zoeal morphological structures could be found on the post-larvae, e.g., the retention of smaller and non-functional naked exopods on maxilliped 2 and 3 and the exopods on the pereiopods are also very reduced but still visible.

At this stage the post-larvae are more pelagic and maintain or adjusts its position in the water column by the sequential beating of the natatory plumose setae on the pleopods. This pelagic behaviour will facilitate distribution, food acquisition and, through occasional forays to the benthos, enable it to find suitable settlement habitat before moulting into a first instar juvenile. The post-larva can alternate between pelagic and benthic behaviour depending on the substrate. If the substrate is found to be suitable it will become more benthic and actively seek food in

the benthic substrate. Post-larvae have sufficient sensory ability to locate food (500 µm diameter pellets) if they are dropped close (5 – 10 cm) to the post-larvae.

The first instar juveniles are benthic, they start showing fine details of colouration in the carapace. They will actively seek food by standing high on their pleopods and using the pereiopods, pump water forwards under the ventral surface of the thorax between the pleopods and past the mouth parts and antennae for sensing food. Any food item touching the legs will be identified and gathered.

Rearing larvae from hatching through their successive larval stages, as in this study, provides the most reliable means to describe larval development in decapod crustaceans. This study has provided detailed descriptions of successive zoeal stages and the post-larval stage. It adds confirmed observations of the larval development to the limited information for the species in the *Metanephrops* genus and outlines the similarities and differences for *M. challengeri* from the other members of the genus.

Chapter 5 : Estimation of the metabolic scope and most advantageous thermal conditions for growth of the New Zealand scampi (*Metanephrops challengeri*).

5.1 Introduction

The scampi, *Metanephrops challengeri* (Balss, 1914), is a deep-water lobster endemic to New Zealand. It can be found primarily at depths ranging from 200 – 600m at densities ranging from 0.02 to 0.1 m^{-2} (Cryer et al., 2005). They are known to occupy regions where the temperature ranges from 6 – 13.5 °C (Cryer et al., 2005), (Hadfield et al., 2007) (Morris et al., 2001; Sutton, 2003). The temperatures increase from 6 – 9 in the south (SCI6A) (Figure 5-1) to 7 – 13.5 °C (SCI1) (Figure 5-1) in the north (Cryer et al., 2005; Hadfield et al., 2007; Morris et al., 2001; Sutton, 2003).

It is an important commercial species that is harvested by bottom-trawling with landings of around 800 t a year valued at over US$20.4 million (MPI, 2018). It has a retail value of US$51/kg (Wahle et al., 2020) making it a good financial prospect for aquaculture.

Wild-caught adult New Zealand scampi have proven to be suitable as captive aquaculture broodstock as they are relatively easy to house, feed and will successfully mate, extrude and incubate their eggs to hatch as larvae (Heasman & Jeffs, 2019). In addition, the larval development of this species is well suited to aquaculture with two lecithotrophic and one pelagic larval stage that feeds and is of short duration, which moults to a reptant post-larva (Heasman & Jeffs, 2019; Jeffs et al., 2020). However, very little is known about the growth of this species either in captivity or in the wild. Attempts to ascertain the growth of this species in the wild using tagged individuals were hampered by low rates of recapture (Cryer & Stotter, 1999; Tuck, 2009).

Knowledge about the growth of New Zealand scampi, especially in relation to environmental variables, such as temperature, is critical for managing productivity in both wild fisheries and aquaculture of this species. The characterisation of the ranges of thermal tolerance and optimal temperature for growth in captivity is fundamental to an initial assessment of the aquaculture potential of New Zealand scampi. Furthermore, the presence of a broad thermal range in marine invertebrates is advantageous in aquaculture because it requires the maintenance of less exacting culture conditions and can provide an opportunity to increase growth performance using higher temperatures (Froehlich et al., 2016). The determination of metabolism (the

biological processing of energy and materials (Brown et al., 2004)) in response to temperature is an effective initial step for investigating the thermal requirements of a species without the need for extensive replicated temperature versus growth experiments, which have the potential to be confounded by other factors, such as food quality and availability (Froehlich et al., 2016; Horodysky et al., 2015; Yuen et al., 2019). Knowledge of the thermal requirements of aquaculture organisms can also guide inferences on other important attributes for aquaculture, such as hypoxia tolerance (Froehlich et al., 2016).

In poikilotherms, body temperature and metabolism vary according to ambient temperature (Daoud et al., 2007; Eckert., 1988; Perera et al., 2007). Temperature is considered the most important environmental factor affecting poikilotherms (Pörtner & Lannig, 2009). The respiration rate, measured as the rate of oxygen consumption over time at a set temperature, is frequently used as a proxy for the metabolic rate (Daoud et al., 2007), with the metabolic rate reflecting the energy utilisation by the organism (Brown et al., 2004). This is important in aquaculture because respiration provides a useful indicator of metabolic expenditure for maintaining vital functions and growth (Brett & Groves, 1979) and assists with understanding the animal's environmental requirements in an aquaculture production setting, especially temperature (Fitzgibbon & Battaglene, 2012). Crustaceans are suggested to have a high thermal sensitivity (Lagerspetz & Vainio, 2006), especially decapods (Matsuda & Yamakawa, 1997). Many other factors influence oxygen consumption in organisms, including body mass (Jensen et al., 2013; Villarreal & Ocampo, 1993), the extent of physical activity, as well as the intake and processing of food (Perera et al., 2007), all of which need to be controlled for when measuring respiration in organisms. Associated with the respiration rate is the Q_{10} or temperature coefficient which measures the velocity of chemical reactions in relation to a 10 °C difference in temperature, i.e., effectively a measure of temperature sensitivity.

The aim of this study was to undertake an initial assessment of the suitable temperature range for culturing *M. challengeri* based on the metabolic difference between the standard and maximum metabolic rates (i.e., MMR - SMR = the metabolic scope) as measured by intermittent flow respirometry methods (Chabot et al., 2016). Furthermore, these data can then be utilised to calculate the Q_{10} which provides an indication of the growth potential of this species.

5.2 Materials and Methods

5.2.1 Collection and transport of scampi

A total of 100 adult scampi (size range 41 to 75 g) were collected from the wild in the designated scampi commercial fishing area SCI3 using commercial trawls of short duration (Figure 5-1). Upon recovery of the trawl to the vessel, undamaged intermoult adult scampi of mixed of sexes, excluding females carrying eggs, were selected and transferred to trays in a purpose-built holding bin incorporating a recirculating seawater system, which was mounted on the vessel deck. Approximately half the seawater in the system was exchanged every 6 h to maintain water quality, which was monitored and found to have no measurable ammonia, nitrite and only traces of nitrate. Seawater within the system was continuously aerated with chilled air to maintain dissolved oxygen saturation. The seawater temperature was maintained with a chiller unit at between 6 and 10 °C , averaging around the ambient water temperature in the scampi collection habitat (9.2 ± 0.05 °C SE). The scampi were returned to port by the vessel within 24 h and the aquaria unit was transferred to a land-based aquaculture research facility near Nelson, New Zealand, which took approximately 2 hours.

5.2.2 Scampi Holding Facilities

At the aquaculture research facility, the scampi were placed in individual tanks (40 × 15 × 20 cm, L × W × H) holding 12 l seawater volume supplied from a recirculating aquaculture system. The system included mechanical and biological filtration, degassing, aeration, UV sterilisation and temperature control. Following arrival at the facility, all scampi were held at a temperature of 10.5 ± 0.2 °C, pH of 8.2 ± 0.05, salinity of 34 ± 0.1 $^{0}/_{00}$, oxygen at 100 % saturation and ammonia at trace concentrations only. The scampi were held under 12:12 h cycle of dim red light (> 915 nm) to avoid light induced stress on scampi accustomed to minimal light in their natural habitat at over 300 m of depth (Raymond & Widder, 2007; Warrant & Locket, 2004). Scampi were allowed to acclimatise to the holding system for a month before being transferred to the experimental unit for acclimation to the test seawater temperatures, in preparation for respirometry measurements.

5.2.3 Determination of the experimentation temperature range

Seawater temperatures in the natural habitat of New Zealand scampi were gathered from data logging thermometers and depth sensors which were attached to the mouth of commercial

scampi trawl nets by researchers from the Cawthron Institute. In addition, data gathered over the last 20 years was supplied by researchers working for the New Zealand Ministry for Primary Industries (i.e., during fishing seasons only) (Table 5-1). Collectively, this temperature sampling was conducted opportunistically with commercial and research trawls for New Zealand scampi in four management areas for commercial fisheries that covered the majority of the latitudinal range of New Zealand waters, i.e., SCI1, SCI2, SCI3 and SCI6 (Figure 5-1). The selected temperatures for respirometry experiments were 7, 10, 13 and 15 °C as these cover the temperature range found within the natural habitat of scampi.

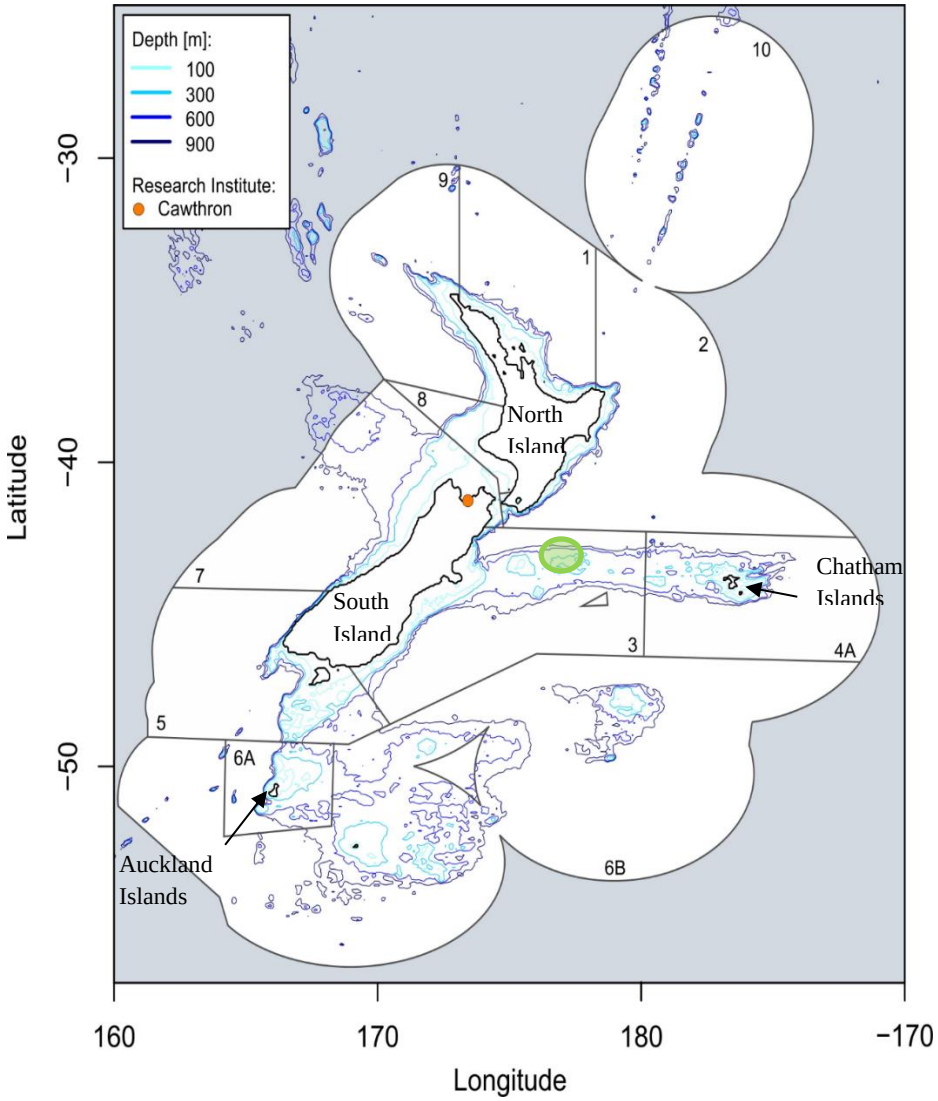


Figure 5.1 Map of New Zealand scampi commercial fisheries management areas showing the four areas where measurements of the seawater temperature in scampi natural habitat were collected, i.e., 1 = SCI 1 in the north, 2 = SCI 2 in the north east, 3 = SCI 3 central & 6A = SCI 6A south west. Live scampi used for this study were captured in 3 (SCI3) and transferred to aquarium facilities at Cawthron Institute's Aquaculture Park (orange dot).

Table 5-1 The mean (±SD), minimum and maximum seawater temperatures recorded in New Zealand scampi habitats measured at four significant commercial fishing management areas covering most of the latitudinal range of fisheries for New Zealand scampi (i.e., SCI 1, 2, 3 & 6 – see Figure 5-1) and including records from a depth range of 192 to 644 m. Source: New Zealand Ministry for Primary Industries (1998 to 2018[†]) and Cawthron data[‡].

Location & Latitude	Period of sample collection	n	Depth (m)			Temperature at depth (°C)		
		Sampling expeditions over 20 years (total samples)	Mean (SD)	Min	Max	Mean (SD)	Min	Max
SCI 1[†] Lat ~35º S	Sept-Apr	14 (792)	387 (80)	195	644	10.3 (1.1)	7.0	13.5
SCI2[†] Lat ~39º S	Jul-May	19 (522)	359 (80)	192	594	9.8 (1.2)	7.0	13.5
SCI3[†] Lat ~ 45º S	Sept -Oct	8 (221)	397 (50)	273	536	8.7 (0.6)	7.2	11.9
SCI3[‡]	October	12 transects (over 10 days) (10664)	367 (9.8)	345	378	9.8 (0.1)	9.2	10.5
SCI6[†] Lat ~ 50º S	Feb-Mar	5 (155)	446 (46)	278	541	7.6 (0.3)	6.7	9.0

The higher temperature range of 15 °C was selected as the highest experimental temperature treatment to determine if there was any tolerance above the highest temperature recorded in their natural habitat (13.9 °C). An increase of 1.1 °C above the highest recorded temperature was considered reasonable at the time of experimental method development.

5.2.4 Respirometry set up

The intermittent flow respirometry method was used for this study (Svendsen et al., 2016) which used four respirometry chambers made of gas-impermeable acrylic plastic of 2 l volume (Figures 5-2 and 5-3). Each chamber could fit within a scampi holding tank to minimise handling stress during transfers to test chambers. An open respirometer chamber was placed into a holding tank with each scampi 24 h before the scampi was walked into the chamber, all remaining gas in the chamber was then replaced with ambient seawater (1 µm and UV filtered), the chamber sealed, so that the respirometry measurements could be initiated. Each respirometry chamber was connected via gas impermeable PTFE (i.e., polytetrafluoroethylene) tubing to a small seawater pump (600 ml min^{-1}) to provide intermittent circulation of seawater through the chamber.

The oxygen demand of the respirometry system was tested by setting up each unit without containing a scampi (the blank) and closing the water flow for 1 h (the approximate time required for an individual scampi assessment). This was repeated three times and the resulting mean provided a baseline of oxygen consumption by the intermittent respirometry system. Any oxygen recorded as consumed in the blank could then be deducted from the oxygen consumed by the scampi during the experiment.

The respirometer chamber was designed to encourage maximum mixing of the water while minimising stress to the scampi. Water was introduced into the horizontal mixing unit positioned on the internal dorsal surface of the respirometer (A1 Figures 5-2 and 5-3) and from there into the respirometer via a series of 2 mm holes (A2 – Figures 5-2 and 5-3) on the lateral surfaces of the horizontal mixing unit. Each 2 mm hole was spaced 20 mm apart horizontally and was pointed at an angle of 10° greater than the adjacent 2 mm hole providing a water discharge direction ranging from 90° for the first hole to 30° for the last hole respectively. Thus, seawater flow was directed down the sides of the chamber until the flows from each side met at the bottom of the chamber and mixed around the scampi. Seawater flow testing using dye visualisation showed that complete mixing occurred within 90 seconds at the flow rate of 600 ml min^{-1}. The seawater from the chamber was collected via a port above the scampi (B1 - Figures 5-2 and 5-3) leaving the respirometer via the dorsal vent (B2 – Figures 5-2 and 5-3) to the pump via the oxygen probe (FireStingO^2, PyroScience GmbH, Aachen - Germany) and then back into the chamber.

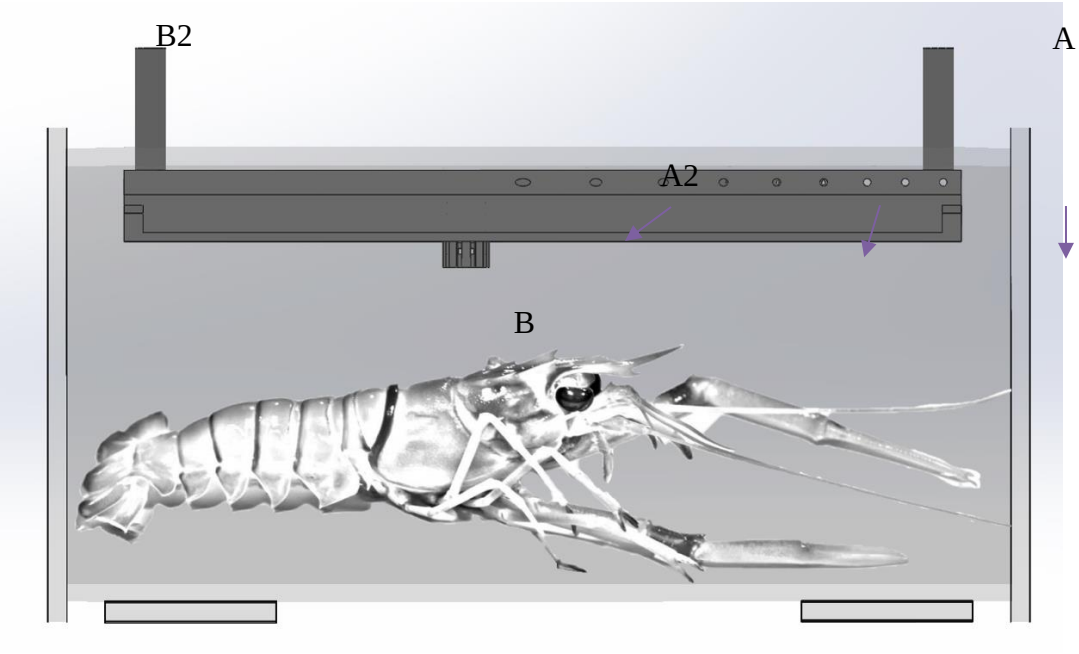


Figure 5.2 The side view of the respirometer used in the experiment showing the increasingly angled water inflow ports to ensure mixing. Arrows indicate water flow direction. A1 = Water inlet into the respirometer. A2 = the 2 mm discharge holes for water distribution and mixing into the chamber. B1 = The water outflow port projecting downward from the dorsal unit. B2 = the water discharge from the respirometer which is directed to pass over the O_2 probe and back into the respirometer via a pump and respirometer entry port A1. (Note scampi not to scale)

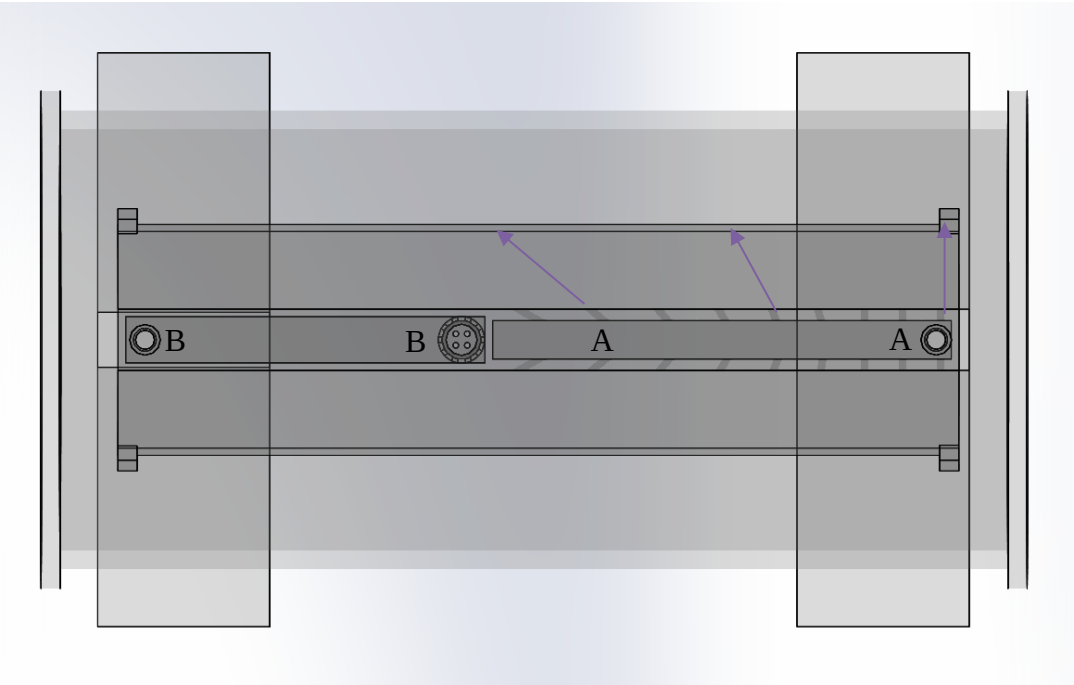


Figure 5.3 Top view of the water inflow ports in the respirometry chamber. Arrows indicate water flow from their respective ports.

Individual oxygen consumption rates (MO_2 in $mgO_2\ h^{-1}$) were determined for scampi and then converted into the metabolic rates (VO_2 in mg $O_2\ h^{-1}\ g^{-1}$ wet weight). The wet mass of the scampi was determined at the completion of each respirometer run. The scampi were taken

from the respirometer, placed on a pillow of tissue paper, the excess water tamped from the surface of the animal and then the scampi was weighed after 30 seconds and sexed (Table 5-2).

5.2.5 Acclimation of scampi

Ten randomly selected scampi ranging in size from 44.4 to 69.8 g wet mass and five females, and five males were held at 10.5 °C for a period of 1 month after transfer from the wild, during which time they were fed to satiation on mussel flesh (*Perna canaliculus*), fish tissue (various species) and squid (*Nototodarus* sp.) supplied from a local fish monger. Tanks were cleared of excess food 24 h after feeding and the walls rubbed down every 3 days. At 7 days prior to undertaking a respirometry measure, a scampi was transferred to an insulated holding tank (43 × 17 × 25 cm, L × W × H) in which an open respirometer chamber was positioned for the scampi to become familiar with the chamber. The pumps circulating the water through the respirometers were turned on so that the scampi could become familiar with the pump noise and water flow. After the settling period of one week, the water temperature in the tanks was adjusted toward the selected test temperature at a rate not exceeding 1 °C per 24 h (i.e., 4 days from 10.5 - 7 °C). After 24 h at the required experimental temperature the scampi were fed fresh squid and given 48 h to clear their gut of the food preventing the digestive process from elevating respiration (Perera et al., 2007). The scampi were then considered to be acclimatised (Aziz & Greenwood, 1981) and ready for respirometry analysis.

5.2.6 The experiment

The respirometry chamber in the scampi holding tanks were exchanged for cleaned and sterile units (Figure 5-4).

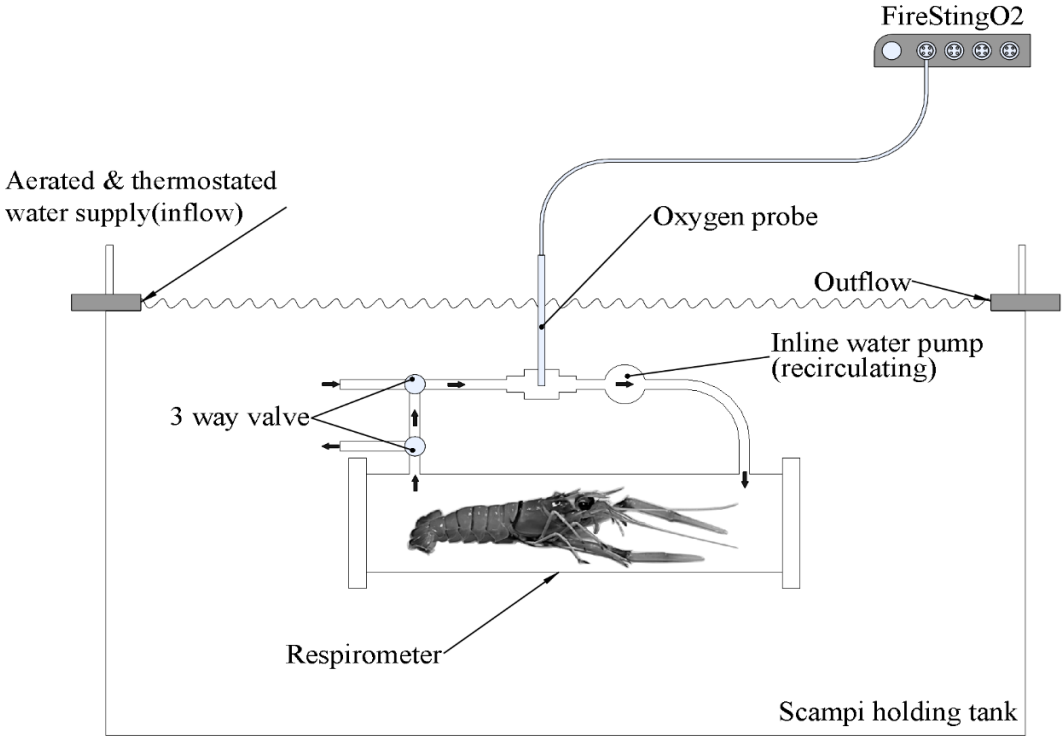


Figure 5.4 Schematic of the respirometry holding chamber showing the respirometer and the water flow options.

The scampi were encouraged to enter the respirometry chamber with minimal disturbance and once settled (generally a period of 15 minutes) the chamber was sealed, residual gas excluded and the water circulation loop closed. The oxygen consumption was then measured every second. If a scampi became agitated the experiment was aborted and the water vented until the scampi settled, at which point the experiment recommenced. If the scampi was continuously agitated or restless during the experiment, it would be left to settle outside of the chamber until the following day when another attempt was made. If this happened for two consecutive days, the scampi would be fed to avoid testing animals stressed from starvation (Armitage & Wall 1982). A minimum of 48 h was allowed for the scampi to clear its gut. Unfortunately, scampi frequently became too agitated whilst enclosed in the chamber, which resulted in long periods between the generation of acceptable data from individuals. Where scampi remained settled in the respirometry chamber they were left until they had depleted the available oxygen by 10 - 20% (Ikeda et al., 2000; Svendsen et al., 2016) or 1 h duration, which ever occurred first. At this time fresh fully oxygenated seawater was allowed to flush through the system. The flush rate was 600 ml min^{-1} which ensured a complete water exchange in the 2 l respirometer in

under 4 mins. This flushing was conducted for 1 hour. If the scampi were relaxed (i.e., normal levels of activity) after this time, another series of oxygen measurements were taken. If the scampi was agitated, then the scampi was left to recover as outlined previously.

Once the oxygen consumption of each scampi at rest had been determined three times at each temperature (each treatment of up to 60 minutes closed flow), the scampi was then tested for active metabolism for which a measurement time of 1 h was used. To determine maximum metabolic rate (MMR), the scampi (while in water) was gently prodded to induce tail flapping, once the flapping ceased the scampi was turned over onto its back and allowed to right itself. This continued until a point of exhaustion was reached and it could no longer right itself. Each scampi was then placed back into the respirometry chamber within a minute, the chamber sealed, the flow valve closed, and the oxygen consumption measured.

The first round of respirometry experiments was conducted at 7 °C. Upon completion of the active respirometry assessment at this temperature the scampi was allowed 12 h to recover undisturbed in a holding tank until the next day. The water temperature was then increased by 1 °C degree per 24 h over the next 72 h to 10 °C. The scampi was then fed, allowed 48 h to clear their gut and the scampi subjected to a repeated respirometry procedure. Once completed at 10 °C the same process for recovery, temperature shift, acclimation and respirometry measures was carried out again for 13 °C, and then again for 15 °C.

5.2.7 Determination of Metabolic Parameters

Standard Metabolic Rate (SMR) per gram and Maximum (or active) Metabolic Rate (MMR) per gram, (i.e., mg O_2 g^{-1} h^{-1}(wet mass)) were calculated in the following manner.

The slope of the linear regression describing the rate of decrease in oxygen saturation per unit time was calculated as per the formula for a closed respirometry system (Equation 1). Only those results with an r^2 value of 0.95 or higher were included in the analyses and no activity during the SEM assessments and the scampi being relaxed post experiment.

Oxygen solubility was defined according to the experimental temperature (FireStingO_2 (FSO_2-4)).

The salinity was measured with a salinometer (Fisherbrand™) and included in the calculation.

Equation 1 - The linear oxygen decline measured in the chamber whilst closed was determined using equation 1. (Steffensen, 1989).

$$y = V_{RE} W_o^{-1} \frac{\delta CO_2}{\delta t}$$

Where:

y = the oxygen consumption (MO_2)

V_{RE} = Effective respirometer volume (respirometer volume + recirculation loop – volume of scampi) (litres) (Equation 2)

W_o^{-1} = wet mass of scampi (kg)

$\frac{\delta CO_2}{\delta t}$ = slope of the linear decrease in O_2 content while the chamber was closed (mg O_2)

Equation 2 - The volume of each respirometer was estimated as per the equation (2) (Svendsen et al., 2016).

$V_{RE} = V_{RT} - W_o P_o^{-1}$

Where:

V_{RT} = total volume of the empty respirometer including the recirculation loop (litre)

W_o^{-1} = mass of the organism (g)

P_o^{-1} = density of the scampi. This was calculated for this experiment as 1.022 kg l^{-1} (± 0.004, n = 10) by weighing 10 individual animals and determining their individual volume by placing them in a full beaker and measuring the water displaced. Most neutrally buoyant marine organisms have a density of 1.025 (Svendsen et al., 2016).

Estimation of the temperature quotient or thermal co-efficient Q_{10}

The thermal co-efficient (Q_{10}), which represents the sensitivity of an organism to temperature variations was estimated using the Van't Hoff Equation:

$$Q_{10} = \left(\frac{k2}{k1}\right) 10/^{(t2-t1)}$$

Where $k1$ and $k2$ are the SMR oxygen consumption of a scampi of standardised mass (g) at temperatures T1 and T2. Q_{10} is an empirical value for which low temperature sensitivity will have a lower Q_{10} value.

In this experiment *k*1 and *k*2 were the mean SMR consumption rates per gram (mg O_2 hr^{-1} g^{-1}) at 7 °C and 10 °C and 10 °C to 13 °C (i.e., T1 and T2 for each range).

5.2.8 Statistical analyses

The effects of temperature, activity level (i.e., resting versus active) and their interaction on oxygen consumption were assessed using a mixed effect linear model, where the individual animal identity was included in the model as a random effect (Zuur, 2009). This approach addressed the non-independence of taking repeated measures from individual scampi. For these analyses, oxygen consumption was natural log transformed to linearize the relationship and reduce heteroscedasticity. The log transformed oxygen consumption data were tested for normality with an Anderson-Darling normality test (Thode, 2002). The marginal means were estimated for the combination of temperature and oxygen consumption and used for comparisons among them. Adjusted p values for the differences were calculated using the Tukey method.

All statistical analyses were performed using the R statistical and programming environment (RCoreTeam, 2019). The R package lme4 (Bates et al., 2015) was used for performing the mixed effect linear models and the package emmeans (Lenth, 2019) was used to estimate the marginal means (least-squares means) for the factor combinations from the mixed effects linear models.

5.3 Results

A total of 151 respirometry experiments were conducted over the three temperatures. Of these 151 experiments, 26, 27 and 27 SMR measurements were completed with acceptable data at 7, 10 and 13 °C, respectively. The scampi held at 15 °C showed increasing listlessness, loss of appetite, lower response to stimulus such as handling and reduced self-cleaning, which if left for more than 2 days resulted in a biofilm beginning to develop on the carapace. As a consequence, all the scampi scheduled for testing at 15 °C were euthanized after 8 days of holding, as they showed a decline in health that was deemed ethically unacceptable. A total of 10 MMR were measured for scampi at each of the 7, 10 and 13 °C temperatures (Table 5-3).

Unfortunately, no scampi remained sufficiently calm to have all the respirometry measurements taken on one day. Therefore, in many instances it took up to 2 weeks to acquire acceptable data from any one individual scampi at a single temperature.

The mixed effect model revealed a significant interaction between activity level and body mass with differences in oxygen consumption among temperature treatments and activity levels, i.e., resting and active (Figures 5-5 & 5-6). The measured SMR at 7 °C was lower than for 13 °C ($p < 0.05$) and the SMR at 10 °C was intermediate and not different to the SMR for either 7 °C or 13 °C. There was no significant difference ($p < 0.05$) between the MMR measured at 7, 10 and 13 °C (Figures 5-5 & 5-6). The SMR was found to increase with greater wet mass of scampi, whereas the MMR decreases (Figure 5-7).

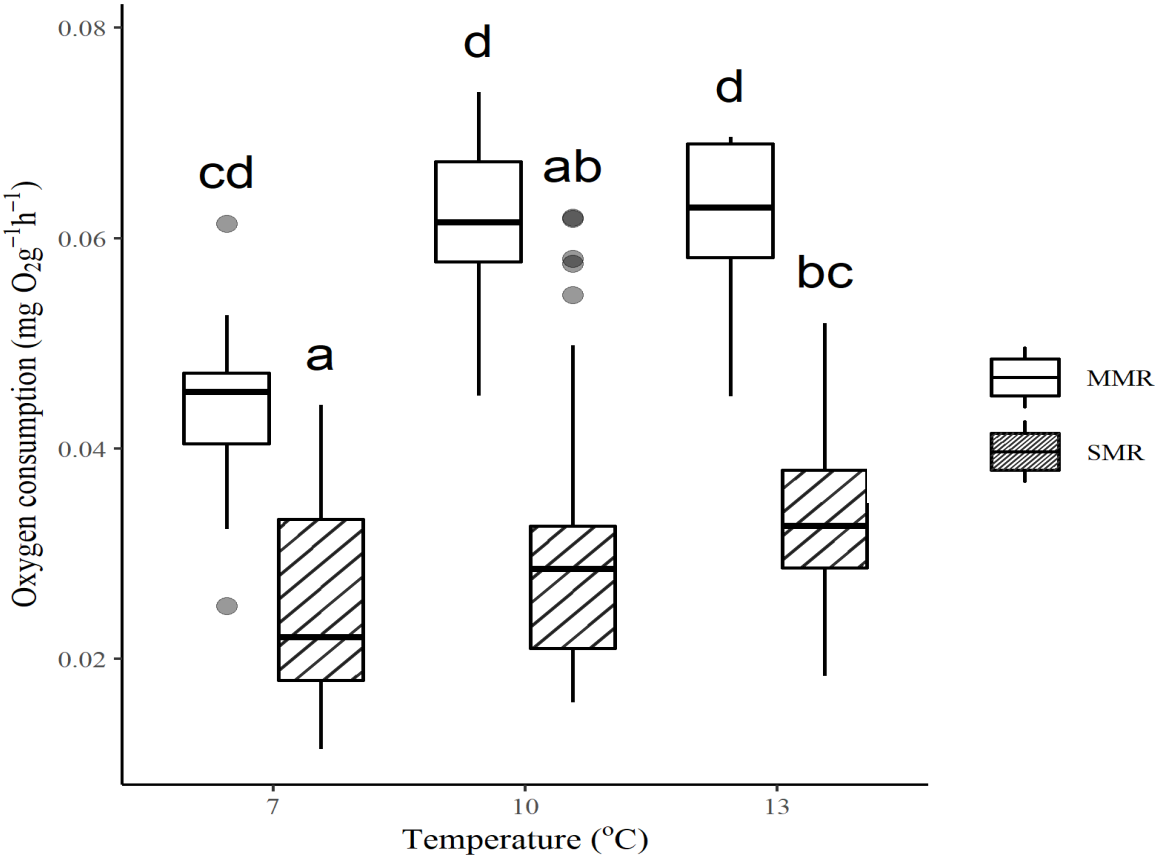


Figure 5.5 Boxplot of mean oxygen consumption for SMR and MMR measured in adult New Zealand scampi at three experimental temperatures 7, 10 and 13 °C. Means with the same letter are not significantly different (P <0.05). (Note: the SMR data is offset to the right of the representative temperature and the MMR is offset to the left of the representative temperature to allow for data points to be seen clearly.)

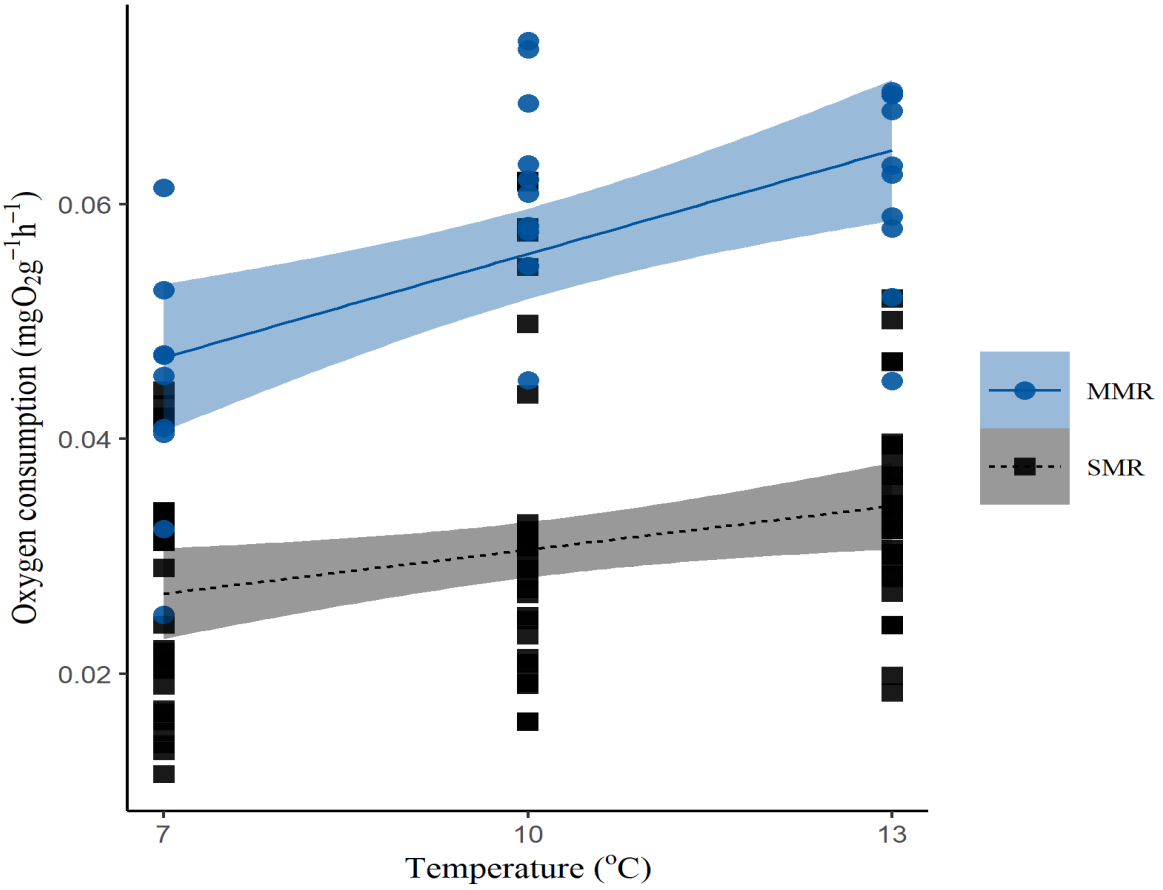


Figure 5.6 Scatter plot of the oxygen consumption measures for SMR and MMR for New Zealand scampi at three experimental temperatures with associated regression lines with 95% confidence intervals.

Table 5-2 Mean SMR and MMR oxygen consumption standardised by wet mass for New Zealand scampi at three experimental temperatures 7, 10 and 13 °C.

Temperature (°C)	(n) SMR	Mean SMR $mgO_2\ g^{-1}\ h^{-1}$ (SD)	(n) MMR	Mean MMR $mgO_2\ g^{-1}\ h^{-1}$ (SD)	Metabolic Scope $mgO_2\ g^{-1}\ h^{-1}$
7 °C	26	0.023 (0.002)	10	0.044 (0.004)	0.021
10 °C	27	0.032 (0.003)	10	0.062 (0.003)	0.030
13 °C	27	0.034 (0.002)	10	0.062 (0.003)	0.028

The SMR increased with increasing wet mass of *M. challengeri*, while the MMR decreased with increasing wet mass (Figure 5-7).

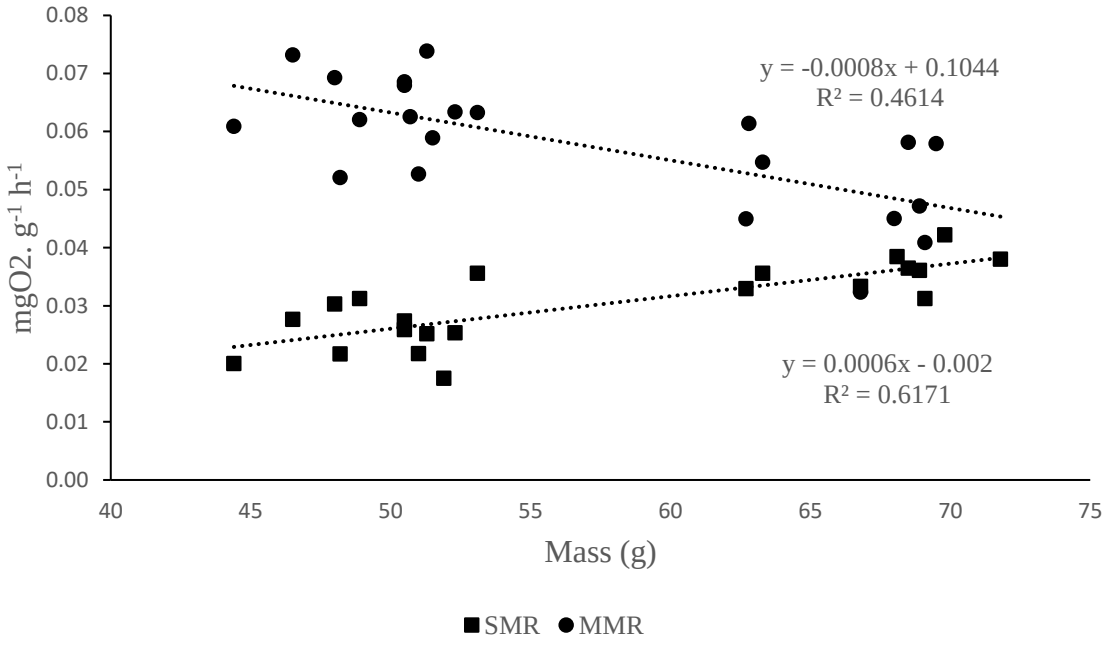


Figure 5.7 The oxygen consumption of scampi (mgO_2 g^{-1} h^{-1}) measured for SMR and MMR versus wet mass of individuals (g) regardless of experimental temperature. Each individual is represented by a SMR and MMR data point.

5.3.1 Q_{10}

The Q_{10} for the SMR measurements taken from 7 and 10 °C is 3.01. The Q_{10} for the SMR measurements taken from 10 and 13 °C is 1.22. The overall Q_{10} results for the measurement of SMR from 7 to 13 °C results in a Q_{10} of 1.92. The temperature of 13 °C was used at the upper maximum experimental temperature for the Q_{10} range given the intolerance of scampi to the 15 °C temperature treatment (Table 5-2).

5.4 Discussion

An important early step required for understanding the aquaculture requirements of an animal is defining its temperature requirements (Fitzgibbon & Battaglene, 2012). Therefore, the aim of this study was to make an initial determination of the temperature range to cultivate *M. challengeri* based on the metabolic difference or scope between the SMR and MMR.

The metabolic scope indicates the proportion of oxygen available for biological activity (Chabot et al., 2016; Farrell, 2016). This measure is the greatest difference between SMR and MMR measured at the same temperature and it can help to define the optimum temperature to culture the animal. The results for New Zealand scampi suggest that the greatest metabolic scope lies between 10 and 13 °C as there are significant differences between 10 °C SMR and

10 °C MMR, and 13 °C SMR and 13 °C MMR. In addition, the means between the SMR and MMR were at their greatest between these two temperatures. Exposure at a temperature of 15 °C resulted in a rapid decline in health and would have ultimately caused the death of scampi if maintained. Therefore, the temperature maxima should be between 13 °C, where scampi remained healthy, and 15 °C. This narrow thermal range may be explained by the natural thermal habitat of this species in deep waters, which is likely to be both narrow in range and relatively stable (Hazel & Prosser, 1974).

The results confirm that SMR oxygen consumption increases with body mass as is widely reported in other studies for marine crustaceans (Table 5-3, Figures 5-7 & 5-8) (Armitage & Wall, 1982; Daoud et al., 2007; Jensen et al., 2013; McClain et al., 2020). While oxygen consumption increases with the mass of the scampi for SMR, it does not for MMR suggesting an upper limit to oxygen uptake in scampi that operates regardless of ambient temperature. The positive increase of oxygen consumption with mass when scampi is at rest is contrary to the results found for many other crustacean species (Bridges & Brand, 1980; McLeese, 1964; Parslow-Williams, 1998) (Figure 5-8, Table 5-4). Whilst scampi are active, the oxygen consumption is at a maximum and therefore follows the trend seen in other studies (Alcaraz & Sardá, 1981; Bridges & Brand, 1980; Parslow-Williams, 1998).

Table 5-3 Relationship between body mass and oxygen consumption in *M. challengeri, N. norvegicus, H. americanus* and *H. gammarus*.

Species	Temperature (°C)	Regression expressions	Author
***Nephrops norvegicus* (SMR)**	10	$y = 0.037x^{-0.139}$	Bridges & Brand (1980)
***Nephrops norvegicus* (SMR)**	10	$y = 0.0534x^{-0.27}$	Parslow-Williams (1998)
***Nephrops norvegicus* (MMR)**	10	$y = 0.206x^{-0.25}$	Parslow-Williams (1998)
***Homarus americanus* (SMR)**	15	$y = 0.073x^{-0.23}$	McLeese (1964)
***Homarus gammarus* (SMR)**	15	$y = 1.357x^{-0.512}$	Thomas (1954)
***Metanephrops challengeri* (SMR)**	10	$y = 0.0037x^{0.545}$	this study
***Metanephrops challengeri* (MMR)**	10	$y = 0.691x^{-0.604}$	this study

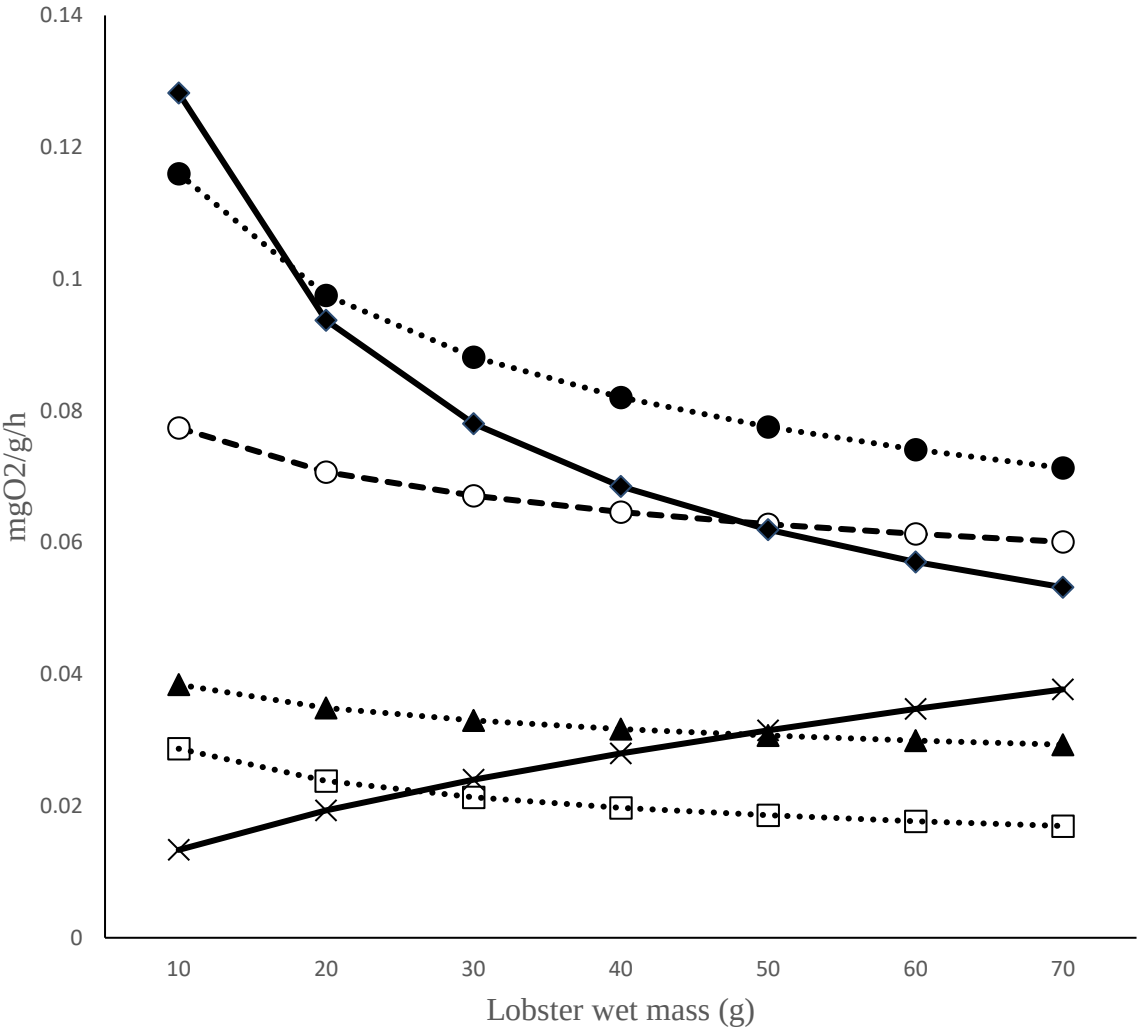


Figure 5.8 The relationship (based on the regression expressions in Table 5-3) between weight specific oxygen consumption for *M. challengeri* SMR (-X -), *M. challengeri* MMR (♦), *N. norvegicus* SMR (·▲·) (Bridges & Brand, 1980) , *N. norvegicus* SMR (·□·) (Parslow-Williams, 1998), *N. norvegicus* MMR (·●·) (Parslow-Williams, 1998) and *H. americanus* SMR (- O -) (McLeese, 1964). Note: *H. americanus* SMR oxygen consumption is shown from animals held at 15 °C, while all other measures are from 10 °C. Equations from referenced papers were converted to $mgO_2/g/hr$ for the purpose of this comparison.

Scampi appears to have oxygen consumption at rest and active at 10 °C similar to *Nephrops norvegicus* of similar sizes (Figure 5-8 and Table 5-3) (Alcaraz & Sardá, 1981; Bridges & Brand, 1980; Hagerman & Uglow, 1985). The oxygen consumption rate of resting *H. gammarus* (Thomas, 1954) appears to be similar to that of active scampi and *N. norvegicus*. Although oxygen demands in juvenile crustacea are expected to be higher than in adult crustacea, Radford et al., (2004) found that juvenile, *Jasus edwardsii* (0.7 to 2.8 g), a non-burrowing shallow water spiny lobster, had a resting oxygen requirement of between 0.063 ± 0.020 and 0.064 ± 0.001 mg O_2 g^{-1} h^{-1} (at 13 °C), which is approximately twice the resting oxygen requirement of scampi. It is suggested that more sedentary burrowing species, such as

scampi, are likely to have a lower metabolism than non-burrowing species (Bridges & Brand, 1980; Company & Sardà, 1998).

It is possible to also interpret the potential drivers of the apparent metabolic rates found in *M. challengeri* from an ecological context (Brown et al., 2004; McClain et al., 2012). It is suggested that in light limited depths (>200 m), metabolic rates and body size are more a reflection of the potential chemical energy (primarily carbon) available to them descending from the more productive surface waters (McClain et al., 2020). Therefore, a species may fit into a metabolic niche according to its energetic priorities. These priorities are influenced by anatomy, physiology and behaviour and essentially controlled by the levels of carbon availability. There may be evolutionary and ecological differences that also contribute to "specific energy regimes" of some species (McClain et al., 2020). Therefore, when considering the generalist feeding behaviour (van der Reis et al., 2018) and the potentially low availability of food resources to scampi at its inhabited depths (200 to 800 m) then a low oxygen consumption relative to other decapods (Foyle et al., 1989) (except for *N. norvegicus* which inhabits a similar habitat) reflects an existence in a constant, low carbon supply and energy environment.

5.4.1 Q_{10}

Using the SMR data, the Q_{10} of the scampi ranges from 3.01 between 7 and 10 °C and 1.22 between 10 and 13 °C providing a mean Q_{10} of 1.92. Using the MMR data the Q_{10} of the scampi ranges from 3.14 between 7 and 10 °C and 1.00 between 10 and 13 °C providing a mean Q_{10} of 1.77. Regardless of the metabolic rate used, the mean is at the lower end of the normal range for crustaceans of between 1.73 and 4.97 (Daoud et al., 2007), and below the typical Q_{10} of 2.2 which has been reported for some shallow water decapods (Belman & Childress, 1973; Clarke, 2017a; Donnelly and Torres, 1988) (Table 5-4).

Table 5-4 A summary of Q_{10} reported in marine crustaceans. For comparison with scampi found in this study.

Crustacea group or species	Temperature range (°C)	Q_{10}	Source
Metanephrops challengeri	7 - 13	1.92	This study
Metanephrops challengeri	7 - 10	3.01	This study
Metanephrops challengeri	10 - 13	1.22	This study
Epipelagic copepods	-1.4 - 30	2	Clarke (2017)
Panulirus interruptus **larvae**	12.5 - 17.4	2.48	Belman & Childress (1973)
Jasus edwardsii	3.5 - 7.5	1.6	Forgan et al., (2014)
Jasus edwardsii	6.3 - 12	3.1	Forgan et al., (2014)
Jasus edwardsii	18 - 20	3.7	Thomas et al., (2000)
Homarus gammarus	10.5 - 14	3.6	Tully et al.,(2000)
Caridae	7 - 20	2.19	Donnelly & Torres (1988)
Penaeidae	7 - 20	2.19	Donnelly & Torres (1988)
Sergestidae	7 - 20	2.22	Donnelly & Torres (1988)
Euphausidae	7 - 20	2.54	Donnelly & Torres (1988)

Both SMR and MMR are very responsive to temperature between 7 and 10 °C (Q_{10} >3) and then both tend to plateau (Q_{10} <1.22) as the scampi enter an optimum thermal region between 10 and 13 °C. While being generally less sensitive to temperature change (mean SMR Q_{10} = 1.92), SMR was seen to increase steadily with warming from 7 to 13 °C, resulting in the greatest measured aerobic scope (and presumed thermal optimum) at between 10 and 13 °C. Aerobic scope declines rapidly below 10 °C, driven by the lowering MMR.

It has been suggested that burrowing crustaceans have a lower oxygen demand than non-burrowing crustaceans (Bridges & Brand, 1980) and therefore *M. challengeri* would be expected to have a lower than "normal" Q_{10}. Prosser (1973) suggested that animals with a Q_{10} value <2 would indicate that some form of metabolic compensation is taking place. Considering the burrow environment that scampi inhabit is likely to have a lower oxygen content than that of the water column, this would probably be a necessity.

In conclusion, *M. challengeri* appears to have evolved to occupy a deep-water low food energy habitat with a narrow and stable temperature range. This is reflected in the narrow band of temperature tolerance and low metabolism rate as indicated by oxygen requirements and low Q_{10}. This species does not appear to have much potential to acclimatise to temperatures outside its habitat range, a feature of species found in stable and constant narrow range habitats. From

an aquaculture perspective, transferring wild adult scampi from its natural habitat at depth does not appear to have a negative impact on their wellbeing making the acquisition of broodstock from the wild feasible. These initial results indicate the optimum temperature for the cultivation of adult *M. challengeri* is between 10 and 13 °C, with temperatures >13 °C affecting well-being. This species has a low Q_{10} when compared with other crustaceans, which indicates scampi is likely to be a slow growing species. The narrow temperature range of *M. challengeri* reduces any prospect of increasing the Q_{10} through increased culture temperature. Any improvements in overall growth rates for aquaculture of scampi would need to come via other avenues, such as nutrition and probiotics (El-Saadony et al., 2021; Prabu et al., 2017; Jeffs et al., 2020), enhancing growth rates, health and moulting success and genetic selection (Regan et al., 2021), selecting for faster growing and gregarious traits.

Chapter 6 : Discussion

6.1 Introduction

Prior to this study, knowledge of the biology of *Metanephrops challengeri* was mostly limited to information derived through research to support the management of the wild fishery (Cryer et al., 2005; Cryer et al., 2001; Cryer et al., 2004; Cryer & Stotter, 1999; Tuck, 2014, 2015; Tuck & Dunn, 2012) with only one published preliminary study focused on larval development (Wear, 1976). There has been some more recent research, which ran simultaneously with this study (Major & Jeffs, 2018; Major, 2017) that also relied on the methods for catching, transporting and holding live scampi that were developed as an essential component of this current research. Using captive held scampi allowed these researchers to examine sensory and feeding behaviour in relation to developing baits for potting scampi. To date, there has been no research focused on the biology and husbandry of scampi in captivity.

The aim of the research presented in this thesis was to examine some key aspects of the biology of *M. challengeri* that can be used in the assessment of the aquaculture potential and fisheries management of this species. The results will also be potentially useful for guiding the potential aquaculture development of other deep-water crustaceans for which little is known.

On commencement of the research there was significant uncertainty regarding the feasibility of capturing live adult *M. challengeri* from great depth (200 - 400 m), bringing them to the surface, transporting them to a land-based facilities, successfully holding them in the facilities, hatching the eggs and rearing the larvae. It was also not known how well scampi would tolerate the experimental conditions. Some assumptions were made based on other species, primarily *Nephrops norvegicus*, *Homarus americanus* and *H. gammarus* (Bridges & Brand, 1980; Carlberg & Van Olst, 1976; de Figueiredo & Vilela, 1972; Drengstig & Bergheim, 2013; Lim et al., 1997; Pochelon et al., 2009; Powell & Eriksson, 2013; Rotllant et al., 2001). There has been a small amount of research on captive rearing of other members of the genus *Metanephrops*, however, these studies provided inadequate information as to the methods of live capture using a trawl (pots have been unsuccessful for the capture of live *M. challengeri* (Major, 2017)), transport and culture protocols (Okamoto, 2008b).

After some experimentation, *M. challengeri* was successfully captured, transported and held in a seawater facility with appropriate water quality and general conditions (i.e., sterilised water, red light, correct temperature and high oxygen etc), which facilitated the development of

holding and cultivation protocols and ultimately enabling this study to progress. After acclimation of the scampi that had been captured and transported from the wild, the mortalities in the live holding facility were low (<5%) in the year following capture. With these protocols established, the study focused on four aspects of the biology of *M. challengeri*; the fecundity of the species (Chapter 2), the influence of temperature on the oxygen usage (respirometry) (Chapter 5) of the adults, the influence of temperature on the development of the embryo (Chapter 3), and the rearing and taxonomic description of the larval and first post-larval stage Chapter 4).

6.2 Reproductive biology

The members of the *Metanephrops* genus are suggested to have originated in Antarctica in the Cretaceous period (Tshudy, 2013) and expanded northwards. *Metanephrops challengeri* is found in the deep waters of the mid latitudes of the southeast Pacific where relatively stable cold temperature and low light conditions prevail. Thus, this species has had considerable time to adapt to the environmental conditions within its current distribution range in New Zealand. This study has confirmed that the temperature range tolerated by *M. challengeri* larvae and adults adheres closely to the relatively narrow thermal range found within its habitat. However, it was found that scampi have little physiological capacity to operate outside of this relatively narrow thermal range. Temperatures over 13 ℃ appear to have a detrimental impact on the wellbeing of adults and larvae, with a temperature of 15 ℃ leading to a chronic decline in health and eventual death. The study did not test the effect of temperatures below 7 ℃ on the adults or larvae and, based on observations and the literature it was determined that activity and embryo development is slower at cooler temperatures, with an estimated critical low minimum at 2.5 ℃ (Cumillaf et al., 2016), the minimum temperature tolerance of *M. challengeri* was not experimentally determined.

Temperature has a significant influence on embryo development time and embryo volume (Chapter 3) (Charmantier & Mounet-Guillaume, 1992; Fischer, 2009; Miller et al., 2016; Mori et al., 1998; Smith, 1987, Yamamoto et al., 2017). The rate of development of the embryo increases with increasing temperatures (Sardà, 1995; Yamamoto et al., 2021). It is estimated that the embryo of *M. challengeri* will take 318 days from extrusion to hatch at 7 ℃ and 226 days from extrusion to hatch at 13 ℃. Survival of the eggs decreased with increasing temperature over the temperature range tested. The volume of eggs taken from wild females also showed an increase in size with the advancement of embryo development. It was also

shown that the volume of the eggs at hatch increased with increasing temperature. Collectively, these findings have implications for both aquaculture and management of the scampi fishery. For example, differences in embryo development times due to ambient water temperatures found at different latitudes can be expected to significantly influence larval productivity and potential recruitment.

Females that were carrying eggs with developing embryos were seen to have developing oocytes in the ovary simultaneously. This is remarkable in that the female will carry eggs externally for an estimated 318 days (at 7 °C) and so in the colder regions of this species' range there is less opportunity for the females to recover from periods of maternal care. During the egg carrying phase they will require nutrient and energy reserves to resource the maturing oocytes and maintain themselves. The reserves may come from active feeding and/or body reserves. Captive female scampi were observed to feed while carrying external eggs but only to a limited extent. It has been suggested that a scampi female that is spawning annually, as occurs in the colder latitudes (e.g., SCI6), will need to gain reserves and condition in the remaining 2 months of the year, before possibly moulting and then spawning at the start of the next annual breeding cycle (Tuck 2010). This seems unlikely given that scampi, are opportunistic scavengers that cannot guarantee the quality and quantity of food resources that will be available in that relatively short window for feeding and recovery. Therefore, it is likely that scampi in this situation will probably only spawn every second year as is suggested to occur in *Nephrops norvegicus* (Bell et al., 2013; Sardà, 1991; Sterck & Redant, 1989). This would have implications with the fishery management in the colder regions in terms of the reproductive output and recruitment for this fishery. However, annual breeding is more likely to be maintained in warmer areas of this species' range (11 to 13 °C) as the embryo development period is an estimated 226 days providing a longer time window for the females to recuperate nutritional condition before initiating the next breeding cycle.

From an aquaculture perspective, maternal recovery should not be an issue in terms of broodstock production and larval output as the female would most likely be held at a temperature between 11 and 13 °C with much better nutrition on demand, which would allow time for conditioning and recuperation before the next cycle. Thus, by manipulating temperature in holding facilities, the management of broodstock, egg and larvae quality and production can be readily adjusted. For example, the timing of larval hatching can be managed to ensure continual production of larvae in an aquaculture facility, to maximize use of larval rearing capacity.

Close examination of the timing of scampi egg hatching in the laboratory during this study strongly suggested it increased at the time of the full moon. This trend became more obvious as the scampi husbandry, transport and holding conditions continued to be improved throughout the duration of this study. This has been seen in other species of benthic decapods (DeVries et al., 1983; Ferrero et al., 2002; Forward, 1987; Haker et al., 2023) and some evidence of this in deeper water species, e.g., *Chionoecetes japonicus* (Yamamoto, et al., 2021) but lunar components are still poorly understood (Mercier & Hamel, 2014). In the benthic environment of the deep sea, it is unlikely that moonlight is perceptible by scampi, however, tidal flows, gravity, or water quality conditions associated with the strength of tidal flow may provide a cue for the timing of scampi hatching, e.g., water temperature, pH or nutrient fluctuations. Mercier et al., (2011) and Aguzzi et al., (2011) suggest that variations in particulate organic matter or phytoplankton deposition and cyclic currents may be a proxy for the moon phase at depth. The fact that this phenomenon persisted in the laboratory indicates that either scampi are maintaining an endogenous lunar clock, or they are capable of detecting gravitational fluctuations, as all the other potential environmental cues would be unavailable in the laboratory situation.

The embryos of scampi and of most other *Metanephrops* species are well developed at hatching, having spent a considerable amount of time as a developing embryo in the egg (Okamoto, 2008b; Uchida & Dotsu, 1973). Upon hatching, the pre-zoea stage of *M. challengeri* is of short duration and even though it may have setae on the maxilla and maxilliped exopods, it cannot swim. The stage I zoea is a swimming stage but is lecithotrophic. Only the stage II zoea is capable of swimming and feeding. This would suggest that the evolutionarily development of the larval stages have been regressed into the egg as suggested by (Williamson, 1982), reducing the length of the free-living larval stages while extending the period of maternal incubation. This trend of regression of larval development is clear in other *Metanephrops* species, such as in *M. japonicus*, which has a pre-zoea stage of short duration that metamorphoses directly into a post-larva (Okamoto, 2008a). This truncation of the pelagic phase of development may benefit the survival of larvae by reducing their exposure to pelagic conditions and predators, however, it could be expected to limit the dispersal of the post-larvae unless they can extend their pelagic existence for a period.

An additional evolutionary consideration is the morphological variations observed in the pre-zoea and stage I zoea (Chapter 4). These may be an artifact of laboratory husbandry but they could also be due to intraspecific variation of which little is known in larval biology (Anger,

2001), particularly in the *Metanephrops* genus. Confirmation of this phenotypic plasticity and possible genetic divergence is of relevance beyond passing taxonomic interest as it may provide information pertaining to evolutionary adaptation for larval biologists.

The stage I post-larvae *M. challengeri* reared during this current study demonstrated behaviour consistent with an ability to extend their pelagic existence, presumably if they were unable to locate suitable benthic settlement habitat. The post-larvae were observed to splay their limbs to reduce their rate of sinking and to use their pleopods to actively swim in the water column. Stage I and stage II zoea are pelagic and were observed to frequent the surface of the upwellers in which they were cultured. This may indicate that larvae undertake a vertical migration as seen in the larvae of *N. norvegicus* (Powell & Eriksson, 2013) and *C. japonicus* (Yamamoto, et al., 2021). This potential vertical migration of the zoeal stages may also be associated with the lunar periodicity of hatching which could act to increase dispersal of the larvae due to spring tidal flows. During an equipment failure in the scampi holding facilities following an earthquake, *M. challengeri* larvae were observed to tolerate 17 °C for 12 hours. This may be reflective of the tolerance of the larvae to higher temperatures they may find if they vertically migrate. The eyes of larvae have been found to have a structure which changes once it seeks to settle at depth (Jinks et al., 2002; Hui et al., 2022). This is thought to facilitate a tolerance of increasing light during larval dispersal.

The moult of the stage II larvae to the post-larvae takes place in the water column at which time the post-larvae can chose to descend to the seabed or remain pelagic. Preliminary experiments showed that if offered a substrate to their liking, the post-larvae were seen to remain benthic and even to start landscaping activities. If the substrate was not suitable, then they would swim up into the water column again for a period. This substrate testing activity seems to be reduced on transitioning to stage II post-larvae when they became more benthic and their landscaping activities increase, as did their carapace colouration. Some preliminary experimentation was also undertaken to assess the burrowing capabilities of the post-larvae and juveniles. A variety of silt/fine grain sand were offered, however, it was found that if the silt was very fine the gills of the post-larvae became clogged and the post-larva either abandoned landscaping efforts or died. By adding larger sediment particles to the benthic substrate, the walls of the developing burrows tended to collapse resulting in the individual giving up or being buried and killed. This would indicate that the post-larvae and juveniles select a very specific benthic substrate type to excavate a burrow (Eriksson & Baden, 1997), unlike *N. norvegicus* (Powell and Eriksson, 2013) which is less discerning. Alternatively,

perhaps *M. challengeri* wait until they are larger to excavate a burrow when they are not as susceptible to their gills becoming clogged. In the interim they may co-inhabit burrows with adults as seen in *N. norvegicus* (Rice & Chapman, 1971; Tuck et al., 1994; Powell and Eriksson, 2013) or perhaps live in depressions in the substrate relying on their smaller size and camouflage for survival. The post-larvae of *N. norvegicus* have been commonly observed to burrow, with activity increasing with age (Eriksson & Baden, 1997; Rice & Chapman, 1971; Powell and Eriksson, 2013).

The longest lived settled post-larvae that was reared in the facility was approximately 22 months. It was held at 10.5 °C and completed 8 moults in that period and yet it was still only weighed approximately 10 g. However, the intermoult period was seen to be highly variable among juveniles and it is suggested that it is linked to providing appropriate nutrition and possibly the gut microbiome. These factors can be manipulated in the future in order to further improve health, moult success and growth rates.

6.3 Temperature and growth

The most important extrinsic factor, excluding nutrition, influencing growth of decapods is temperature (Anger, 2001) and therefore an important aspect of this study. The temperature dependant aerobic growth curve is defined as the difference between the standard and maximum metabolic rate (Stoffels et al., 2016). This provides the ability to assess the scope for growth and to compare it to other species. The results of research presented in Chapter 5 shows that the respiration rates of adult *M. challengeri* are not very different from other lobsters (e.g., *H. americanus*, *H. gammarus*, and *N. norvegicus*) at the temperature range of 7 to 13 °C (Bridges & Brand, 1980; McLeese, 1964; Parslow-Williams, 1998; Thomas, 1954). However, unlike many other lobster species, *M. challengeri* was found to have a narrow thermal range. It has been suggested by Cumillaf et al., (2016) that deep sea crustacea have a critical minimum tolerance of 2.5 °C and therefore it is suggested that *M. challengeri* can tolerate cooler temperatures than were examined in this current study (i.e., <7 °C). However, the species cannot tolerate prolonged (days) exposure to 15 °C.

As shown in the research presented in Chapter 5, the Q_{10} of the scampi ranges from 3.01 between 7 and 10 °C and 1.22 between 10 and 13 °C providing a mean Q_{10} of 1.92. This is at the lower end of Q_{10} measures from other crustaceans in general (Clarke, 2017a; Forgan et al., 2014; Tully et al., 2000) and is associated with a slow growth rate. Since *M. challengeri* has a

narrow temperature range with a low upper limit to its thermal range of approximately 14 °C there is little room to improve the Q_{10} in a culture situation through manipulation of rearing temperature. It is suggested that the low maxima expressed by scampi is not due to the amount of oxygen available in the water but that it has thermally limited enzyme variants, as found in poikilotherms that inhabit cold narrow range habitats (Clarke, 2017b). In cold conditions it has also been suggested that cold-adapted ectotherms increased mitochondrial function and density which may increase scope for growth (Fangue et al., 2009; Johnston et al., 1998; Lucassen et al., 2006; Stoffels et al., 2016). This would facilitate metabolism at cooler temperatures, however, as temperatures increase there will be a point where the extra mitochondrial activity demands more oxygen than can be provided. Therefore, in this instance the scampi may be reaching an oxygen delivery issue to the tissues which is the key constraint to a higher thermal range of the species. Siebel and Drazen (2007) have an alternative view on the drivers of metabolic rates of deep-water species. Although their focus was on pelagic species, they suggest that the metabolic rates of deep-water species is related to the requirements of survival, i.e., locomotion capacity associated with visual predation/escape activity. As scampi are burrowing species, they can gain refuge in their burrows reducing predation risk and therefore have evolved for reduced energy expenditure. This theory does not entirely explain the low Q_{10} of the scampi but perhaps it has an influence. Regardless of the mechanisms, these potential stenothermic characteristics means that the habitat availability is confined by substrate and temperature. It also means that there are limited prospects of using temperature increases to improve growth potential for aquaculture in scampi unless there is sufficient genetic diversity and plasticity within the population which may expand this tolerance. Genetically there does not appear to be a phylogeographic partition between the remote southern SCI6 (Auckland Island) population of scampi and the more northern SCI1 (Bay of Plenty) population. However, some genetic variation has been found among other locations within the extensive range of the scampi populations (Verry, 2017). Therefore, there may be some *M. challengeri* individuals found in shallower waters or at lower latitudes that may have a greater tolerance to higher temperatures, which could enhance growth prospects under aquaculture conditions. Essentially this is part of the process of selective breeding and domestication for aquaculture (Fujaya et al., 2016; Gjedrem & Baranski, 2009; Hoa, 2009) which *M. challengeri* will have to go through to improve growth performance for aquaculture.

6.4 Diet

The fact that *M. challengeri* is a scavenger (van der Reis et al., 2018; van der Reis, 2021), and if it mimics *N. norvegicus* in filter feeding on organic particulates (Santana et al., 2020) then this can be an advantage to its husbandry and culture. There has been considerable research into crustacean nutrition (Bordner et al., 1986; D'Abramo et al., 1997) in general and there are many studies which focus on individual species, e.g., *H. americanus* and *H. gammarus* (Daniels, 2011; Fiore & Tlusty, 2005; Tlusty et al., 2005), individual aspects of diets (Haryanti et al., 2021; Sales, 2010) and morphological adaptation to feeding capability, such as suspension feeding (Lavalli & Barshaw, 1989; Loo et al., 1993, Santana et al., 2020) and feeding appendages (Sahlmann et al., 2011).

As this study was the first to look at rearing *M. challengeri*, the goal was to achieve survival of larvae, juveniles and adults, each of which had different challenges. The adults, being scavengers were the easiest to feed and maintain, choosing to consume the types of tissue naturally found and consumed in their habitat (e.g., fish, shellfish) (van der Reis, 2021). This does not disregard the prospect of significantly improving the diets for broodstock and growout in the future. The initial effort in terms of the larvae and juvenile diets were simply directed at particle size and palatability. Fortunately, the stage II larvae will capture and consume live *Artemia* as well as frozen enriched *Artemia* and organic particles. This eager acceptance of *Artemia* by the scampi larvae readily facilitates the development of the enhancement of larval diets through experimentally varying enrichment formulations. To improve the diet of larvae and juveniles an artificial pelleted diet was formulated based on the requirements outlined in the literature (Council, 2011; D'Abramo et al., 1997) for crustacean larvae and juveniles. (Table 6-4). This diet was designed to provide an appropriate amino acid profile through the provision of protein from different sources. The polychaete meal was determined to be a strong appetite enhancer in this species. Calcium was added to ensure there was sufficient calcium for the formation of the carapace. Minerals and vitamins were added based on the perceived requirements of other marine crustaceans. Alginate and kelp were added as a binder. Fish oil provided an source of marine lipids. The ultimate energy levels were consistent with the requirements of other crustacea as found in the literature (Council, 2011; D'Abramo et al., 1997). Pellets were cold extruded in a desk top extruder. Pellet sizes were from 0.5 to 3 mm.

Table 6-1. The experimental diet tested with post-larvae and juveniles

Ingredients	Actual g/kg
Fish meal	340
Krill meal	250
Polychaete meal	150
Squid meal	160
Seaweed – Kelp	20
Calcium carbonate	20
Mineral and Vitamin mix	10
Cholesterol	2.6
Fish oil	20
Alginate – Binder	27.4
Proximate composition (per kg)	
Dry Matter, g	963
Gross Energy, MJ	15.1
Protein, g	553
Lipid, g	102
Ash, g	182

The diet seemed to be highly attractive to the post-larvae and juveniles, however, it appeared to clog the mouth parts and excessive cleaning behaviour followed after a period of feeding. Although scampi did consume reasonable quantities of the experimental diet, there were obvious issues which resulted in poor larval survival. Therefore, commercial pellets (Prochaete™) were tested which resulted in improved survival. Adding cultured live microalgae (*Isochrysis galbana, Chaetoceros calcitrans, Pavlova lutheri*) to the rearing waters of the larvae and post-larvae enhanced health and survival as has been found in other rearing studies of decapod larvae (Daniels, 2011; Fiore & Tlusty, 2005; Mente, 2010; Powell & Eriksson, 2013; Tlusty et al., 2005). Recent research by Santana, et al., (2020) suggests that the diet of *N. norvegicus* consists of 47% suspended organic matter. Based on the fine assemblage of the seta on the mouth parts of both adults and larvae (Chapter 4) it is quite possible that *M. challengeri* could also potentially consume more particulate matter than previously considered. However, based on the diet analysis by van der Reis et al., (2018) this does not seem to be the case. The larval and juvenile survival and health were further enhanced through improved holding methodology and bespoke system design. Overall, while this study has been able to raise numbers of larvae and juveniles experimentally, there remains significant scope to

improve the survival and health of larvae and juveniles through further research into more effective diets.

6.5 Aquaculture

Metanephrops challengeri is a relatively high value seafood species but the trawl fishing methods used for harvesting this species from the wild generate considerable sustainability concerns, including seabed damage, liberation of carbon sequestered in the seabed, high fossil fuel use, and high bycatch (Anderson, 2012; Anderson & Edwards, 2018; Ballara, 2009; Hartill et al., 2006). Therefore, the production of scampi through land-based aquaculture has the potential to improve the sustainability performance and offer a higher quality product, such as supplying live seafood markets with a premium product. The high value of this species would offset some of the costs of the biological constraints of this species identified through this current study, i.e., low fecundity, long egg incubation periods, low Q_{10} and therefore slower growth. However, the growth rates and survival rates could be enhanced through improved diets, development of optimal rearing conditions, broodstock management and selective breeding.

6.6 Advantages for aquaculture

Through the handling and husbandry of scampi for this current study, it has been observed that scampi is a robust species that is very tolerant of aquaculture conditions, provided that suitable environmental conditions are maintained. The larval stages of scampi are of short duration and there are only three larval stages with minimal feeding required and an absence of cannibalism. This is in contrast to many of the challenges of rearing larvae of other lobster species being developed for aquaculture, such as the clawed and spiny lobsters, which have longer duration, multiple stages of larval development, exacting feed requirements, and cannibalism, (Fotedar & Phillips, 2011; Jeffs et al., 2020). Scampi larvae have high survival and can be raised in high densities (30 to 45 larvae per l) in relatively simple upweller larval tanks. In contrast, larval densities in *N. norvegicus* are reported to be between 3 and 25 per l (Pochelon et al., 2009; Smith, 1987). The larvae of *H. americanus* are suggested to be even more aggressive than *N. norvegicus* (Powell & Eriksson, 2013) and to require much lower densities again. The larvae of scampi, like the adults, readily capture and consume live and prepared diets making diet formulation straightforward. Similarly, all of the life stages of scampi will consume prepared diets making future diet formulation much more achievable compared to species which are

constrained by behaviour propensity for acceptance of live or fresh feed (Jeffs, 2020). Mating of scampi was observed in captivity during the course of the study therefore there appears to be the potential to study and/or develop breeding strategies of this species. Observations made by Katoh (2011) on *N. norvegicus* tend to support the potential for this development also being successful in scampi.

6.7 Disadvantages for aquaculture

Females spend a large proportion of their mature life in an ovigerous state and so are not good candidates for aquaculture as their somatic growth is curtailed as resources are directed towards egg production (Cavalli et al., 2001). The number of eggs produced by a single female is relatively low compared to other lobster species (Burton, 2003; de Figueiredo & Thomas, 1967). The embryos have a long development period, and the eggs must be raised on the females for this period to ensure survival. Thus, maintaining sufficient brooding females to supply an aquaculture operation with juveniles will require significant operational resources. Juveniles and adults are slow growing and therefore will require grow-out facilities to be extensive in order to hold multiple cohorts of scampi for extended periods of time until they reach sufficient size for harvesting. It may be possible to develop an egg incubation system for eggs separated from the female scampi soon after extrusion to greatly improve efficiency of egg production. However, egg incubation systems for brooding invertebrates are frequently challenged by pathogen infection and can be difficult to develop to achieve high hatching success (Spreitzenbarth, 2021).

6.8 Recommendations for future research

This study has provided some valuable new knowledge on the fundamental biology of *M. challengeri*. Further research is required on this species to refine the knowledge generated through this study but also to investigate additional areas, such as nutritional requirements, possible methods of enhancing growth in culture (e.g., selective breeding, generation of single sex populations for culture), the mechanisms which allow tolerance/conformance of variable water quality conditions (e.g., oxygen, pH, nitrate etc) found inside and outside their deep-water burrows on the sea floor. The influence of future climate change factors (e.g., pH, marine chemistry) on this deep, cold water species also warrants research attention.

The *M. challengeri* egg is large and provides resources to support a developing embryo for a considerable period of time as well as the subsequent lecithotrophic larval stages. Therefore, the development of effective broodstock diets will be critical for ensuring female scampi can accumulate and allocate sufficient nutritional resources toward producing well-resourced, high quality eggs.

The prominent cause of mortality in the larvae and juveniles is at the moult. Either insufficient energy is being provided to the life stage to support moulting to the next stage or there is one or more nutrients lacking in the diet or seawater to facilitate successful moulting. This may include precursors (e.g., cholesterols and sterols) to hormones required for moulting, or phospholipids, which are known to be implicated in moulting success (D'Abramo et al., 1984; Hammond, 2006; Irvin et al., 2010; Noordin et al., 2020; Yeap et al., 2022). Integrating some types of lipids into diets for *M. challengeri* may be challenging given the changes in physical characteristics of lipids at the lower temperatures in which *M. challengeri* are cultured. Research aimed at eliminating mortalities at the moult would greatly improve the efficiency of any aquaculture production of *M. challengeri*.

During this study *M. challengeri* larvae were observed to tolerate 17 °C for 12 hours during an equipment failure in the scampi holding facilities following an earthquake. This indicates that the larvae may well be able to tolerate near sea surface temperatures during a vertical migration for at least short periods. The extent of the tolerance of the larvae and juveniles to higher temperatures and the implications for ongoing growth, health and survival need to be examined further.

The suggestion that the larvae undertake an upward vertical migration needs further testing. It is possible that the larval eyes will be damaged if they encounter the higher light levels present in the photic zone near the sea's surface. Therefore, the limiting factor (light or temperature) should also be determined as this will provide a better understanding of larval dispersal capabilities in *M. challengeri*.

Larval nutritional requirements need to be investigated so as to facilitate the production of healthy post-larvae through larval culture. Such research would extend from an understanding of broodstock provisioning of the eggs and lecithotrophic larval stages. The nutrients provided to a well-resourced egg, their utilisation by the embryo and the influence of temperature on nutrient usage will inform what the larvae will require at first feeding.

During this study *M. challengeri* juveniles and adults were offered a commercially prepared prawn feed that contained high protein and lipid levels. A number of *M. challengeri* consuming the feed were observed to lose appetite and were euthanised and dissected. It was found that there was a bolus of what appeared to be feed material blocking the stomach. It is possible that this is caused by carbohydrate binders commonly used in commercial prawn feeds being indigestible by *M. challengeri*, as it would seem unlikely they would encounter significant amounts of complex carbohydrates in their diet in the wild. Further, research will be required on the formation and presentation of prepared diets for scampi if their performance under aquaculture conditions is to be maximised.

Observations of *M. challengeri* juveniles indicated they appeared to consume sand particles, possibly to assist with their digestion. Particulate organic matter (POM) has also recently been shown to make up a considerable percentage of the diet of *N. norvegicus* (Santana et al., 2020). If this is the case (the necessity for both sand and POM), then they will need to be provided with POM and sand particles of a suitable size in culture. The size of sand particles that is ideal to assist with digestion would need to be determined. Incorporating sand grains to the feed would be preferable as rearing animals with substrate provides extensive substrate for bacterial proliferation and adds to the complexity of cleaning culture systems and potentially compounding scampi health issues.

It is acknowledged that the use of consecutively increasing temperatures to determine the SMR and MMR of *M. challengeri* may have influenced the corresponding respirometry results as it may have introduced a degree of thermal acclimation. Future research into respiration would benefit from randomising the treatment temperature to ensure acclimation does not influence results.

The respiration of larvae and scope for growth among smaller scampi needs to be better assessed to aid in the understanding of this deep water species and the potential influences of climate warming on this species.

6.9 Conclusions

Metanephrops challengeri has been a challenging species to research with little to compare it to in terms of laboratory research of other *Metanephrops* species. Its adaptation to its deep-water habitat has left it relatively stenothermic in terms of tolerance to variations in temperature. Although it has been shown to have a low fecundity when compared to other

lobster species, and a long egg development duration, the larvae are advanced on hatching. Based on the Perkins eye index, the development period of the embryos can be significantly decreased with increasing temperatures to an upper limit estimated to be 13 °C. Embryo development appears to be optimal in terms of survival at approximately 10 °C. Egg volume also increased with increasing temperature. Upon hatching, the life stages include pre-zoea (which can be considered a late embryo stage), a stage I zoea and a stage II zoea before moulting into the post-larvae. All these stages have a short duration (hours or days). Adults cannot tolerate 15 °C or above for extended periods. Temperature has a significant effect on oxygen consumption of the adults and the Q_{10} is low when compared to other lobsters.

With these parameters in mind, as an aquaculture species New Zealand scampi, *M. challengeri*, has some potential, but it is limited mainly by the extended duration of egg development and slow growth rates. It might be possible to improve the growth rate with correct diet, selective breeding, and husbandry methods, however, collectively the undertaking of this research and development is considerable, and the extent of the likely gains to aquaculture performance are somewhat constrained. However, there remain significant gaps in the knowledge of this species and advances could arise with future research. With the husbandry information derived from this preliminary study, future research should be significantly easier. The animal itself is a good candidate for research in that, if held in the right conditions, is robust and appears to be conducive to husbandry and laboratory experiments.

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