Energetics of successful settlement in spiny lobsters

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Abstract

Spiny lobsters have an oceanic larval phase (i.e. phyllosoma) that can persist for over a year in some species. These larvae are active and specialised predators of macrozooplankton. The phyllosomal phase in spiny lobsters ends when they metamorphose into a transitional post-larval puerulus, a nektonic, non-feeding phase that relies on energetic reserves accumulated during the previous phyllosomal phase, a phenomenon known as secondary lecithotrophy. Pueruli actively swim towards the coast, and upon arrival in shallow coastal waters they seek out suitable benthic habitat to settle and moult into benthic juveniles, and recommence feeding. Secondary lecithotrophy limits the energy available to make this onshore migration, and it has been postulated that increasing seawater temperatures increase the bioenergetic requirements for the directed movement of pueruli.

To test this hypothesis, the energetics of the nektonic phase and early juvenile stages of two species of spiny lobsters were studied: *Jasus edwardsii* and *Sagmariasus verreauxi*.

Wild caught pueruli of *J. edwardsii* were experimentally swum for periods of up to 5 days whilst measuring changes in their total lipid and protein content. Using this method pueruli of this species were estimated to use 96 J per day while in transit. A similar experiment was done for wild pueruli of *S. verreauxi* at three seawater temperatures (i.e., 17, 20 and 23 °C) with the pueruli using 23.1 J, 42.4 J and 48.6 J per day respectively. The time that pueruli of *S. verreauxi* spent swimming decreased markedly with increasing temperature with a 27% reduction in swimming when temperature increased from 17 to 23 °C.

Swimming experiments of pueruli of both species showed them matching the speed of the currents inside the tanks (0.15-0.17 m s^{-1}) and exhibiting rheotaxis. Pueruli would swim both during the night and the day.

Starvation experiments were performed in both species to investigate post-pueruli energetics in first instar juveniles. Once pueruli had moulted into first instar juveniles they were not allowed to feed until they perished, with their lipid and protein content compared to levels in control pueruli pre metamorphosis. It was estimated that first instar juveniles of *J. edwardsii* use 25.0 J per day and *S. verreauxi* use 11.7 J per day during starvation. There was no difference in the energy use of first instar juveniles of *S. verreauxi* starved at 17, 20 or 23 °C. For *S. verreauxi* the recovery time post-starvation was also examined, this was calculated to be 30.4 days, if refed within this time frame 50% can recover and continue developing.

The results of this study indicate the lowest levels of lipid that are viable for survival and continued development in pueruli and first instar juveniles of *S. verreauxi* is around 6% (dry weight), and when they approach this threshold lipid is spared and protein catabolism is used to meet metabolic demands.

Collectively the results of this research indicate that the transport success of pueruli into inshore and coastal waters is likely to be negatively affected by increasing seawater temperatures. However, once pueruli moult to the first instar juvenile stage they become much more tolerant of elevated temperatures. This can result in reduced recruitment of lobsters into the population.

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Chapter 1. Introduction

1.1. Economic importance of spiny lobsters

Spiny lobsters are decapod crustaceans belonging to the family Palinuridae comprising at least 12 genera with over 60 species (Chan 2010). Spiny lobster species are found in coastal areas within temperate and tropical seas over much of the world, with their northernmost limit of distribution in Scotland (*Palinurus elephas*) (Ansell and Robb 1977), and the southernmost distribution in the south of New Zealand (*Jasus edwardsii*) (Jeffs et al. 2013). Spiny lobsters are highly valued as seafood around the world, being commercially harvested in more than 90 countries and with all fisheries fully exploited (Booth 2006). The global seafood market demand for spiny lobsters has been increasing rapidly in recent years as a result of growth in Asian economies where the consumption of lobsters is traditionally associated with luxury and elevated social status (Hart 2009, Jeffs 2010). For example, in China the import of lobsters increased by 10 fold from 2001 to 2014 (Pereira et al. 2017), with market prices over this period showing a continual upward trend. For example, the average export wholesale price for live lobsters from New Zealand to China was NZ$79 in 2010 compared to NZ$105 in 2017 (New Zealand Seafood Export Database).

Two species of spiny lobster have natural ranges that cross the Tasman Sea and form the basis of important fisheries in both southern parts of Australia, and in New Zealand. The red rock lobster, southern rock lobster or koura, *Jasus edwardsii*, is found around the coast of all of New Zealand and parts of southern Australia, from Western Australia to New South Wales (NSW) and including Tasmania (Jeffs et al. 2013). The annual commercial landings of *J. edwardsii* in Australia during 2014 was 2940 t with a gross value of production of AUD$207.5 million (Plagányi et al. 2017). In New Zealand the average yearly catch from 2010-2015 was 2,839 t, and the export revenue of *J. edwardsii* in 2016 was NZD$286 million (Williams et al. 2017). The packhorse lobster, eastern rock lobster or pawharu, *Sagmariasus verreauxi* is found along the coast of most of New South Wales with low numbers in northern Tasmania. In New Zealand

the population of this lobster is centred around the north-eastern tip of the country, with individuals occasionally found as far south as Stewart Island (Booth 2006). In New South Wales, the annual commercial catch of this lobster is around150 t with a gross value of AUD$11 million in 2014 (Plagányi et al. 2017). In New Zealand the catch of *S. verreauxi* is valued at around NZD$1.2 million a year (Williams et al. 2017).

During the period of 2005-2009 there was a 20% decrease in global spiny lobster landings that was likely to be caused by a combination of overfishing, climate change, disease, habitat destruction coastal pollution and a marked decrease in the recruitment of post-larval lobsters for several species of spiny lobster that support substantial commercial fisheries (Jeffs, 2010). Climate change can affect the oceanic larval phase of the spiny lobster lifecycle indirectly by changing ocean circulation, potentially making their journey longer or trapping them in water masses with low prey (Chiswell and Booth 1999, Yeung and Lee 2002, Cai et al. 2005, Caputi et al. 2010b, O'Rorke et al. 2014), or directly by increasing metabolism and thermal stress (Tong et al. 2000a, Fitzgibbon and Battaglene 2012a, Fitzgibbon et al. 2014b, 2014a, 2017). Any of these environmental changes, or a combination of them, would likely reduce the number of post-larval lobsters that can reach the coast and recruit to the population, ultimately impacting the sustainability of the fishery.

There is evidence that the temperature in the Tasman Sea has increased due to the intensification of the East Australian Current, which brings warm water with less nutrients into the Tasman Sea region (Ridgeway 2007). These changes in temperature are causing reduction in phytoplankton (Thompson et al. 2009) and macroalgae, expansion in the distribution of sea urchins, and slower growth rates in abalone along the eastern coast of Tasmania (Johnson et al. 2011). The use of models to hindcast the recruitment of *J. edwardsii* in Tasmania using catch and effort data, growth data, and population size structure, and relating it to sea surface temperatures have found that rising sea temperatures has negatively affected recruitment (Pecl et al. 2009). Furthermore, future projections using climate change scenarios indicate that the recruitment in this spiny lobster species will continue to decrease.

In New Zealand *J. edwardsii*, post-larvae have been found to be energetically compromised (Wilkins and Jeffs 2011) which could affect recruitment. Models of larval dispersal have found that the settlement of *S. verreauxi* in south-eastern Australia will be negatively affected by changes in ocean circulation due to climate change, although it also thought that warming sea temperatures may lead to increased larval survival (Cetina et al. 2015). Other studies of *S. verreauxi* have found that post-larvae are already likely to be facing recruitment failure due to energetic shortcomings attributed to climate variation (Fitzgibbon et al. 2014).

1.2. Importance of temperature in spiny lobsters

Some of the consequences of climate change are large scale changes in ocean circulation, increases in the frequency, intensity and duration of heat waves, incremental increases in ambient seawater temperature means, as well as increasing temperature in superficial oceanic and coastal waters (Meehl et al. 2007). Spiny lobsters are ectothermic, and as such operate within a specific range of temperature dictated by their metabolic tolerances that in turn also restricts their geographic range (Pörtner et al. 2005). Ectotherms operating at temperatures below their lower thermal tolerance have greatly reduced mitochondrial function that prevents their metabolic energy demands being met. In ectotherms the rate of metabolism normally increases with temperature (Clarke and Johnston 1999, Gillooly et al. 2001), while at temperatures above the upper thermal tolerance of a species the oxygen demand cannot be met by normal respiratory function (Pörtner 2002).

The thermal tolerance and thermal optimum for a species of spiny lobster can change throughout its development. For example, in *S. verreauxi* the early and mid-stage larvae (known as phyllosomas) have optimum growth at 23 °C, but late stage have their optimal growth at 21 °C (Fitzgibbon and Battaglene 2012b). Pueruli of this species has an optimal temperature range of 15 to 24 °C (Fitzgibbon et al. 2014b), whereas for 60-day-old juveniles the optimal temperature is 21.5 °C (Fitzgibbon et al. 2017). Pueruli and juveniles of *S. verreauxi* are metabolic conformers such that when water temperature increases from 15 to 27 °C the standard and resting metabolic rates for both pueruli and juveniles increase proportionately (Fitzgibbon et al. 2014b, 2017). In contrast the ambient seawater temperatures

throughout the distribution range of adult *S. verreauxi* varies from 11 to 27 °C (Condie and Dunn 2006).

In *J. edwardsii* the optimal temperature for growth of early larval stages is 18.2 °C (Bermudes and Ritar 2008). In comparison the growth in juveniles was found to be optimal at 20.6 °C (Thomas et al. 2000), while in adult lobsters optimal growth has been found at 18 °C (Hooker et al. 1997). In contrast the ambient water temperatures throughout the distribution range of adult *J. edwardsii* varies from 8 to 23 °C (Hooker et al. 1997).

Given the differences in the physiological responses to temperatures at different developmental stages of spiny lobsters, it is important for research aimed at determining their possible responses to environmental temperature changes, to use the appropriate developmental stage for any experimental determinations.

1.3. Biology of two Australasian spiny lobster species

1.3.1 *Jasus edwardsii*

Female *J. edwardsii* reach sexual maturity at 60–120 mm carapace length (Annala et al. 1980) and males at 55-85 mm (MacDiarmid 1989). Most males moult between October and November and in northeastern New Zealand the females moult between April and May, with the larger lobsters molting earlier that the smaller ones (MacDiarmid 1989). Mating activity is at its highest in June, and most of the resultant larvae hatch in the period of September-November (MacDiarmid 1989). In more southern latitudes with colder waters, the females moult around 2 to 3 months earlier and the egg incubation period lasts 5 months (Street 1969), whereas in the northeast of the country the incubation period averages 111 days (MacDiarmid 1989). In culture the larval duration averages 322 days (Kittaka, 1994), but in the wild the larval duration is estimated to last on average 18.2 ± 1.6 months(Bradford et al. 2015).

1.3.2 *Sagmariasus verreauxi*

Females of *S. verreauxi* reach maturity at 150-170 mm carapace length and typically release the eggs from September to January (Montgomery 1992). When raised under culture conditions, phyllosomas (i.e., larvae) of this species usually pass through 17 moults in 234-263 days to metamorphose to become pueruli, which then take an average of 25.5 days to moult to become first instar juveniles (Kittaka 1997). In NSW the pueruli arrive to settle on the coast mostly from September to January, so the oceanic larval duration is thought to last 240-365 days (Montgomery and Craig 2005). Very little information is known about larval and puerulus biology of *S. verreauxi* in New Zealand waters with only a tiny number of individual pueruli ever captured (Booth 2011).

1.3.3 *Spiny lobster early development*

Spiny lobsters have separate sexes, paired gonads, and have gonopores at the base of the fifth pair of pereiopods in males and in the third pair in females (Meglitsch and Schram 1991). During mating the males deposit a spermatophore near the female's gonopore. Females extrude their eggs and then use the spermatophore to complete external fertilization of the eggs (Phillips 2006).

1.3.3.1 Phyllosoma stage

The embryos of spiny lobsters are externally brooded under the abdomen for many weeks and are released as hatching free-swimming larvae, known as naupliosomas, that moult to become a stage I phyllosoma larvae within hours of hatching (Gilchrist 1913, Harada 1958). Phyllosomas are transparent, thin, and flat, hence their name (phyllosoma is Latin for "leaf-like"). They appear to migrate rapidly offshore into oceanic waters where they complete their subsequent larval development (Lesser 1978, Bruce et al. 1996). Phyllosomas progress through 6 to 29 instars, in 6 to 13 morphologically distinct developmental stages, depending on the species (Lewis 1951, Kittaka 2000, George 2005, Phillips 2006). The phyllosoma of the two species of spiny lobster with an Australasian range, *J. edwardsii* and *S. verreauxi*, have 11 phyllosoma developmental stages involving 17 instars (Lesser 1978, Kittaka 1994, Kittaka et al. 1997).

The pelagic larval duration for spiny lobsters is among the longest in marine invertebrates but varies enormously among species, ranging from around 4 months in some tropical species to up to 18 months in some temperate species (Kittaka, 2000; Phillips, 2006). For example, the phyllosoma phase can last over 18 months in *J. edwardsii,* from 9 to 11 months in the Western Australian spiny lobster, *Panulirus cygnus,* and from 9 to 12 months in *S. verreauxi* (Montgomery and Kittaka 1994, Kittaka et al. 1997, Bradford et al. 2015). Stage I-III phyllosoma of *J. edwardsii* and *S. verreauxi* catch soft-bodied prey, such as fish larvae and gelatinous zooplankton that comes in contact with their pereiopods, by spearing them and bringing them to their mouth (Cox and Bruce 2003). As phyllosoma develop, the size and firmness of their prey is thought to increase (Johnston and Ritar 2001). Phyllosomas are opportunistic predators that can feed on zooplankton of a wide range of taxa and sizes, such as cnidarians, arrow worms, gastropods, fish larvae and crustaceans (Saunders et al. 2012, O'Rorke et al. 2013, Connell et al. 2014).

Early and mid-stage phyllosoma are considered poor horizontal swimmers but can adjust their vertical position in the water column (Kittaka 1994). Mid-stage phyllosoma of *J. edwardsii* have been found in the upper 20 m of the water column, while late stage phyllosoma are only at this depth during the night, whilst at day they are mainly found between 20 and 100 m below the ocean surface (Bradford et al. 2005). Late stage phyllosoma have well-developed pleopods that are thought to provide them with some horizontal swimming ability that may enable them to start their migration toward the coast prior to metamorphosis to puerulus (Chiswell and Booth 1999, Wells et al. 2001).

1.3.3.2 Puerulus stage

The transition from phyllosoma to the post-larval (puerulus) phase is a unique life-history stage in spiny lobsters. Metamorphosis of final stage phyllosoma appears to occur at a wide range of locations offshore, with the trigger(s) for metamorphosis yet to be identified (Jeffs et al. 2005, Phillips and McWilliam 2009). Recently metamorphosed pueruli of *J. edwardsii* (Fig. 1) have been found from 24 to 216 km offshore of New Zealand with their location bearing no connection with water depth or sea surface temperature (Jeffs et al. 2001a). Once phyllosomas metamorphose into pueruli they use their well-developed pleopods to actively swim towards

the coast, crossing the continental shelf to settle in shallow coastal waters (Phillips and McWilliam 1986, Jeffs et al. 2005). Pueruli do not feed, instead their onshore migration is fuelled by extensive lipid stores that have been accumulated during the prior lengthy larval phase, i.e., they exhibit secondary lecithotrophy (Lemmens 1994a, Jeffs et al. 1999). Recently metamorphosed pueruli of *J. edwardsii* caught offshore have been found with 29.7 % lipid content on average (Jeffs et al. 2001a). There have been theoretical estimations of the energy needed for pueruli to cross the continental shelf based on differences in the lipid content of pueruli found inshore compared to offshore (Jeffs et al. 2001a, 2001b, Phillips et al. 2006, Wilkin and Jeffs 2011), but these estimates fail to take into account the actual transport pathway and are therefore likely to be incorrect due to possible resistance or assistance from wind, waves and currents. While it is known the pueruli have high metabolic capacity to potentially support sustained swimming activity (Wells et al. 2001), very little information is available on the swimming behaviour of pueruli, such as whether, they can sustain swimming for long periods of time, or if there are circadian behavioural patterns. Recent experimental observations in the field indicate that pueruli swimming occurs intermittently both during the day and the night in open waters, i.e., 6 to 8 km offshore (Kough et al. 2014). However, most of the information about the swimming behaviour of pueruli has come from opportunistic field observations or short term observations in laboratory conditions (Phillips and Olsen 1975, Calinski and Lyons 1983, Jeffs and Holland 2000, Booth 2001, Hayakawa and Nishida 2002). Pueruli appear to have directed swimming towards the coast, and can modify their direction in response to tide changes (Jeffs et al. 2005, Kough et al. 2014), as well as chemical, and physical cues (Goldstein and Butler 2009, Stanley et al. 2015, Hinojosa et al. 2016).

There have been measurements of metabolic rates of pueruli and juveniles of spiny lobsters, but these have tended to have been done for inactive lobsters, and only for short periods of time (Lemmens 1994b, Fitzgibbon 2010). Short term metabolic measures provide an unreliable basis for estimating the overall energy requirements of pueruli migrating from the point of metamorphosis offshore through to an inshore coastal settlement location.

Once pueruli reach the coast they generally look for microhabitats, such as crevices or thick macroalgae, where they can hide from predators. Pueruli subsequently undergo significant morphological changes associated with a transition to a benthic lifestyle, including the commencement of feeding and the loss of pleopods which are used for horizontal swimming in the pueruli (Deshmukh 1966, Wolfe and Felgenhauer 1991, Abrunhosa and Kittaka 1997, Guerao et al. 2006). The changes are operationalised when the pueruli moult to first instar juvenile and the new carapace becomes pigmented and hardened by mineralisation (Guerao et al. 2006). Like the preceding pelagic puerulus phase, the post-settlement transition of the pueruli to first instar juvenile is also energetically highly demanding (Barclay et al. 1983, Musgrove and Geddes 1995, Oliver and MacDiarmid 2001). Consequently the energy stores of the pueruli need to be sufficient to fuel both the pelagic migratory stage, as well as the subsequent transition to first instar juvenile when feeding can resume (Jeffs et al. 1999, 2001b). The puerulus stage can last from 19 days in culture and up to 70 days in the wild for *J. edwardsii* (Kittaka 1990, Phillips and Booth 1994). In *S. verreauxi* the pueruli stage is reported to last for around 25.5 days in culture conditions (Kittaka et al. 1997). During these periods basal metabolic demands of pueruli also need to be met from the energy stores of the pueruli (Wilkin and Jeffs 2011).

It has been postulated that a significant proportion of pueruli have insufficient energy reserves to facilitate their migration from the point of metamorphosis offshore through to their establishment as benthic first instar juveniles. For example, recently metamorphosed pueruli of *J. edwardsii* have been found from 24 to 216 km offshore, with a mean lipid content from 6.2 to 48.0 mg (Jeffs et al. 2001a), which is estimated to be capable of providing sufficient energy for 40.1 to 312.2 km of swimming (Jeffs et al. 1999). Judging from the location of the collection of these pueruli it was estimated that 16.5% of them had insufficient energy reserves to complete their migration to the coast unassisted (Jeffs et al. 2001a). These estimations suppose all the lipid content is available to use as fuel, and do not include the pueruli arriving with insufficient lipid to fuel the subsequent transition to first instar juvenile. Therefore, if these estimates are correct then the proportion of pueruli failing to survive to first instar juveniles would be significantly more than 16.5%.

Chapter 1. Introduction

It has been proposed that recent marked declines in pueruli settlement and subsequent recruitment observed in some major spiny lobster fisheries may be due to more rapid exhaustion of the energy reserves of pueruli as a result of increased metabolic demand from warming oceans (Thomas et al. 2000, Bermudes and Ritar 2008, Fitzgibbon and Battaglene 2012b, Fitzgibbon et al. 2014b, 2014a, 2015). Pueruli are active in surface waters and coastal environments which tend to be more affected by heat waves and are generally warming much faster than the open ocean (Meehl et al. 2007, Lima and Wethey 2012, Frölicher and Laufkötter 2018). The evidence in support of this theory relates to differences in metabolic rates measured in cultured pueruli at different water temperatures, and correlations between recruitment and ambient water temperature at the time of pueruli settlement (Fitzgibbon et al. 2013, 2014, 2015). Should this theory prove correct it would be of particular significance to the future outlook and management of spiny lobster populations and fisheries. Consequently, the energetic requirements of the puerulus phase of the lifecycle of spiny lobsters is worthy of further investigation.

Therefore, the aims of the research presented in this thesis are:

1) To provide more precise measures of the energetic costs of swimming in the pueruli of spiny lobsters in order to improve our understanding of the likely effects of any increases in seawater temperatures on the energetic reserves of this phase of the lifecycle, which is critical to subsequent recruitment. Energetic costs will be determined experimentally for pueruli of *J. edwardsii* and *S. verreauxi*, by swimming challenges in kreisel flume tanks, held at different temperatures where possible, and measuring changes in energetic condition of the pueruli through time. These experiments will also provide insight on the swimming behaviour of pueruli.

2) To provide more precise measures of the energetic limitations of first instar juveniles of spiny lobsters, in order to improve our understanding of the point at which the depletion of reserves are likely to negatively affect the survival and subsequent recruitment of individuals. This was done by the combination of 3 approaches. The first approach was by calculating the time needed for first instar juveniles to resume feeding to survive and moult to second instar

juveniles. The second approach was by calculating the lipid and protein utilization rate of inactive starved juveniles of *J. edwardsii* and *S. verreauxi*, and how it's affected by an increment in temperature where possible. The third approach was by determining the amount of lipid and protein that are unavailable for catabolism. These results would help us understand how these stages are affected by increasing temperatures on coastal waters, and better understand the yearly settlement fluctuations.

Together the results of this research are expected to provide better insight to the likely impact of rising seawater temperatures on the energetics, nutritional condition, and survival of spiny lobsters during the transition from pelagic to benthic existence. The result of the research will also provide more precise evaluations of the energetic prospects of future wild pueruli and juvenile monitoring efforts.

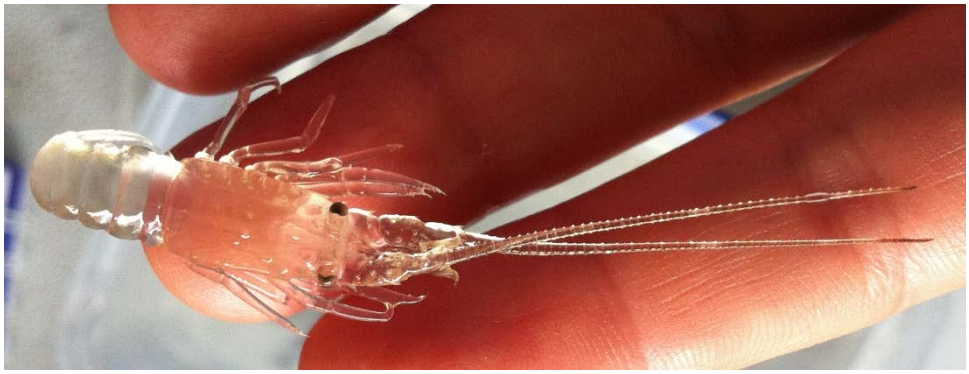

Figure 1. Puerulus of *J. edwardsii* collected in Castle Point, Wairarapa, New Zealand

Chapter 2. Swimming energetics of the post-larvae of the spiny lobster *Jasus edwardsii* in New Zealand

2.1. Introduction

Many spiny lobster populations around the world have marked interannual fluctuations in the settlement of their post-larvae or pueruli, which greatly influences the subsequent recruitment of juveniles into the population (Jeffs 2010). However, substantial declines in pueruli settlement over a number of years have recently occurred in the three largest spiny lobster fisheries in the world; the Caribbean spiny lobster (*Panulirus argus*) in Florida (Ehrhardt and Fitchett 2010), the western rock lobster (*Panulirus cygnus*) in Western Australia (Feng et al. 2011), and the southern rock lobster (*Jasus edwardsii*) in southeastern Australia (Linnane et al. 2010c). One hypothesis for these declines is that changes in oceanic conditions are impacting the ability of larvae to gather sufficient nutritional resources to support the non-feeding puerulus stage which follows (Fitzgibbon et al. 2014a).

The puerulus is thought to have a high demand on energy stores whilst actively migrating a long distance from offshore waters over the continental shelf to reach shallow coastal waters where they settle and subsequently become juveniles. Relatively small increases in seawater temperatures have been shown to reduce food intake in larval spiny lobsters while increasing their metabolic energy demand (Fitzgibbon and Battaglene 2012b). This results in a potential reduction in their accumulation of nutrient reserves, which are critical for supplying the energy demands during the cross-shelf migration of the subsequent puerulus stage. Furthermore, elevated seawater temperatures increase oxygen consumption in decapod crustacean larvae (Belman and Childress 1972, Iguchi and Ikeda 1995) and greatly diminish aerobic capacity and increase energy demands on the finite energy reserves of pueruli (Fitzgibbon et al. 2014b). Warmer waters are also associated with diminished settlement in the northern region of the distribution of *S. verreauxi* and *J. edwardsii* (Fitzgibbon et al. 2014b, Johnson et al. 2011).

Scientific inferences about the puerulus stage in the lifecycle in spiny lobsters are commonplace due to the difficulty of studying pueruli in the field, largely as a result of their extremely low abundance, combined with their cryptic appearance and behaviour (Jeffs et al.

2005). Pueruli have transparent bodies and appear to be nektonic at night, but their behaviour during daytime is unknown (Phillips et al. 2006a), although the metabolic enzymes of pueruli suggest a capacity for maintaining swimming activity for extended periods (Wells et al. 2001). Active daytime swimming has been observed in experimental field observations (Kough et al. 2014). It is thought that spiny lobster larvae (i.e., phyllosomas) metamorphose to pueruli near the continental shelf break based on the capture of pueruli at sea with large zooplankton nets (Jeffs et al. 2001a) and if they metamorphose beyond the slope region of the continental shelf they are unlikely to recruit to the population (Phillips and McWilliam 2009).

From metamorphosis pueruli have directed movement shoreward by directed horizontal swimming at speeds of 8 - 45 cm s^{-1} based on observations of pueruli swimming on the surface of the sea at night time and in tanks (Phillips and Olsen 1975, Calinski and Lyons 1983, Jeffs and Holland 2000). Active swimming over a number of days is thought to be the primary mode of transport for the lecithotrophic pueruli to cover the tens to hundreds of kilometres required to reach shallow coastal reefs where they settle, then moult to become juveniles so they can re-commence feeding (Jeffs et al. 2005, Phillips et al. 2006b, Feng et al. 2011).

From larval metamorphosis through to moulting to become a juvenile, the puerulus phase of the lifecycle typically lasts 10 to well over 30 days depending on the spiny lobster species and water temperature (Limbourn et al. 2008, Stanley et al. 2015). This lengthy and highly active post-larval period places an enormous demand on the stored energy reserves of the puerulus, with some evidence that a significant proportion of pueruli deplete their reserves to the point of compromising their subsequent survival (Jeffs et al. 2001a, 2001b, Phillips et al. 2006b, Wilkin and Jeffs 2011, Fitzgibbon et al. 2014a). This proposition has been based on changes in the nutritional condition of pueruli caught at different locations offshore, and on theoretical calculations of the bioenergetics of pueruli swimming. Therefore, in this study we undertook direct measures of energy consumption by experimentally swimming wild-caught pueruli for 2, 3 and 5 days at ambient seawater temperatures (19-22 °C) and comparing their final biochemical condition. Our experiments used wild-caught pueruli of the Australasian red spiny lobster, *Jasus edwardsii*, for which changes in nutritional reserves, especially storage lipids, have previously been well described and used to infer a shortfall that would jeopardise survival

in as many as 16.5% of wild pueruli settling on the coast (Jeffs et al. 1999, 2001a, 2001b, Phleger et al. 2001, Wells et al. 2001).

2.2. Materials and methods

2.2.1 *Study area and sampling*

Pueruli were collected at Castle Point, Wairarapa, New Zealand (40° 54.2' S; 176° 13.8' E), from the 7 to 11 February 2016. Crevice collectors (Booth and Tarring 1986) were deployed in the nearshore during low tide and left submerged overnight. The collectors were cleared of pueruli during low tide each day. The pueruli were immediately sorted into developmental stage following the schema of Booth (1979) and only stage I pueruli that would have arrived in the collector overnight from nektonic swimming were selected for swimming experiments. It is assumed that the short period of contact of the pueruli with the collector would not affect their subsequent swimming behaviour once transferred to the swimming experiment. Pueruli of stages I and II were selected as baseline controls for biochemical analyses. Stage I pueruli advance to stage II after 24-36 hours at the ambient seawater temperatures (19 to 22 °C) in which the pueruli were caught, such that any additional energy reserves consumed by stage II pueruli during this additional period of development would provide a conservative estimate of the baseline for any comparisons of nutritional status.

2.2.2 *Swimming experiments.*

A total of 71 pueruli were collected and divided into 4 groups**.** Eleven pueruli were swum for a period of 2 days, 13 were swum for 3 days, 24 were swum for 5 days, and 24 pueruli were used as controls and were frozen on the day of their collection.

Pueruli to be swum were placed in a specialised 7.36 l kreisel tank (Fig. 2), a cylindrical aquarium with circular water flow, with ambient seawater (19 – 22 °C) tangentially injected into the tank to create a continuous rotating water current of 0.15-0.17 m s^{-1} in the tank. This speed is in the middle of the range of the swimming speed previously recorded for pueruli of *J. edwardsii* (Jeffs and Holland 2000). The water current was measured with a Hoentzsch vane

wheel flow probe (ZS 25) at the position in the tank in which pueruli most often occupied whilst actively swimming. Water exited the tank behind the water jets to prevent pueruli from leaving the tanks. A dye test was performed to confirm homogenous circulation pattern.

The tanks were made of transparent acrylic to facilitate visual observations with red light to avoid disturbing their behaviour (Weiss et al. 2006). No more than four pueruli were placed in a single tank at one time to decrease interactions between pueruli. Every 30 min during the day and every 3 h at night (due lo logistical reasons) the pueruli were checked to confirm they remained suspended in the water current within the tank and were not holding on to the edges of the tank or to each other. Any pueruli that were clinging on were gently detached by nudging with a cable tie. The tanks were kept in continual darkness to simulate night time which was observed to promote swimming activity in the pueruli and is consistent with their natural behaviour of remaining out of the photic zone in the oceanic pelagic environment (Phillips et al. 2006a). At the end of their assigned swimming duration each puerulus was removed from the kreisel tank and frozen by storing in a freezer. Samples were freeze dried before morphological measurements were taken and biochemical analyses performed.

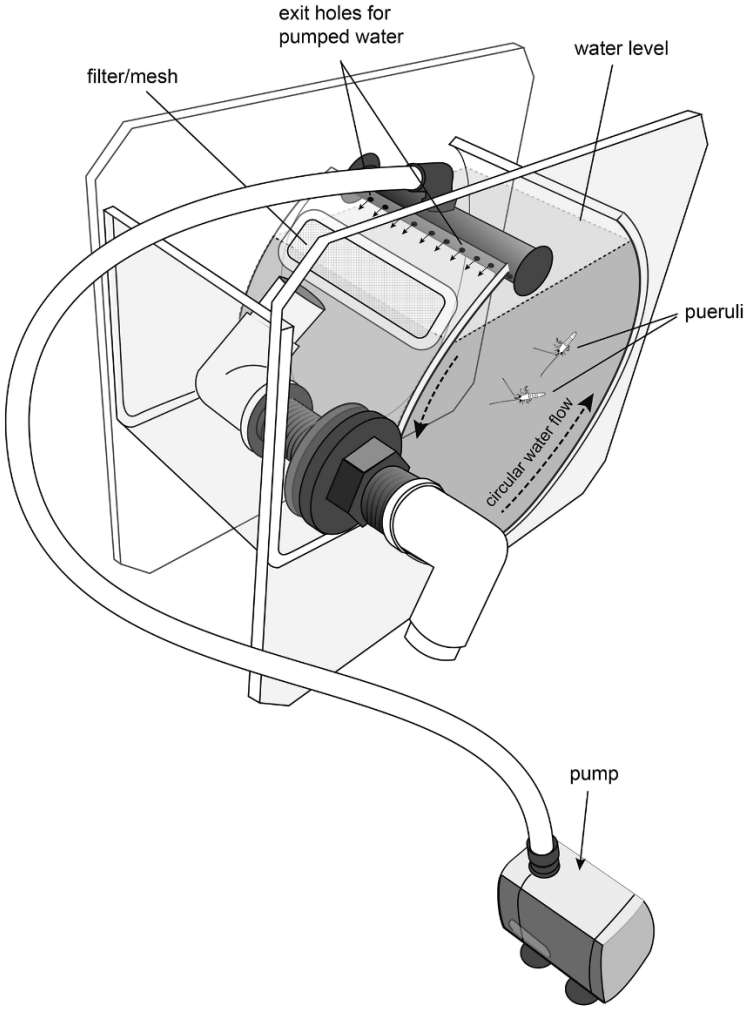

Figure 2. Diagram of the kreisel tank with arrows indicating the circular water flow that allows for continual swimming behaviour in pueruli of *Jasus edwardsii*

2.2.3 *Biochemical analyses*

The carapace length (CL) and the wet weight (WW) of each puerulus was measured, and then the pueruli were lyophilised and re-weighed to determine their dry weight (DW). Total lipid (TL) of each puerulus was determined using a modified Bligh and Dyer protocol (Bligh and Dyer 1959) which uses lipid's high solubility in chloroform to extract lipid from samples. The remaining puerulus tissue was then used for total protein (TP) determination, using a Micro

BCA protein assay (Thermo Scientific, Rockford, Illinois, USA) based on the bicinchoninic acid determination method for protein quantification by colorimetric techniques (Walker 1994).

2.2.4 *Statistical analyses*

Comparisons of the mean carapace length, dry weight, total protein, and total lipid of pueruli were made with a one-way ANOVA for each data set with the time of swimming as a fixed factor (i.e., 0, 2, 3 and 5 days). Prior to analyses the equality of variance of the data was confirmed with a Brown–Forsythe test, and normality of the data with a D'Agostino-Pearson test. Where ANOVA revealed significant overall differences among means Tukey's multiple comparisons tests were used to identify differences among pairs of means. A linear regression analysis was used to estimate the rate of lipid utilization from the collective data set for total lipid. All mean values are presented with standard errors. The slope of the regression line (i.e., rate of lipid consumption) is reported with a 95% confidence interval. One-way ANOVA test was performed using SigmaPlot version 13.0, from Systat Software, Inc., San Jose California USA, www.systatsoftware.com.

2.3. Results

2.3.1 *Pueruli behaviour*

Once placed in the kreisel tank the pueruli would initially adopt a sinking posture with spread antennae, and outstretched abdomen, tailfan and pereiopods whilst initially drifting in the water current circulating in the tank. Typically the pueruli would then move to adopt a swimming posture by pulling the antennae together directly in front, tucking in the pereiopods to the side of the cephalothorax and lifting the abdomen whilst actively paddling with the pleopods. Pueruli consistently showed positive rheotaxis by positioning themselves at the location of the higher current speed, near the bottom of the tank and swimming into the water current so they maintained their position in relation to the tank. Pueruli were observed to alternate between extended periods of swimming at the speed of the current and drifting with the water flow. At

night time, the duration of periods of swimming appeared to be longer and the correspondingly periods of drifting shorter. Extended observations at night indicated that pueruli were capable of swimming continuously at the speed of the water current in the kreisel for more than 3 hours at a time.

2.3.2 *Pueruli size*

Carapace length among all the pueruli sampled ranged from 9.33 to 12.07 mm, with a mean of 11.10 mm (SE = 0.07 mm) and did not differ among the groups of pueruli swum for different durations ($F_{3,70}$ = 0.37, P = 0.82). The wet weight of all pueruli sampled was highly variable and ranged from 377.0 to 806.0 mg, with a mean of 570.1 mg (SE = 9.4 mg) and did not differ among the groups ($F_{3,70}$ = 1.52, P = 0.19). The dry weight of all pueruli sampled was highly variable ranging from 85.0 to 181.0 mg, with a mean of 135.2 mg (SE = 2.3 mg) and did not differ among the groups ($F_{3,70}$ = 1.89, P = 0.11).

2.3.3 *Total protein*

Total protein content of all the pueruli sampled ranged from 32.1 to 63.5 mg and differed among the groups of pueruli swum for different durations ($F_{3,70}$ = 2.64, P = 0.05). There was a trend for mean protein to decrease with increasing swimming duration, however, the only significant difference was between pueruli not swum and those swum for 5 days (P = 0.05). The control group of pueruli that were not swum had a mean TP of 50.8 mg (SE = 6.8 mg) while the pueruli swum for 5 days had 45.1 mg (SE = 8.3 mg), an average decrease in protein of 5.7 mg (SE = 10.6 mg).

Regression analysis of all of the sampled pueruli showed the rate of protein utilisation by pueruli was linear over the course of the 5 day period within which the animals were swum ($F_{1,59}$ = 7.6, P = 0.009) with number of days pueruli were swum explaining 11% of the variability in total lipid content of pueruli (i.e., R^2 = 0.11, Fig. 3)

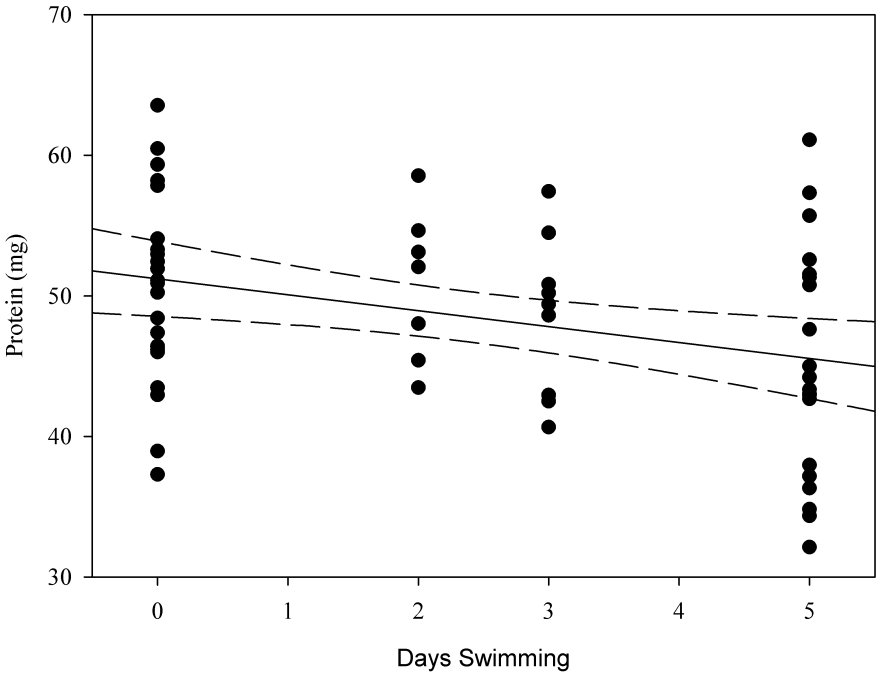

Figure 3. Linear regression analysis of total protein content of pueruli swum experimentally for different durations. Confidence intervals (95%) around the slope are indicated by dashed lines (n = 59).

2.3.4 *Total lipid*

Total lipid content of all pueruli sampled ranged from 3.5 to 30.1 mg and differed among the groups of pueruli swum for different durations ($F_{3,70}$ = 13.95, P = <0.0001). The mean TL of pueruli was not different between the control group and the pueruli swum for 2 days, however, the control pueruli had significantly more TL than pueruli swum for both 3 and 5 days (P = 0.006 and P > 0.0001 respectively), and pueruli swum for 2 days had significantly more TL than those swum for 5 days (P = 0.022). The TL of the control (unswum) pueruli ranged from 12.8 to 30.1 mg (mean of 15.9 mg, SE = 1.1 mg), while the TL of pueruli swum for 5 days ranged from 4.3 to 13.4 mg (mean of 7.8 mg, SE = 0.6 mg). On average pueruli used 8.14 mg day^{-1} (SE = 0.58 mg day^{-1}) over the 5 day period.

Regression analysis of all of the sampled pueruli showed the rate of lipid utilisation by pueruli was linear over the course of the 5 day period within which the animals were swum ($F_{1,69}$ = 42.9, P <0.0001) with number of days pueruli were swum explaining 38% of the variability in total lipid content of pueruli (i.e., R^2 = 0.38, Fig. 4) despite the high inherent variability in the TL of pueruli arriving on the crevice collectors. Regression analyses indicated the mean rate of lipid utilisation by pueruli was 1.63 mg day^{-1} (SE = 0.24).

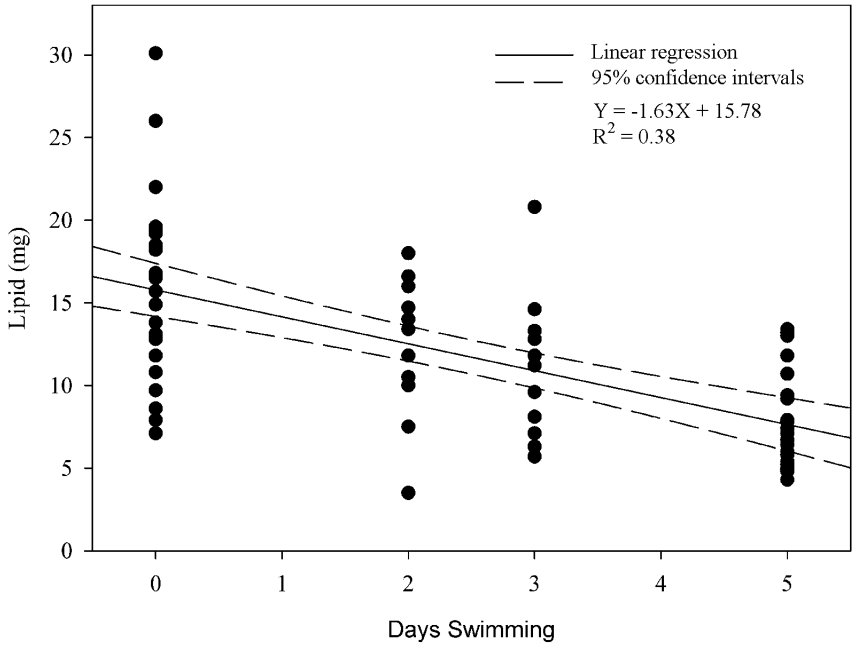

Figure 4. Linear regression analysis of total lipid content of pueruli swum experimentally for different durations. Confidence intervals (95%) around the slope are indicated by dashed lines (n = 71).

2.4. Discussion

Previous attempts to experimentally swim the pueruli of spiny lobsters in flume tanks have had limited success and as a result it has been assumed that pueruli do not express rheotaxis behaviour (Jeffs and Holland 2000), in the same manner as is commonly found in fish (Montgomery et al. 1997). However, there are a number of reports of observations of pueruli

in the wild swimming directly into water currents which are indicative of rheotaxis; i.e., pueruli of *Panulirus cygnus* (Phillips and Olsen 1975) and *P. argus* (Kough et al. 2014). In this present study pueruli consistently exhibited rheotaxis behaviour in the circular flume tanks, including adjusting their swimming speed to match the current speed in the tank so as to maintain a stationary position in the tank. This behaviour may have been facilitated by matching the current speed in the kreisel tanks with the mean swimming speed (0.161 m s^{-1}) previously measured in the pueruli of this species (Jeffs and Holland 2000). The rheotaxis in the pueruli must have been mediated by non-visual stimuli as the pueruli were capable of successfully maintaining their position in the kreisel tanks whilst in total darkness. This may involve water current detection by external mechanoreceptors, which are abundant in pueruli (Jeffs et al. 1997), in the same manner that the lateral line plays a role in sensing flow dynamics for mediating the expression of rheotaxis in the blind cavefish, *Astyanax mexicanus* (Montgomery et al. 1997). Where rheotaxis has previously been identified in crustaceans it is usually expressed in combination with other sensory cues such as locating upstream food odours (e.g., Zhou and Rebach 1999), rather than in response to water flow alone. The potential benefits of such behaviour in spiny lobster pueruli is uncertain given that a primary goal for pueruli is to traverse the continental shelf as quickly as possible to reach settlement habitats in coastal waters. Expressing rheotaxis into prevailing oceanic currents or in response to water velocities created by Stokes drift or the flow of surface waters by winds would often be counterproductive for pueruli wishing to orientate and move toward the coast from offshore waters (Jeffs et al. 2005). Alternatively, rheotaxis may only be expressed by pueruli once in coastal waters in an effort to maintain or advance their position relative to the coast in the presence of coastal tidal flows, as has been observed in a number of coastal crustacean species (Warman et al. 1993). It is also possible that rheotaxis is expressed in the absence of other sensory cues used to provide shoreward orientation, such as underwater sound and chemical cues (Hinojosa et al. 2016, 2017). This is supported by observations of swimming pueruli of the Caribbean spiny lobster within 20 km of the Florida Keys which altered mean orientation direction between ebb and flood tides consistent with adjusting their movement towards the coast in response to cues associated with tidal flow but not to directly counter the direction of flow as occurs in rheotaxis (Kough et al. 2014).

The mean TL and TP of stage I pueruli collected in this current study were higher (27.2% higher, P = 0.0075 and 130% higher, P = 0.0001 respectively) than for pueruli taken from crevice collectors in the same location in 1997-1998 (Jeffs et al. 1999). Mean total lipid of the pueruli from collectors in this study were similar to those captured 20 km offshore in 1997-1998 (P = 0.82) and were yet to migrate to the coast to settle, however, protein levels were still much higher in 2016 than at 20 km offshore (117.1% higher, P = 0001). Such differences indicate the natural level of interannual variability in pueruli nutritional condition and also indicate that the pueruli used in the swimming experiment in this study were not energetically depleted at the outset of the experiment. Previous studies have shown marked interannual and geographic variation in the nutrition of pueruli arriving on the coast which has been related to differences in feeding conditions for the preceding phyllosoma stages, and changes in water temperatures leading to differences in metabolic rates in the consumption of stored energy reserves in the lecithotrophic pueruli.

In this current study the total protein content of pueruli tended to decrease with the increasing number of days that pueruli were swum, however, this decrease was only statistically significant between pueruli swum for 0 and 5 days, with a mean decrease of 5.7 mg of protein. Similarly the total protein content of pueruli caught 20 km offshore from Castle Point tended to be higher than for pueruli caught onshore in December 1997 to February 1998 but was not significantly different. Other lecithotrophic Decapod larvae use lipid as a primary source of stored energy, but can switch to protein if lipid reserves become depleted (Limbourn and Nichols 2009, Anger and Schuh 1992, Fitzgibbon et al. 2013). For example, cultured pueruli of the eastern rock lobster were found to increasingly utilise protein versus lipid after settling and whilst completing development toward moulting to a juvenile, possibly as a result of limitations in the lipid reserves accumulated during the larval culture process (Pearce 1997, Fitzgibbon et al. 2014b). Analyses of large numbers of pueruli of *J. edwardsii* of all developmental stages taken from crevice collectors in Tasmania showed that TL remained at similar levels throughout the period of pueruli development over stages I to III , and only decreased once pueruli moulted to first instar juvenile (Pearce 1997). Although protein content was not analysed in this previous study, it implies this phase of development of the pueruli is

primarily fuelled by the catabolism of protein, whilst the nektonic and early juvenile phases are primarily fuelled by lipid (Jeffs et al. 2001a, 2001b).

In this current study, captive nektonic pueruli that spent much of their time actively swimming used on average 1.61 ± 0.24 mg of lipid per day. Using lipid caloric equivalents (Winberg 1971) 1.61 mg of lipid is equivalent to 63.2 J of energy, and assuming a similar basal metabolic rate as in western rock lobster (13.0 J per day, Lemmens 1994a) a day in the water column, including swimming activity, is 50.2 J more energetically demanding than for a day once settled for pueruli of *J. edwardsii*.

Previous measures of the change in the TL content of pueruli over a 20 km transect offshore to onshore at Castle Point estimated the rate of lipid catabolism by actively migrating pueruli of *J. edwardsii* at 0.154 mg km^{-1} assuming pueruli swam in a straight line shoreward without encountering resistance or assistance from any physical processes (Jeffs et al. 1999). At our measured rate of 1.61 mg lipid per day, pueruli would need to swim at their average speed of 0.161 m s^{-1} for 75% of each day in order to match this previous indirect estimate of 0.154 mg km^{-1}, and in so doing cover a distance of 20 km in two days. In the current study systematic observations of the periodicity of swimming by individual pueruli were, unfortunately, not recorded. However, in general pueruli appeared to spend much of their time in the kreisel tanks actively swimming, including during the day time. It is possible that our experimental pueruli spent less time swimming than newly metamorphosed nektonic pueruli given that previous observations of the extent of swimming activity in fish larvae (Leis et al. 2011) and in the eastern rock lobster (Fitzgibbon et al. 2014a) have observed decreases in activity with their progression of development.

The rate of energy consumption in pueruli of *J. edwardsii* (i.e., 0.154 mg km^{-1}) is more than double that previously estimated for western rock lobster (i.e., 0.065 mg km^{-1}) (Phillips et al. 2006b). However, onshore winds and possibly associated Stokes drift have been identified as important processes assisting in the delivery of pueruli to inshore settlement areas of Western Australia (Feng et al. 2011, Weller et al. 2012, de Lestang et al. 2015) The size of the difference in estimated rates of energy consumption of pueruli between these two species of spiny lobster,

possibly indicates the importance of favourable onshore physical processes in influencing the extent of pueruli settlement and recruitment. The potentially important role of these physical processes on recruitment have also been identified in a number of recent studies correlating the settlement and recruitment of spiny lobsters with oceanographic and climatological variables including wind forcing (Briones-Fourzán et al. 2008, Linnane et al. 2010, Feng et al. 2011, Weller et al. 2012, de Lestang et al. 2015, Hinojosa et al. 2016, 2017).

The concordance of our direct measure of energy expenditure of swimming pueruli of *J. edwardsii* with previous indirect measures adds further weight to the hypothesis that a significant proportion of spiny lobster pueruli perish as a result of exhausting their energy reserves during their active cross shelf migration. An estimated 16.5% of 360 pueruli of *J. edwardsii* captured during zooplankton sampling offshore of the east coast of New Zealand were estimated to have insufficient reserves to reach inshore settlement grounds based on the estimated use of their measured TL content (Jeffs et al. 2001a). The marked difference in the mean TL content of pueruli caught in collectors in 1997-1998 versus those taken in this current study in 2016, shows the extent of interannual variability in nutritional condition of pueruli at settlement. On average pueruli sampled in 2016 had sufficient lipid to fuel the equivalent of two additional days of active swimming. This much higher nutritional condition could be expected to greatly increase overall pueruli survival and the subsequent establishment of early juveniles recruiting into the population.

The possible causes of interannual differences in the nutritional condition of spiny lobster pueruli are unclear, however, elevated seawater temperatures have been shown to disproportionately increase metabolic energy demands in both phyllosoma and pueruli of eastern rock lobster (Fitzgibbon and Battaglene 2012b, Fitzgibbon et al. 2014b). Increased seawater temperatures also reduce food intake in cultured phyllosoma which could also be expected to compromise their ability to accumulate nutritional reserves to fuel the subsequent puerulus. Phyllosoma of *J. edwardsii* are opportunistic macroplankton feeders spending much of their extensive larval period in oligotrophic waters (Jeffs et al. 2004, Connell et al. 2014, O'Rorke et al. 2014). It is possible that relatively small interannual changes in zooplankton availability or quality could also greatly influence the ability of phyllosoma to accumulate

sufficient nutritional reserves, as has been suggested for western rock lobster (Wang et al. 2015a, O'Rorke et al. 2015, Wang et al. 2015b). Differences in physical processes (such as wind, storms, temperature and ocean circulation) impeding or assisting cross shelf migration in pueruli, also have large capacity to influence the nutritional condition of pueruli arriving into coastal settlement grounds (Linnane et al. 2010, Feng et al. 2011, de Lestang et al. 2015). The relative importance of these processes with the potential to create high interannual variability in pueruli nutritional condition need to be examined further if we are to improve our understanding of the variability in recruitment into spiny lobster populations.

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Chapter 3. Increasing temperature impacts the survival of post-larval spiny lobsters

3.1. Introduction

Spiny lobsters have an extended oceanic larval phase that can last well over a year in some species (Lesser 1978, Montgomery and Craig 2005, Bradford et al. 2015). During this time the larvae, or phyllosoma, grow in size and gradually accumulate considerable energetic reserves by feeding on a wide range of macro-zooplankton, including, cnidarians, chaetognaths, crustaceans and fish larvae (Jeffs 2007, Suzuki et al. 2008, Saunders et al. 2012, O'Rorke et al. 2013, Connell et al. 2014, Wang et al. 2014). For example, from egg hatch until the end of the 18 month larval phase in the Australasian red spiny lobster, *Jasus edwardsii*, the dry biomass of the larvae increases by more than an order of magnitude, and the proportion of lipid in the larvae increases more than six fold from 5% to over 33% (Phleger et al. 2001). The accumulated reserves are used for fuelling the active migration towards the coast by the subsequent lecithotrophic post-larval or puerulus stage (Phillips and Olsen 1975, Phillips and McWilliam 1986, Kough et al. 2014). The metamorphosis into a puerulus occurs at a wide range of distances offshore and newly metamorphosed pueruli start their migration towards shore with widely varying levels of accumulated energy reserves (Booth and Chiswell 2005, Jeffs et al. 2005). This ultimately results in the migrating pueruli arriving on the coast with highly variable levels of remaining energetic reserves which is thought to greatly affect their prospects for establishing as benthic juveniles (Fitzgibbon et al. 2014a). Pueruli are believed to use chemical and physical cues to migrate directly toward coastal settlement habitats (Goldstein and Butler 2009, Stanley et al. 2015, Hinojosa et al. 2016) but their swimming behaviour while migrating onshore is largely unknown. Short term observational experiments tracking pueruli swimming in situ found that they swim towards the coast, during both day and night (Kough et al. 2014). They have been observed to exhibit rheotaxis and to use non visual cues, such as chemical and sound cues, to orient themselves (Hinojosa et al. 2016, 2018, García-Echauri and Jeffs 2018) however, it is unclear how much of their time is spent swimming. Pueruli have been observed

preferentially swimming at night and are believed to use water currents and onshore winds to aid their shoreward migration (Calinski and Lyons 1983, Caputi and Brown 1993, Phillips and Pearce 1997, Acosta and Butler 1999, Caputi et al. 2010, Linnane et al. 2010).

Ocean temperatures are increasing (Hansen et al. 1997, Levitus et al. 2000), with the oceans in the Southern Hemisphere heating four times faster than in the Northern Hemisphere (Wijffels et al. 2016). One of the fastest warming ocean regions in the world is in south-eastern Australia (Cai et al. 2005, Wu et al. 2012, Oliver and Holbrook 2014), within which is the natural range of the eastern rock lobster, *Sagmariasus verreauxi*. In this region the mean sea surface temperature is projected to increase by 2 °C by 2050 compared to the average temperature for 1990-2000 (Hobday and Lough 2011). The phyllosoma of *S. verreauxi* have an optimum growth and development at 23 °C (Fitzgibbon and Battaglene 2012). Cultured pueruli have their peak aerobic scope at 24.9 °C with an upper temperature pejus of between 24 and 27 °C, while temperatures above this are critical and result in poor oxygen supply to tissues (Pörtner 2010). Within the range of 15 to 27 °C the standard and routine metabolic rate in pueruli increase exponentially with temperature (Fitzgibbon et al. 2014b). These metabolic measures suggest that regional increases in water temperature will make the onshore migration of pueruli more energetically costly, potentially resulting in fewer pueruli retaining sufficient energetic reserves to survive through settlement and establish into the coastal population as benthic juveniles.

Therefore, the aim of this research was to determine how increases in water temperature affects the swimming behaviour and the amount of energy used by migrating pueruli of *S. verreauxi*.

3.2. Materials and methods

3.2.1 *Study area and sampling*

Recently arrived pueruli (i.e., stage I and II, sensu Booth 2001) of *S. verreauxi* were collected from the coast of New South Wales, in Sydney and Ulladulla, from collectors deployed by the Department of Primary Industries in Australia, from August to November in both 2015 and 2016 (Phillips and Booth 1994). Seawater temperatures during pueruli collection ranged from

17 to 20 ºC. Immediately after collection pueruli were held in ambient seawater and transferred to the nearby aquarium facilities at the Sydney Institute of Marine Science.

3.2.2 *Swimming experiments*

Wild pueruli are difficult to capture in large numbers simultaneously, hence it was necessary to rely on smaller numbers of pueruli collected at multiple occasions from two sites over a two year period. The smaller numbers of pueruli from each collection event were then used in sequential experiments, each with a limited range of temperature treatments due to the constrained numbers of post-larvae available at any one time. However, the pueruli from every collection event were allocated to three experimental treatments; control, swimming, and holding. Control pueruli were frozen immediately upon collection, while pueruli used for swimming and holding were kept in the experimental conditions for either 3 or 6 days, and at either 17, 20 or 23 °C. In total 428 wild pueruli were collected from 12 separate collection events for experiments (Table 1).

For the swimming treatment small cylindrical kreisel tanks with a slow rotating flow (0.15-0.17 m s^{-1}) of filtered seawater (UV and 5 µm) were used to re-create pelagic conditions by maintaining pueruli in continuous suspension (see description of tank design and operation in Garcia-Echauri and Jeffs 2018). The behaviour of each puerulus was observed every half hour during the day and every three hours at night. The recorded behavioural categories were: swimming, clinging onto the walls of the tank, drifting with the water current, and clinging onto other pueruli whilst drifting together as "clumps". If a puerulus was holding on to the walls of the tanks or another puerulus at the time of observation they were gently detached by nudging with a cable tie. The number of pueruli was kept to four or less per tank in an effort to reduce clinging behaviour.

Pueruli in the holding treatment were kept in aquaria that were supplied with continuously flowing filtered seawater but did not recreate pelagic conditions and allowed pueruli to adopt a reptant lifestyle consistent with their behaviour following settlement into coastal habitats.

The aquaria were kept in continual darkness, with behavioural observations made under dim red light, since pueruli are thought to remain out of the photic zone in the wild (Phillips et al.

2006a). When each respective experimental period ended the pueruli were removed from their swimming or holding tanks and immediately frozen for later biochemical analyses.

3.2.3 *Biochemical analyses*

The carapace length (CL) and the wet weight (WW) of each puerulus was measured, before being lyophilised and re-weighed to determine their dry weight (DW). Total lipid (TL) of each puerulus was determined using a modified Bligh and Dyer (1959) protocol. The residual puerulus tissue was then used for total protein (TP) determination, using a Micro BCA protein assay (Thermo Scientific, Rockford, Illinois, USA) (Walker 1994). The quantity of lipid used by each puerulus whilst undergoing their experimental treatment (i.e., swimming or holding for 3 or 6 days) was estimated by subtracting their measured TL from the mean TL of the control pueruli at capture. The same approach was also used to estimate protein use. The estimates of lipid and protein expenditure were converted to estimates of biochemical energy (joules) by using calorific equivalents (Winberg, 1971).

3.2.4 *Statistical analyses*

To determine if results could be pooled for analyses from sequential experiments run on smaller numbers of pueruli that were collected at different times and from two sites, the morphological and biochemical parameters of all the control pueruli were compared to determine if they were similar. Two-way ANOVAs were used to compare each of the parameters, CL, DW, TL and TP for control pueruli, using collection site and date as factors. If the controls from different collections were equivalent for all the morphological and biochemical parameters they were pooled as a separate group, and the experimental results from those collections were subsequently analysed together as a group.

It was not possible to use multi-factorial ANOVA for data comparisons because not all combinations of experimental treatments were present for each group of pooled results. Therefore, for each group of pooled experimental results a one-way ANOVA was used for comparing each of the four morphological and biochemical parameters of experimental pueruli (i.e., CL, DW, TP, and TL) from all of the possible treatment combinations; control, holding, and the completed combinations of the six available for both swimming and holding (i.e., for

3 or 6 days at 17, 20 or 23 °C). Where an ANOVA revealed significant overall differences among the means a two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli (Benjamin et al. 2006) was used to perform multiple comparisons tests to identify differences among pairs of means. Prior to analyses the equality of variance of the data was confirmed with a Brown–Forsythe test, and normality of the data with a D'Agostino-Pearson test. If the normality test failed, data transformations were performed. If data transformations did not parametrise the data then a Kruskal Wallis test was used as a non-parametric alternative for the analyses.

For each distinct group (pueruli that on collection had similar morphological and biochemical parameters), the amount of lipid and protein used by pueruli in swimming or holding treatments was calculated by subtracting the TL or TP at the end of the experimental interval (i.e., 3 or 6 days, at 17, 20 or 23 °C) from the mean TL or TP of the control pueruli at capture, with the resulting difference providing an estimate of the biochemical substrate consumed during the experimental interval. A one-way ANOVA was then applied for TL and TP to determine if there were differences among the treatment combinations. For each group the lipid and protein spent from each temperature, length of experiment, and type of activity (swimming or holding) was then standardised to lipid and protein utilisation per day by dividing by the experimental duration in days (i.e., 3 or 6 days) and compared with a one-way ANOVA.

To calculate total energy spent in each treatment, the lipid and protein spent was transformed into joules (Winberg 1971). The joules resulted from lipid and protein used was added to calculate total energy used in each treatment. The joules spent by pueruli for each treatment combination was then compared to the other treatments in the same group and in other groups with a one-way ANOVA.

A logistic regression model was used to identify differences in swimming behaviour at different temperatures, with swimming behaviour expressed as a binomial dependent variable (i.e., swimming or not swimming) with experimental temperature (i.e., 17, 20 or 23 °C), diurnal period (i.e., periods of day and night at collection site), and number of days swimming used as independent variables (i.e., predictor variables). The behaviours of drifting, clinging to the walls, and clinging to another puerulus were also fitted to the binomial models in a similar

manner. To analyse swimming behaviour pueruli were separated in the same groups used for the biochemical comparisons, and within groups the different collection dates were analysed with estimated marginal means tests and compared with Tukey's test. Since the model tests the proportion of pueruli displaying a behaviour at a given moment, observations from pueruli in the 3 days swimming experiment were pooled with pueruli in the 6 days swimming experiment. All means are reported with their accompanying standard errors.

3.3. Results

3.3.1 *Wild pueruli*

Control wild pueruli were highly variable in their morphometric and biochemical measurements among the different collection months and locations (Table 1). A two-way ANOVA of CL in control pueruli using collection site and date as factors found CL differed among collection dates ($F_{(2, 183)} = 12.26$, $P < 0.001$) (Table 1). However, neither collection site ($P = 0.35$), or the interaction of collection site and date were significant ($P = 0.36$) as main effects in the ANOVA. A two way ANOVA of the DW of control pueruli using collection site and month as factors found differences for the date of collection ($F_{(2, 179)} = 12.82$, $P = 0.001$) (Table 1). Collection site did not have an effect on the mean DW either alone ($F_{(1, 179)} = 1.9$, $P = 0.17$) or as an interaction with collection date ($P = 0.22$).

A two way ANOVA of the TL of control pueruli using collection site and date as factors found a difference among the dates of collection ($F_{(5, 177)} = 9.56$, $P < 0.001$), but not between collection sites ($F_{(1, 177)} = 3.29$, $P = 0.07$) (Table 1).

A Kruskal Wallis test of TP found significant differences among both collection dates and sites ($H = 43.95$, $P = 0.0001$) (Table 1).

The results of these preceding analyses enabled the control pueruli collected at different sites and dates to be pooled into three groups for which there were no differences for any of the four morphological and biochemical parameters (Table 1). The pooled data for these three groups were then used for subsequent comparisons of the experimental results within the groups.

Table 1. Differences in mean morphological and biochemical parameters of control *S. verreauxi* pueruli sampled from either of two sites in New South Wales, Australia, on 12 separate occasions. Syd = Sydney, Ulla = Ulladulla, Exp = experimental. Different letters indicate statistical differences within columns ($P < 0.05$). The column marked "Experimental groups" indicates the control pueruli for which there were no differences in morphological or biological parameters and therefore could be pooled for subsequent analyses of results of experimental treatments of pueruli.

Site	Date	n	CL	DW	TL	TP	Exp Group
Syd	9/2015	24	9.8 ± 0.1^{a}	$71.2\ 1.9^{a}$	6.5 ± 0.5^{a}	26.5 ± 1.3^{a}	1
Ull	9/2015	4	9.9 ± 0.1^{a}	77.2 ± 0.7^{a}	7.1 ± 0.9^{a}	30.2 ± 1.9^{abc}	1
Syd	10/2015	5	10.3 ± 0.3^{ab}	79.5 ± 8.7^{ab}	9.2 ± 3.0^{abc}	30.8 ± 3.4^{abc}	1
Ull	10/2015	16	10.2 ± 0.1^{ab}	78.2 ± 2.5^{ab}	7.8 ± 0.6^{ab}	25.3 ± 2.8^{ab}	1
Syd	11/2015	4	10.4 ± 0.2^{ab}	83.3 ± 5.4^{abc}	5.4 ± 1.0^{ab}	24.7 ± 4.9^{abc}	1
Ull	11/2015	6	10.3 ± 0.2^{ab}	86.9 ± 3.1^{abc}	8.3 ± 1.1^{abc}	32.9 ± 3.3^{abc}	1
Syd	9/2016	17	9.9 ± 0.1^{ab}	76.9 ± 2.3^{a}	7.0 ± 0.7^{a}	33.1 ± 2.47^{abc}	1
Ull	9/2016	18	10.0 ± 0.1^{a}	77.2 ± 2.3^{a}	7.2 ± 0.8^{a}	33.1 ± 1.8^{abc}	1
Syd	10/2016	19	10.1 ± 0.1^{ab}	79.7 ± 2.5^{b}	9.51 ± 1.1^{bc}	37.9 ± 2.5^{bc}	2
Ull	10/2016	26	10.4 ± 0.1^{bc}	89.1 ± 2.5^{c}	10.8 ± 0.6^{bce}	34.9 ± 1.1^{c}	2
Syd	11/2016	20	10.5 ± 0.1^{c}	92.3 ± 2.3^{c}	12.8 ± 1.7^{d}	38.2 ± 2.0^{c}	3
Ull	11/2016	27	10.6 ± 0.1^{c}	91.2 ± 1.74^{c}	10.2 ± 0.6^{de}	36.1 ± 1.7^{c}	3

3.4.1.1 Experimental Group 1 (n = 94)

The DW of pueruli from the control and all treatments ranged from 44.1 to 114.9 mg, with no difference between any combination of treatments and the control ($F_{(8, 202)} = 1.3$, $P = 0.22$ Fig. 5) with an overall mean of 76.36 ± 0.81 mg.

Overall there was a significant difference in the mean TL among experimental treatment combinations ($F_{(7, 207)} = 6.52$, $P < 0.0001$), with control pueruli having higher TL than pueruli swum for 6 days at 17, 20 and 23 °C ($P = 0.008$, $P < 0.0001$ and $P = 0.0002$ respectively), and higher TL than pueruli held for 6 days at 23 °C ($P < 0.0001$) (Fig. 6A).

There was an overall significant difference in the TP among experimental treatment combinations ($H = 30.78$, $P < 0.0001$), with TP being higher in control pueruli than for

pueruli swum for 6 days at 20 and 23 °C (P = 0.001, P = 0.0002 respectively), and pueruli held for 6 days at 23 °C (P = 0.001) (Fig. 6B).

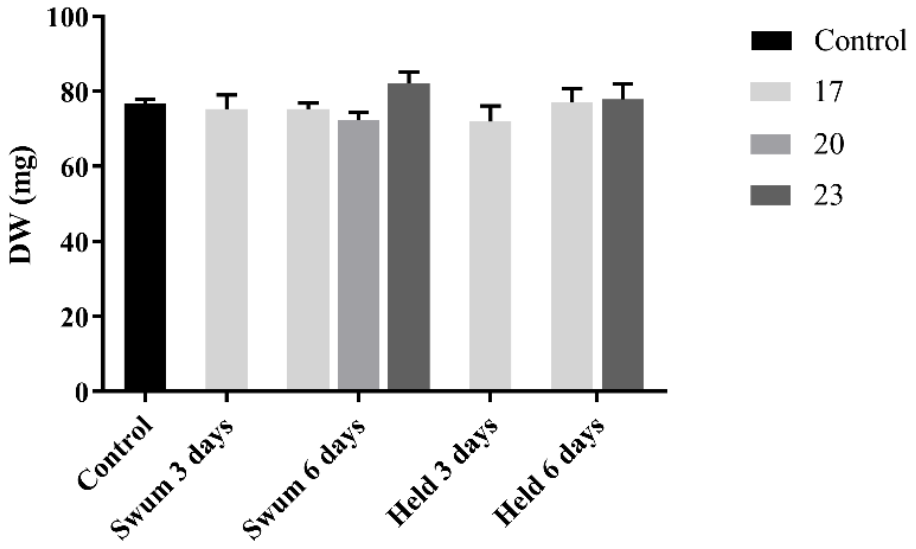

Figure 5. Mean DW of pueruli from experimental group 1, with data for pueruli that were swum and held for 3 and 6 days at 17 °C, swum for 6 days at 17, 20 and 23 °C, and held for 6 days at 17 and 23 °C.

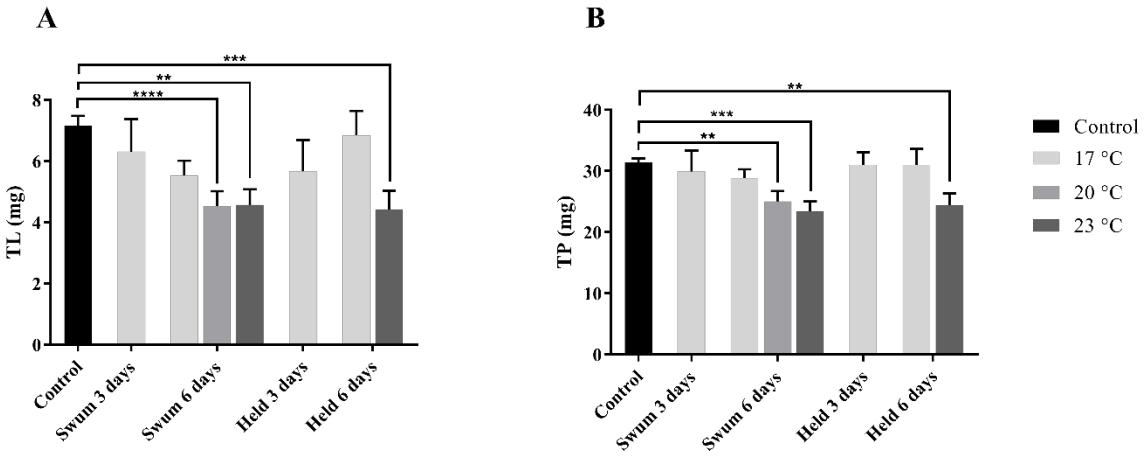

Figure 6. Mean TL (A) and TP (B) of pueruli from experimental group 1, with data for pueruli that were swum and held for 3 and 6 days at 17 °C, swum for 6 days at 17, 20 and 23 °C, and held for 6

days at 17 and 23 °C. Asterisks represent statistical differences (**** $P \leq 0.0001$, *** $P \leq 0.001$, ** $P \leq 0.01$).

3.4.1.2 Experimental Group 2 (n = 45)

There were no differences in DW, TL or TP between control and treatments ($F_{(8, 112)} = 1.0$, $P = 0.44$; $H = 12.93$, $P = 0.11$; $H = 5.41$, $P = 0.71$ respectively) (Fig. 7, 8A and 8B).

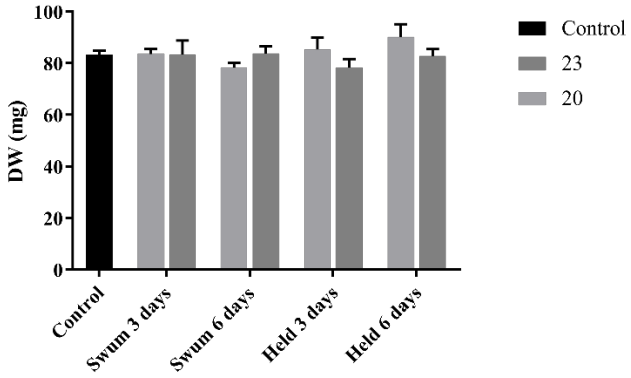

Figure 7. Mean DW of pueruli from experimental group 2, with data for pueruli that were swum and held for 3 and 6 days at 20 and 23 °C.

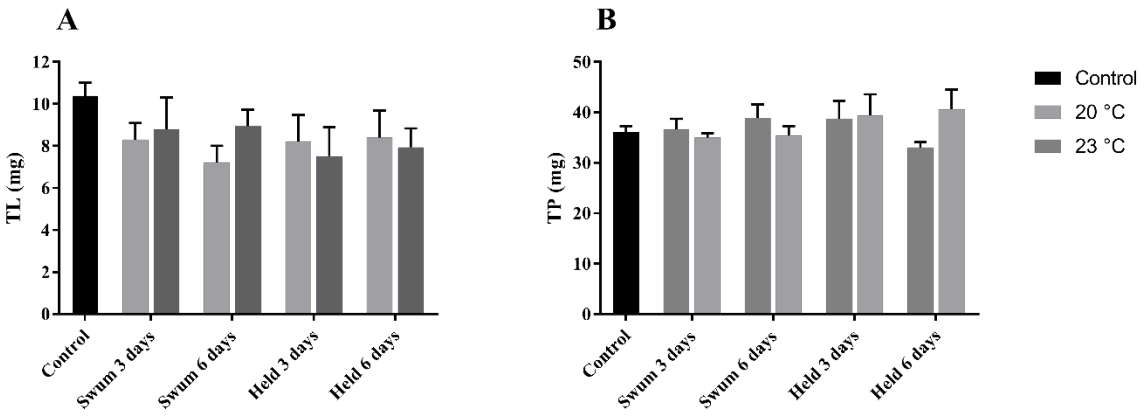

Figure 8. Mean TL (A) and TP (B) of pueruli from experimental group 2, with data for pueruli that were swum and held for 3 and 6 days at 20 and 23 °C.

3.4.1.3 Experimental Group 3 (n = 27)

The mean DW of pueruli was different among experimental treatment combinations ($F_{(8, 77)}$ = 2.47, P = 0.02) (Fig. 9), but multiple comparisons tests did not find differences between pairs of treatments.

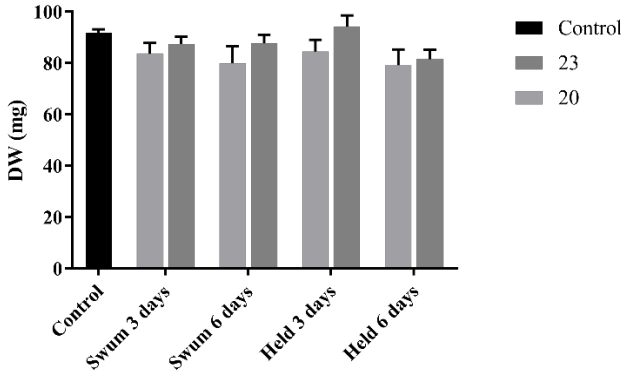

Figure 9. Mean DW of pueruli from experimental group 3, with data for pueruli that were swum and held for 3 and 6 days at 20 and 23 °C.

There were overall differences in the mean TL in pueruli of among the experimental treatment combinations ($F_{(8, 85)}$ = 9.39, P < 0.0001), with the control pueruli having higher TL compared to pueruli swum for 6 days at 20 and 23 °C (P < 0.0001) as well as when compared to pueruli held for 3 days at 20 °C (P = 0.0002) and held 6 days at 20 and 23 °C (P = 0.0001 and P = 0.004 respectively) (Fig. 10A). There were no differences in the TP of pueruli from the different treatments (H = 8.18, P = 0.42) (Fig. 10B).

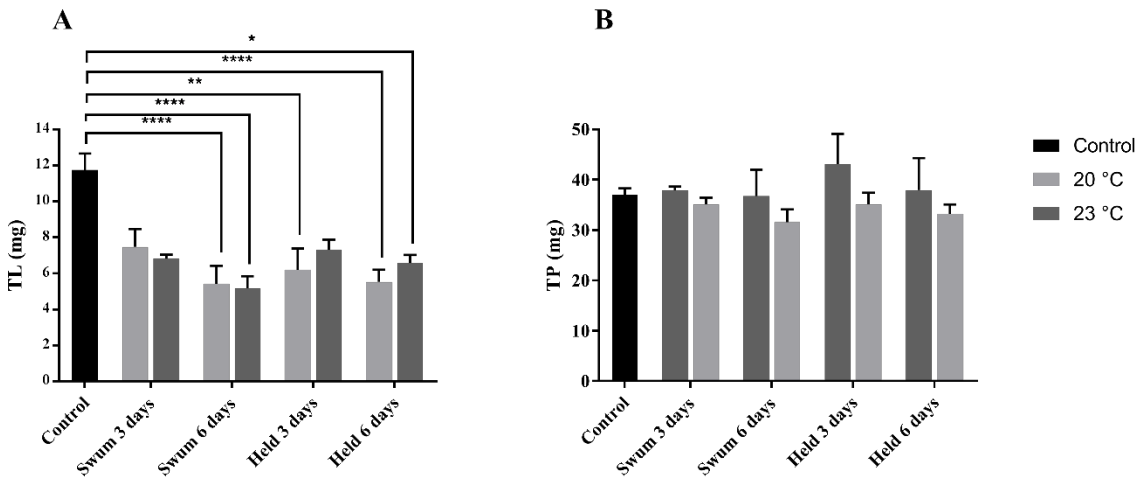

Figure 10. Mean TL (A) and TP (B) of pueruli from experimental group 3, with data for pueruli that were swum and held for 3 and 6 days at 20 and 23 °C. Asterisks represent statistical differences (**** $P \leq 0.0001$, *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$).

3.3.2 *Energy Spent*

There were no differences in the amount of lipid used by pueruli among the treatment combinations in experimental groups 1 and 3 ($F_{(6,116)} = 1.58$, $P = 0.16$ and $F_{(7, 39)} = 1.29$, $P = 0.28$ respectively) (Table 2 and Table 3).

There was a significant difference in the amount of protein used by pueruli among the treatments in group 1 ($H = 15.97$, $P = 0.01$), pueruli swum or held for 3 and 6 days used less protein than pueruli swum or held for 6 days at 20 and 23 °C, there was no difference in the protein used within treatments at 17 °C, or between 20 and 23 °C, either swimming or being held (Table 2).

In group 1 the total energy spent was lower in pueruli swum or held for 3 and 6 days at 17 °C compared to pueruli swum or held for 3 and 6 days at 20 and 23 °C ($F_{(6, 179)} = 13.06$, $P < 0.0001$, table 4), in group 3 there was no difference in the energy spent among treatments ($F_{(7, 39)} = 1.29$, $P = 0.28$, Table 4) (Fig. 11). Comparing the energy spent in matching treatments between group 1 and 3 resulted in no differences ($F_{(5, 118)} = 0.77$, $P = 0.57$) (Table 4).

Table 2. Amount of lipid and protein (mg) used by pueruli of *S. verreauxi* in group 1 after swimming or being held for 3 and 6 days at 17, 20 and 23 °C and the equivalent in joules of the lipid and protein used in the 6 days treatments. Different letters indicate differences within rows, i.e., $P < 0.05$.

Temperature	17 °C		20 °C	23 °C	
Treatment	Swimming	Held	Swimming	Swimming	Held
Lipid used in 3 days	0.9 ± 1.1^{a}	1.5 ± 1.0^{a}	N/A	N/A	N/A
Protein used in 3 days	1.5 ± 3.5^{a}	0.3 ± 2.0^{a}	N/A	N/A	N/A
Lipid used in 6 days	1.6 ± 0.5^{a}	0.3 ± 0.8^{a}	2.6 ± 0.5^{a}	2.6 ± 0.5^{a}	2.8 ± 0.6^{a}
Protein used in 6 days	2.8 ± 1.3^{a}	0.4 ± 2.6^{a}	5.7 ± 1.4^{b}	8.0 ± 1.6^{b}	7.0 ± 1.9^{b}
J from lipid used in 6 days	79.9 ± 20.6^{a}	12.7 ± 31.2^{a}	104.0 ± 19.0^{a}	102.8 ± 20.2^{a}	109.1 ± 24.5^{a}
J from protein used in 6 days	58.84 ± 32.61^{ac}	9.69 ± 61.91^{a}	150.29 ± 39.70^{bc}	188.80 ± 37.81^{b}	164.46 ± 45.37^{bc}
Total J in 6 days	138.7 ± 19.3^{ab}	22.5 ± 34.7^{b}	254.3 ± 22.0^{c}	291.6 ± 21.4^{c}	273.6 ± 25.8^{c}

Table 3. Amount of lipid (mg) spent by pueruli of *S. verreauxi* from group 3 after swimming or being held for 3 and 6 days at 20 and 23 °C and the equivalent in joules of the lipid used in the 6 days treatments. There were no differences within rows, i.e., $P > 0.05$.

Temperature	20 °C		23 °C	
Treatment	Swimming	Held	Swimming	Held
Lipid used in 3 days	4.28 ± 0.99	5.58 ± 1.21	4.94 ± 0.22	4.45 ± 0.57
Lipid used in 6 days	6.34 ± 1.01	6.21 ± 0.66	6.58 ± 0.67	5.19 ± 0.46
J from lipid used in 6 days	250.68 ± 39.94	245.54 ± 26.10	260.17 ± 26.49	205.21 ± 18.19

Table 4. Average joules spent per day by pueruli of *S. verreauxi* from group 1 and 3 after swimming or being held for 6 days at 17, 20 and 23 °C, letters in superscript represent differences in means among treatments within columns, i.e., $P > 0.05$, there were no statistical differences within rows, i.e., $P \leq 0.05$.

Temperature °C	Treatment	Group 1	Group 3
17	Swimming	23.1 ± 3.2^{a}	N/A
20	Swimming	42.4 ± 3.7^{b}	32.6 ± 1.5^{a}
23	Swimming	48.6 ± 3.6^{b}	41.8 ± 6.7^{a}
17	Held	3.8 ± 7.8^{a}	N/A
20	Held	N/A	29.3 ± 3.8^{a}
23	Held	45.6 ± 4.3^{b}	41.0 ± 4.3^{a}

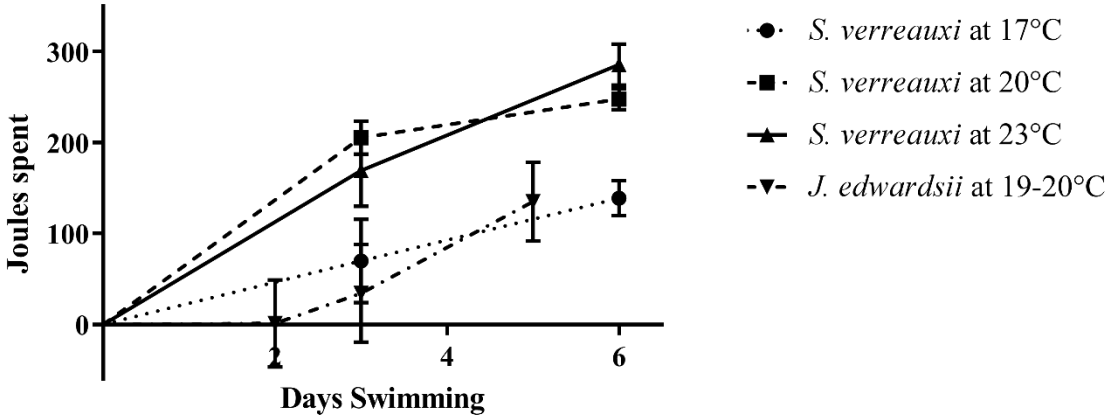

Figure 11. Mean energy (joules) spent by pueruli of *S. verreauxi* swimming from experimental group 1 and 3, and *J. edwardsii* from chapter 2.

3.3.3 *Behavioural results*

3.4.3.1 Group 1

Because of differences in proportion of time spent swimming among collection dates in group 1 ($P < 0.0001$) the group had to be subdivided into two sub-groups for the analyses of

behavioural observations, group 1A is the data collected in September, October and November 2015 and group 1B is the data collected on September 2016. For group 1 the behavioural observational data could only be collected for the experiments at 17 °C due to logistic constraints. During the swimming experiment at 17 °C in group 1A pueruli over 3 days and 6 days combined spent 39.63% ± 1.65% of the time swimming (Fig. 12), the proportion of time spent clinging to the tank was 8.42% ± 0.85%, drifting was 52.21% ± 1.75%, and the proportion of time spent clinging to pueruli was 0.22% ± 0.16%. During the day the time spent swimming was 38.14% ± 5.25% and at night was 39.81% ± 5.76% (Fig. 13).

In group 1B the overall mean proportion of time pueruli spent swimming at 17 °C was 34.18% ± 0.79% (Fig. 12), the proportion of time spent clinging to the tank walls was 19.31% ± 1.08%, time spent drifting was 36.41% ± 0.80%, and the time spent clinging to another puerulus was 4.44% ± 0.57%. The proportion of time spent swimming was 27.45% ± 0.96% during the day and 36.77% ± 1.49 % during the night (Fig. 14).

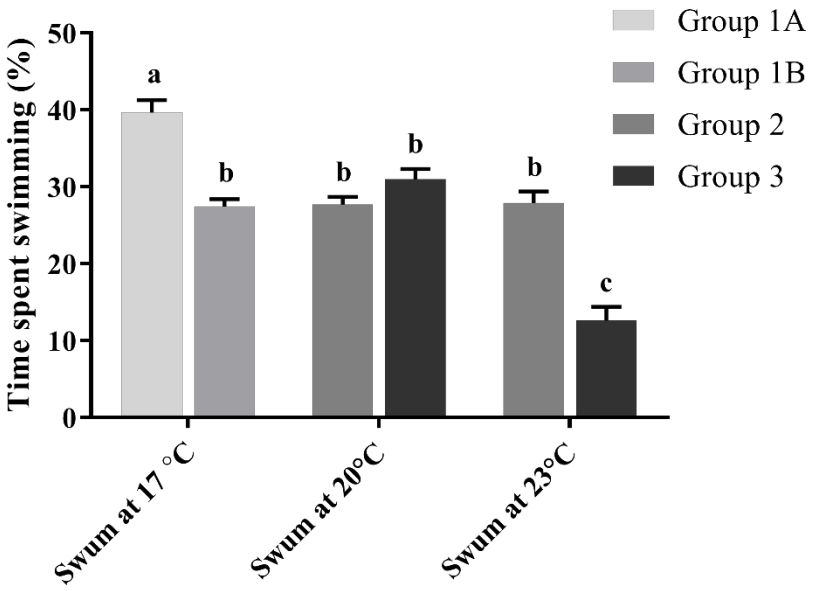

Figure 12. Overall proportion of time spent swimming by *S. verreauxi* pueruli from groups 1, 2, and 3 swum at 17, 20 and 23 °C. Different letters on bars represent differences between means ($P < 0.05$).

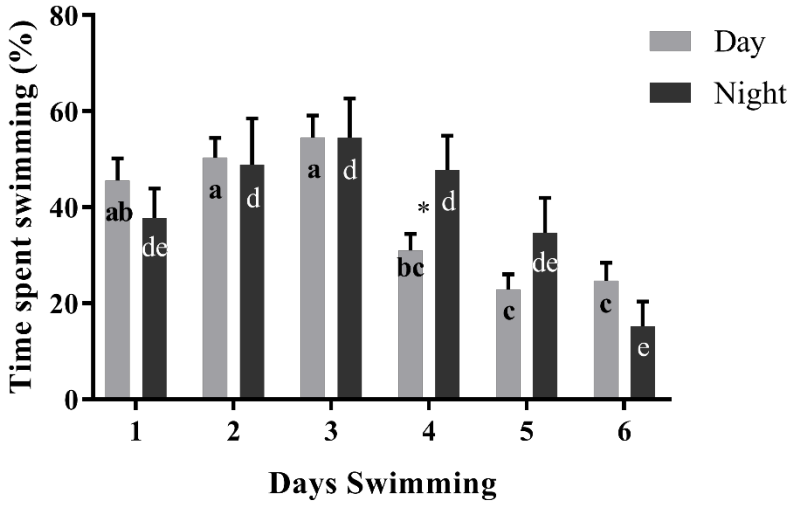

Figure 13. Proportion of time spent swimming by *S. verreauxi* pueruli for each successive day of the experiment for group 1A at 17 °C. Letters in bars indicate differences among means during the day or night ($P < 0.05$). Asterisks represent significant differences between day and night measurements on the same day ($P < 0.05$).

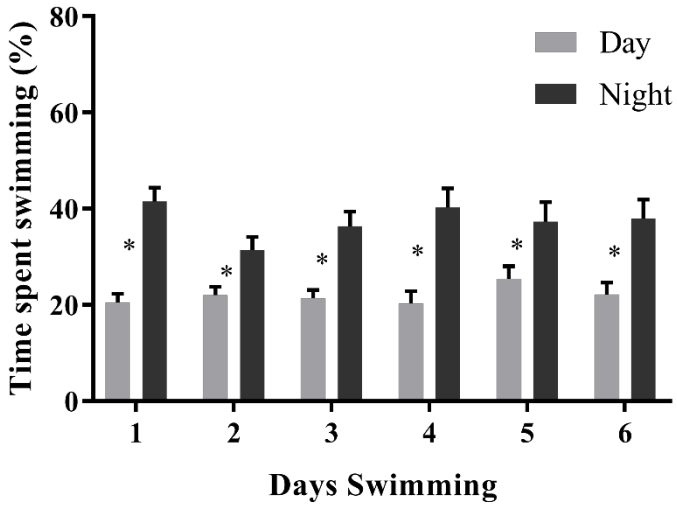

Figure 14. Proportion of time spent swimming by *S. verreauxi* pueruli for each successive day of the experiment for group 1B at 17 °C. Asterisks represent differences between night and day on the same experimental day ($P \leq 0.05$).

3.4.3.2 Group 2

At 20 °C the mean proportion of time pueruli spent swimming was 27.67% ± 1.02% over 3 days and 6 days combined (Fig. 12), drifting time was 24.39% ± 0.98%, the time spent clinging to the tank walls was 32.51% ± 0.0%, and the time spent clinging to another puerulus was 15.70% ± 0.24%. At 23 °C the proportion of time spent swimming was 27.88% ± 1.51%, the proportion of time spent drifting was 31.94 ± 1.57%, the proportion of time spent clinging to the tank walls was 24.62 ± 1.69% and the time spent clinging to another puerulus was 14.95% ± 0.16%.

At 20 °C during the day the mean proportion of time spent swimming was 27.07 % ± 5.35% and during the night was 30.40 % ± 4.64%. At 23 °C during the day the mean proportion of time spent swimming was 34.42% ± 6.15% and during the night 40.81% ± 5.26% (Fig. 15). They were swimming 1.37 times more during the night versus day (P = 0.0003).

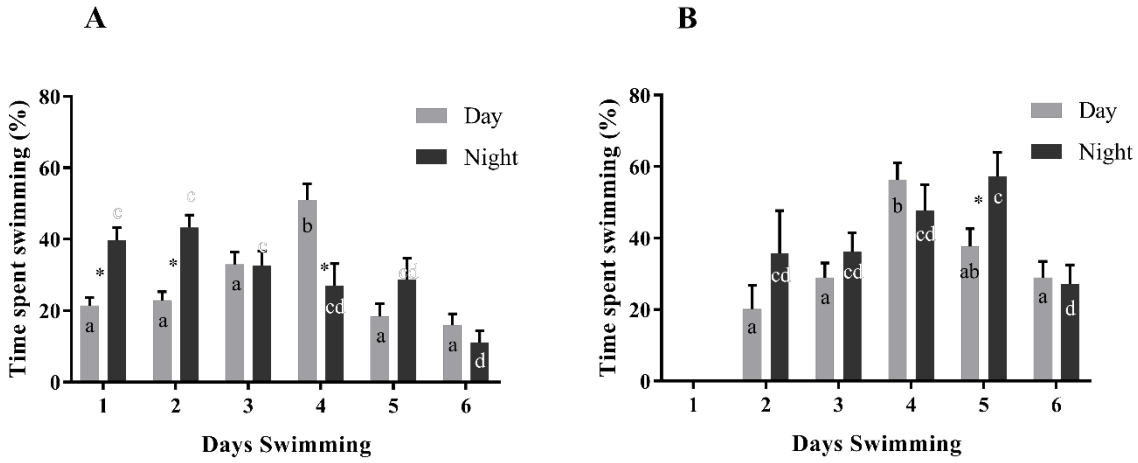

Figure 15. Proportion of time spent swimming by *S. verreauxi* pueruli for each successive day of the experiment for group 2 at 20 °C (A) and 23 °C (B). Letters in bars indicate differences among means during the day or night ($P \leq 0.05$). Asterisks represent differences between day and night measurements on the same day ($P \leq 0.05$).

3.4.3.3 Group 3

At 20 °C the mean proportion of time spent swimming over 3 days and 6 days combined was 30.98% ± 1.32% (Fig. 12), the proportion of time spent clinging to tank walls was 32.72% ± 0.0%, the drifting time was 34.92% ± 1.36%, and the time spent clinging to another puerulus was 1.20% ± 0.0%. At 23 °C the proportion of time spent swimming was 12.61% ± 1.77%, the proportion of time spent clinging to the tanks walls was 9.86% ± 1.66%, the drifting time was 77.08% ± 2.25%, and the mean proportion of time spent clinging to another puerulus was 0.56% ± 0.01%.

At 20 °C during the day the mean proportion of time spent swimming was 25.61 % ± 4.02 % and at night 40.41% ± 5.01 % (Fig. 16). At 23 °C during the day the mean time spent swimming was 6.15% ± 3.44%, at night the mean time spent swimming was 1.55% ± 0.64%. At 23 °C pueruli were swimming 1.72 times more during the night versus day ($P < 0.0001$). Pueruli at 20 °C were almost 10 times more likely to be swimming compared to pueruli at 23 °C ($P < 0.0001$).

There was no difference in the proportion of time pueruli spent on each of the four different behaviours between groups 2 and 3 in swimming experiments at 20 °C ($P = 0.27$), but at 23 °C the pueruli from group 3 were 2.68 times less likely to be swimming than in group 2 ($P < 0.0001$).

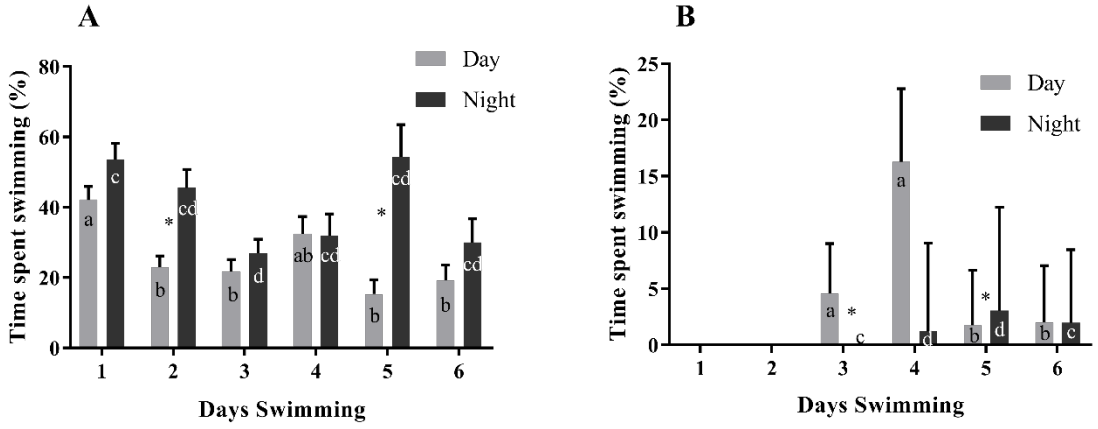

Figure 16. Proportion of time spent swimming by *S. verreauxi* pueruli for each successive day of the experiment for group 3 at 20 °C (A) and 23 °C (B), scaling in graph B is smaller to show data, but the

data from night observations on day 3 are too small to show on the graph. Letters in bars indicate differences among means either during the day or night ($P \leq 0.05$). Asterisks represent differences between day and night measurements on the same day ($P \leq 0.05$).

3.4. Discussion

3.4.1 *Lipid and protein use*

Migrating pueruli primarily use lipid to fuel their swimming (Jeffs et al. 1999, 2001a, 2001b, 2002, Limbourn et al. 2008, Limbourn and Nichols 2009) and start using protein once lipid reserves become depleted (Fitzgibbon et al. 2014a). Control pueruli in group 3 had the greatest starting levels of lipids (12.82%) and proteins (40.34%) and pueruli in this group subsequently spent more lipid than pueruli in groups 1 and 2 during both swimming and holding treatments at all temperatures. Pueruli in group 3 did not use proteins during these treatments. Control pueruli in group 1 had mean TL of 9.33%. Pueruli from this group had 7.20% lipid or more at the end of the treatments (swum and held for 3 and 6 days at 17 °C) did not spend protein during the experiments. However, those pueruli with lower TL of 5.73% to 5.95% also had low protein levels (swum for 6 days at 20 °C, and swum or held for 6 days at 23 °C) consistent with a switch to the catabolism of protein once pueruli reach between 7.20% to 5.95% lipid. Pueruli in group 3 at the end of all treatment combinations had from 5.90% to 7.75% lipid, and had not used significant amounts of protein. It could be that pueruli from the treatment with 5.90 % lipid (swum 6 days at 23 °C) had only recently reached the critical lipid concentrations and were starting to catabolise protein, but their protein use was insufficient to be detected. The total energy spent in joules between groups 1 and 3 was similar when swimming or held at the same temperature.

A minimum of 5% lipid of body mass has been suggested as essential for meeting requirements for structural and biochemical functioning for krill (Hagen et al. 2001). This minimum is consistent with the apparent threshold in pueruli of *S. verreauxi* when they switch from catabolising lipid to protein. The effect of temperature on the amount of lipid used in the different treatments was not detected in group 1, since lipid was being spared, but these effects

are evident in the protein used. In contrast, in group 3 the effect of temperature was seen in the lipid used, but not in protein content. These differences in the results also highlight the high variability in the biochemical condition of pueruli arriving to the coast, especially among different times.

Pueruli in holding treatments used the same amount of lipid and protein as those swimming for the same duration at the same temperature. It is likely that the development toward first instar juvenile was suppressed in pueruli that continued swimming in the experimental flumes, in comparison to the pueruli that were in reptant holding conditions where development appeared to be accelerated (pers. obs.). Extensive morphological changes are required for the moult to first instar juvenile, which are also likely to require substantial rearrangement of body proteins, and the extensive use of remaining energy reserves as an energy source at a similar rate to that measured for swimming pueruli.

3.4.2 *Energy spent swimming*

Wild pueruli in Group 1 had a daily average use of 23.12 J ± 3.22, 42.38 J ± 3.66 J, and 48.6 J± 3.57 J when swimming at 17, 20 and 23 °C respectively, using a combination of lipid and protein. Group 3 used 32.56J ± 1.46 J per day when swimming at 20 °C and 41.78J ± 6.65J when swimming at 23 °C, in both cases using only lipids. Experimental measures of energy using similar experimental methods in swimming pueruli of *J. edwardsii* found that they consumed 63.2 J per day of lipid and 32.14 J per day of protein at 19-22 °C, (i.e., total of 96 J per day) which is considerably higher than measured for pueruli of *S. verreauxi* at a range of temperatures. However, the body mass of *J. edwardsii* pueruli is approximately double that of *S. verreauxi* and their physical body dimensions are larger, which would greatly increase the hydrodynamic drag during active swimming, which would explain the greater energy use by pueruli of this species.

Estimations of the resting metabolic rates in pueruli of *P. cygnus* have found they spend 13.0 J per day (Lemmens 1994a), and in *S. verreauxi* metabolic rate was measured at 7.7 to 26.4 J per day (Fitzgibbon et al. 2014), depending on the moult cycle and metabolic state (standard,

active, or routine metabolic rate). These measures are all smaller than the equivalent rate of energy utilisation measured in this current research.

3.4.3 *Swimming behaviour*

The clinging behaviour observed in the experimental pueruli is believed to be a settlement seeking behaviour (Kittaka and Ikegami 1988, Kittaka and Kimura 1988, Kittaka 1990) and may be a consequence of the wild pueruli being removed from collectors where they had already begun to adopt a reptant clinging behaviour. The behaviour of pueruli clinging to one another has also been reported for *P. argus* (Calinski and Lyons 1983), where pueruli would clump in groups up to 50-60 individuals when captured and held together. When swimming behaviour decreased the drifting behaviour increased. Comparing the drifting proportion of group 1 at 17 °C and group 3 at 23 °C, drifting went from 52.2% to 77.1%, while the clinging behaviour only increased in small percentages (clinging to tanks 8.4% to 9.9%, clinging to other pueruli 0.2% to 0.6% from 17 to 23 °C respectively). The drifting behaviour has also been previously observed in pueruli of *P. argus* in an experimental field situation (Kough et al. 2014). Drifting by pueruli in the experimental tanks in this current study could also reflect a resting period needed to recover after the use of anaerobic metabolism to support elevated energetic demands (Ellington 1983, Jensen et al. 2013), which can explain the significant reduction in swimming behaviour observed at 23 °C. Oxygen stress has been observed to decrease swimming activity with a concomitant increase in hovering behaviour in krill larvae (Kils 1982). Likewise, in fish larvae drifting has been proposed as an effective means of saving energy when subjected to strong currents (Hogan and Mora 2005). Drifting could also be used as passive transport, by relying on currents or Stoke's forces to advance towards the coast (George 2005, Jeffs et al. 2005).

In the swimming experiments wild pueruli would swim to match the speed of the current in the tanks while continuously displaying rheotaxis, as previously seen in *J. edwardsii* (García-Echauri and Jeffs 2018). In this study, although pueruli were not continuously active, they would swim both during the day and night, as has also been observed in the wild (Phillips and Olsen 1975, Kough et al. 2014). Pueruli mostly spent a greater proportion of time swimming during the night as has also been observed in fish larvae (Champalbert and Castelbon 1989,

Forward et al. 1996, Fisher and Bellwood 2003), but in some experimental groups of pueruli this difference was not maintained throughout the duration of the experiment. The swimming experiments in this study were performed in darkness, and there is evidence that fish larvae can adjust their circadian patterns of swimming to the timing of artificial lights (Champalbert and Castelbon 1989), so it is possible that day and night differences observed in the swimming pattern of pueruli may not be representative of pueruli in the wild. It has been reported that fish larvae swim less after settlement (Leis et al. 2011). Therefore, it is also possible that the experimental use of pueruli from collectors that have already completed a migration to coastal settlement habitat may under represent the swimming capabilities of pueruli, compared to newly metamorphosed pueruli in the pelagic environment. In this study there is a reduction in time spent swimming towards the end of the 6 days swimming experiment on group 1, these observations were on pueruli swimming at 17 °C, which had over 7.20% lipid, and were not yet sparing lipid.

3.4.4 *Effect of increased temperatures on pueruli migration*

For a puerulus of *S. verreauxi* to swim 1 km at 17 °C it would require on average about 4.5 J and take around 4 hours, while at 20 °C it would require10.6 J and take 6 hours, and at 23 °C about 24 J and about 15 hours based on the estimates of the time spent swimming, the swimming speed, and the rate of energy being used, from experimental results from groups 1 and 3. The pueruli of *J. edwardsii* have been estimated to use 96 J per day when migrating onshore based on the same experimental methods as this current study and taking into consideration both lipid and protein (García-Echauri and Jeffs 2018). Indirect estimations have calculated that pueruli of *J. edwardsii* use 5.7 J km^{-1} (Jeffs et al. 1999) and *P. cygnus* uses 1.6 J km^{-1} when crossing the continental shelf, but with the aid of currents and wind (Phillips et al. 2006b). The estimations of distance capable of being swum by pueruli of *S. verreauxi* in this study are the distances that pueruli can traverse autonomously, without the assistance or hindrance of physical processes.

The increment in temperature from 17 to 23 °C increases the energy used by pueruli two times and reduces the average swimming time by three times. Increasing water temperature could be expected to prolong the shoreward migration of pueruli, increasing the chances of predation,

ultimately reducing the number of pueruli that reach the coast because of an energetic shortfall, or arrive with insufficient reserves to subsequently successfully transition to benthic juveniles.

This study confirms the potential negative effects that warming oceans can have on the recruitment of spiny lobsters, and particularly for *S. verreauxi*, a species that is distributed in a hot spot for climate change.

Chapter 4. Landing in hot water: Recruitment of spiny lobsters in warming coastal waters

4.1. Introduction

Increasing ocean water temperatures that are thought to be due to changes in global climate have been identified as the likely cause of a consistent pattern of recent declines in the recruitment in several important spiny lobster fisheries in different parts of the world (Fitzgibbon et al. 2014a). The elevated temperatures are thought to increase the catabolism of nutritional reserves in the lecithotrophic post-larval or puerulus stage of spiny lobsters, ultimately resulting in a shortfall among those individuals with lesser reserves accumulated during the prior larval phase of the lifecycle (Fitzgibbon et al. 2014a). The pueruli of spiny lobsters have limited cardiorespiratory capacity (Fitzgibbon et al. 2014b) and place exceptional demands on their energy reserves due to their active and lengthy shoreward migration from oceanic waters, where they undergo the majority of their extended larval development (Phleger et al. 2001, Booth 2006, Fitzgibbon et al. 2014a). For this onshore migration the pueruli use large stores of polar lipids as their primary fuel, which at the outset of their shoreward journey typically make up 86–96% of the total lipid content of pueruli and comprise around a third of their dry body mass (Jeffs et al. 1999, 2001a, 2001b, 2004, Fitzgibbon et al. 2014a). Protein may also be utilised, but to a much lesser extent (Jeffs et al. 2002, Fitzgibbon et al. 2014a). After arriving in coastal waters pueruli need to have sufficient remaining nutritional reserves to sustain routine metabolism, as well as providing resources for the morphological and physiological changes associated with the transition into a benthic first instar juvenile (FIJ), which also includes a moult (Wells et al. 2001, Phillips et al. 2006b). These changes include the development of hardened mouthparts and fully functioning digestive tract that allows feeding, and therefore the subsequent resumption of a reliance of exogenous nutritional resources. Environmental variations, such as fluctuations in the strength and direction of ocean currents, can delay pueruli from reaching suitable coastal settlement locations, thereby further increasing the demand on energy reserves needed to reach coastal waters (Jeffs et al. 2005,

Wilkin and Jeffs 2011). Such events are thought to result in energetically compromised settling pueruli and FIJs that subsequently expire due to insufficient reserves. It has been postulated that this situation is occurring more frequently because of increasing surface water temperatures encountered by these lobsters, which results in more energetic reserves being used by these lobster stages as they are metabolic conformers (Fitzgibbon et al. 2013). This scenario is suggested as a possible explanation for recent declines in recruitment of major spiny lobster populations in several parts of the world (Ehrhardt and Fitchett 2010, Linnane et al. 2010a, Feng et al. 2011, Fitzgibbon et al. 2014a).

Starvation in crustacean larvae typically delays the progression of development, and digestive enzymes are downregulated and growth diminishes or is halted (Liddy et al. 2003, Calvo et al. 2012, 2013, Guerao et al. 2012). Starved larvae of spiny lobsters, known as phyllosomas have an increase in protease activity and decrease in lipase activity (Johnston et al. 2004), while lipid and protein content diminishes as a result of catabolism to maintain routine metabolism (Ritar et al. 2003). Under-fed juvenile spiny lobsters also show a marked reduction of growth as nutritional resources are used to maintain homeostasis (Robertson et al. 2000).

During prolonged starvation in crustacean larvae, besides lipid reserves typically decreasing, irreversible changes commonly occur in the ultrastructure of cells in the hepatopancreas involved in the storage of lipid, glycogen and trace elements (i.e., autolysis in the endoplasmic reticulum and the mitochondria become swollen), and degeneration in epidermal and muscle tissues also occurs (Loizzi 1971, Storch and Anger 1983, Al-Mohanna and Nott 1987, Guerao et al. 2012). Once food deprivation has caused irreversible damage (Anger 1995) the larvae are considered to have passed their point of no return (PNR) (Blaxter and Hempel 1963). The median PNR (PNR_{50}) is defined as the time when 50% of the starved animals can no longer recover if subsequently fed (Paschke et al. 2004).

The point at which larvae reach their PNR depends on the extent of the endogenous structural damage to the lobster caused by starvation, as well as the extent of the remaining nutritional reserves. In particular, the sufficiency of these reserves to meet the requirements for progression into the next stage of development, can be expected to vary in relation to temperature and rate of oxygen consumption (Ross and Quetin 1989). Previous studies have

hypothesised that a critical point in the early life of spiny lobsters is the nutritional condition of pueruli upon settling in coastal waters, and whether they retain sufficient remaining energy reserves from their prior migration from offshore waters to successfully moult and fully establish as FIJs that are capable of independent benthic feeding (Fitzgibbon et al. 2014b). For example, the PNR_{50} has been calculated at 22.5 days in *P. cygnus* juveniles, a subtropical spiny lobster of Western Australia (Limbourn et al. 2008), and in the tropical *P. argus* at only 9.5 days in the cold season and 12 days in the warm season in the Caribbean coast of Mexico (Espinosa-Magaña et al. 2017). *Jasus edwardsii* and *S. verreauxi* are species of spiny lobsters that both occur in temperate waters with overlapping ranges across parts of Australasia (Jeffs et al. 2013). Their distribution occurs within a global ocean region characterised by both past and predicted future rapid increases in seawater temperatures that have been associated with climate change (Blasiak et al. 2017). The optimal temperature for *J. edwardsii* juveniles is 19-21 °C (Thomas et al. 2000) and for *S. verreauxi* juveniles is 21.5 °C (Fitzgibbon et al. 2017). Climatic perturbations and changes in oceanographic processes, including increasing coastal seawater temperatures, have been implicated in marked fluctuations in recruitment of both spiny lobster species (Linnane et al. 2010b, Fitzgibbon et al. 2014b, Hinojosa et al. 2017).

Therefore, the aim of this work was to experimentally determine the resilience of FIJs of these two spiny lobster species by determining their nutritional status and survival for a range of temperatures. In addition, the PNR_{50} of *S. verreauxi* was experimentally determined to assess their capacity to recover from low nutritional status.

4.2. Materials and methods

4.2.1 *Experimental animals*

4.3.1.1 Starvation in *Jasus edwardsii*

Pueruli of *J. edwardsii* (stages II and III sensu Booth 1979) were collected in crevice collectors (Booth 2001) deployed in shallow coastal water at Castle Point, on the Wairarapa coast, New Zealand (40° 54' 07"S, 176° 13' 44"E) during January 2016. Crevice collectors were checked

daily and any pueruli that had arrived in the collectors overnight were carefully removed and staged according to the schema of Booth (1979).

4.3.1.2 Starvation in *S. verreauxi*

Pueruli of *S. verreauxi* (stages II and III sensu Booth 1979) were collected at two sites in shallow coastal waters of New South Wales, near Sydney (34° 4' 14.92"S, 151° 10' 56.45"E) and Ulladulla (35° 24' 40.51"S, 150° 27' 12.21"E). Seaweed-type pueruli collectors (Phillips and Booth 1994a) were deployed by the New South Wales Department of Primary Industries, at both sites from August to November in 2015, and in October 2016. Pueruli collectors were cleared every month within the week after the new moon, weather permitting (Montgomery and Craig 2003). Seawater temperature during collection ranged from 17 to 20 ºC. Pueruli were placed in buckets filled with ambient seawater from the collection sites and aerated via an airstone, and transported back to the aquaria facilities at the Sydney Institute of Marine Science.

4.2.2 *Experimental setup*

4.3.2.1 Food withholding experiments in *J. edwardsii*

A total of 19 stage II pueruli of *J. edwardsii* were frozen immediately after collection for subsequent biochemical analyses to establish a baseline for comparison of nutritional condition among pueruli. The stage III pueruli were retained for a food witholding experiment as these pueruli would belong to the same cohort of pueruli as the stage II, all having arrived at the collectors within a few days of one another (Kittaka et al. 1997). Nine stage III pueruli were placed in individual containers within buckets filled with ambient seawater (19 to 22 °C) aerated with an air-stone, and then transported back to the Leigh Marine Laboratory. Once at the laboratory, the containers holding individual pueruli were transferred to aquaria supplied with settled and filtered seawater (200 µm) held at 21 ± 0.5 °C. The lobsters were held without food and observed every 12 hours until they died. When pueruli moulted to first instar juveniles (FIJs) their exuviae were removed from their individual containers and the lobsters were retained under the same holding conditions. When a lobster was found dead it was frozen immediately for later biochemical analyses. A record was kept of the date on which all pueruli moulted to become FIJs, and the subsequent period before the lobster expired.

4.3.2.2 Food withholding experiments in *S. verreauxi*

Stage II pueruli of *S. verreauxi* were frozen immediately upon collection for later determination of nutritional condition. Twenty-eight, 21 and 10 Stage II pueruli were collected in September, October and November 2015 respectively. As before, stage III animals from the same cohorts of pueruli arrivals were retained for food withholding experiments, but at different temperatures.

Fourteen stage III pueruli collected in September 2015 were held at 17 °C, nine collected in October 2015 were held at 20 °C, and eleven collected in November 2015 were held at 23 °C. The seawater supply for pueruli in the 23 °C treatment was slowly raised from 20 °C (i.e., the ambient temperature at capture) to 23 °C over two days. Lobsters were housed in individual containers in aquaria in a darkened room supplied with filtered seawater (20 µm) at controlled water temperatures (± 0.5 ºC) with 100% replacement per hour.

Subsequent observations of development and mortality proceeded in the same manner as for pueruli of *J. edwardsii* in the preceding section.

4.3.2.3 Point of no return in *S. verreauxi*

In October 2016 a total of 114 stage II and III pueruli of *S. verreauxi* were recovered from collectors, and then 55 pueruli were randomly selected shortly after collection and frozen for subsequent biochemical analyses to determine their initial nutritional condition. The remaining 59 pueruli were housed in individual containers in aquaria at the Sydney Institute of Marine Science and supplied with filtered seawater (UV and 5 µm) at 17 °C until they moulted to FIJs. The FIJs were then randomly allocated to six starvation duration treatments, i.e., 0, 15, 20, 25, 30 and 35 days after moulting to FIJs. Following each period of starvation, the lobsters were fed ad libitum with fresh oyster flesh and their subsequent survival was tracked until they perished or transitioned into second instar juveniles (SIJs). The experimental determination of PNR_{50} for *J. edwardsii* could not be undertaken due to the limited number of animals available for experimentation because of the difficulty in their collection.

4.2.3 *Biochemical analyses*

The carapace length (CL) and the wet weight (WW) of frozen lobsters were measured, and after being lyophilised they were re-weighed to determine their dry weight (DW). Total lipid (TL) of each lobster was determined gravimetrically using a modified Bligh and Dyer (1959) protocol. After extracting the lipids the residual tissue was assayed for total protein (TP) using a Micro BCA protein assay (Thermo Scientific, Rockford, Illinois, USA) based on the bicinchoninic acid determination method for protein quantification (Walker 1994, Jeffs et al. 1999). The lipid and protein composition of lobsters as a percentage of DW were calculated to facilitate comparison between the two species of lobsters, which have different body sizes. The rate of catabolism of lipid and protein by FIJs of *S. verreauxi* and *J. edwardsii* starved to death was calculated by subtracting the TL or TP at expiration from the mean TL or TP of the control pueruli at capture, the resulting difference provides an estimate of the lipid utilised between capture and expiry. This estimate was then divided by the total number of days it took for the puerulus to go from stage II until it perished. The transition time for stage II pueruli to FIJs was set as 14 days for *J. edwardsii* (Kittaka 1990, Phillips and Booth 1994) and 12 days for *S. verreauxi* (Kittaka et al. 1997).

4.2.4 *Statistical Analyses*

For *J. edwardsii* the mean CL, DW, TP and TL of lobsters sampled at the time of collection was compared with the corresponding mean for lobsters held without food until expiration using a *t*-test.

For the food withholding experiment for *S. verreauxi*, one-way ANOVAs were firstly used to compare mean CL, DW, TP and TL, of the control lobsters sampled from collectors on different dates and locations to confirm there were no differences in the morphological or biochemical condition among the different sources of lobsters at the outset. Following this confirmation, the data were pooled and one-way ANOVAs were then used to compare the TL and TP in *S. verreauxi* sampled at collection and after being held at three different temperatures without feeding. The durations of survival of lobsters held without feeding were compared for the three different temperatures using a Kruskal-Wallis test.

For the point of no return experiment in *S. verreaux*i a logistic regression model was used to determine the PNR_{50} from the percentage of FIJs recovering from the different food withholding treatments sufficiently to successfully moult to SIJs. One-way Poisson ANOVAs were used to compare the length of survival of FIJs exposed to different durations of food withholding prior to feeding commencing, and the time taken by FIJs to successfully progress to SIJs after different durations of prior starvation.

The mean rate of catabolism of lipid and protein by starved *S. verreauxi* FIJs lobsters was compared for the three temperature treatments using a one-way ANOVA. The mean proportions of remaining lipid and protein in starved lobsters was compared between *J. edwardsii* and *S. verreauxi* using a *t*-test.

Prior to all analyses, any percentage data were arcsine transformed, and the normality of the data was confirmed with a D'Agostino-Pearson test, and the equality of variance of the data was confirmed with a Brown–Forsythe test. Variation around means is presented as standard error throughout the results.

4.3. Results

4.3.1 *Food withholding experiment in J. edwardsii*

The 19 lobsters sampled immediately after removal from crevice collectors were highly variable in all morphological and biochemical measures (Fig 17). For example, their DW varied by more than 200% ranging from 78.0 to 170.0 mg, and TL by over 400% ranging from 6.2 to 30.1 mg.

All lobsters held without food moulted to FIJs, but none transitioned to second instar juvenile. The lobsters held without feeding survived from 25 to 48 days (mean = 34.44 ± 3.44 days) post-moult to FIJs and were less variable in their morphological and biochemical measures than the lobsters collected upon removal from collectors (Fig. 17). For example, DW of the starved lobsters ranged from 105.0 to 170.0 mg, and TL ranged from 0.9 to 3.3 mg.

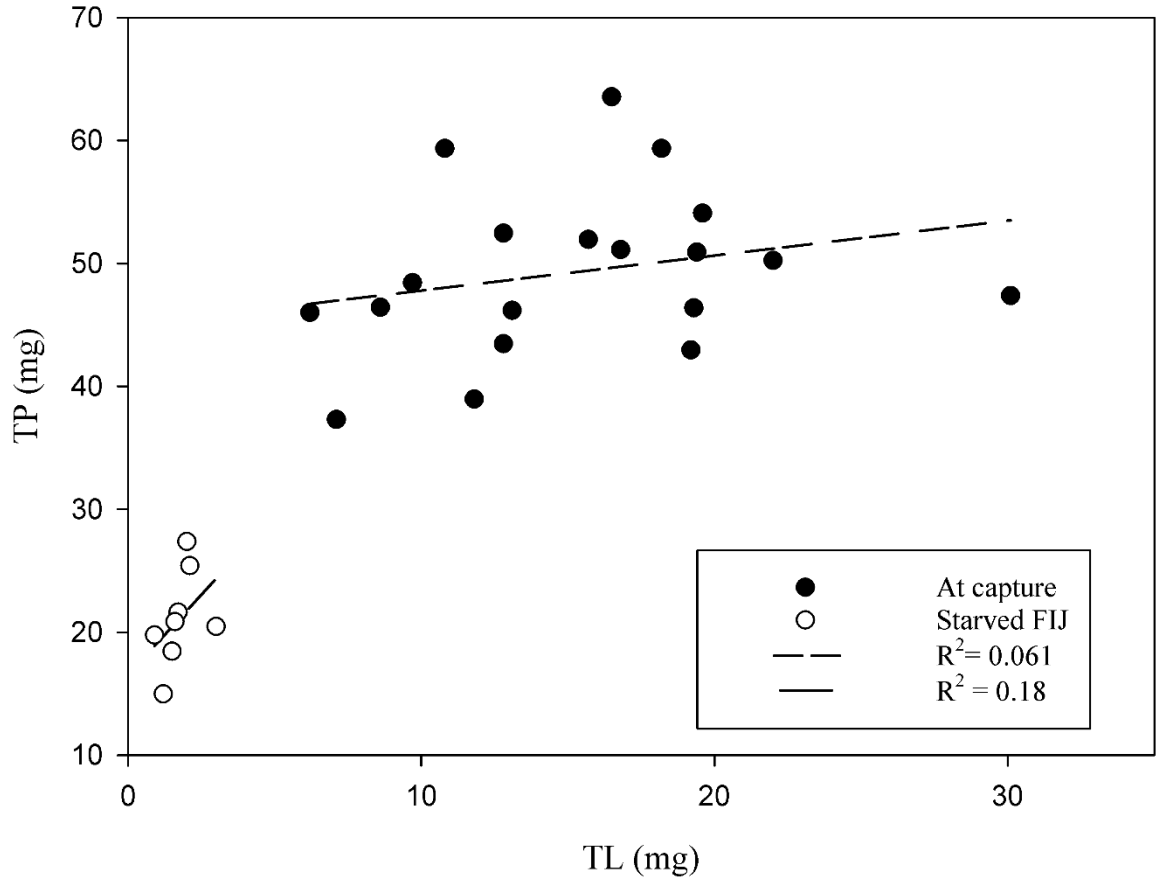

Figure 17. Measured total lipid and total protein content for individual *J. edwardsii* at capture and after being held at 21 ± 0.5 °C without feeding until expiration.

There were only small increases in both morphometric measures (i.e., CL and DW) of the lobsters sampled from the collectors and those lobsters that were held at 21± 0.5 °C without feeding until expiration (Table 5). In contrast, there were marked decreases in all biochemical measures, with TP more than halving, and TL decreasing by more than 80%. On average TP in the lobsters decreased by 28.7 ± 0.9 mg and TL by 13.1 ± 1.2 mg (Table 5).

Table 5. Mean (±SE) morphometric and biochemical measures of *J. edwardsii* at capture and after being held at 21 ± 0.5 °C without feeding until expiration. Within row significant differences of means, * ≤ 0.05, **** ≤ 0.0001.

Mean Measure (± SE)	Treatment	
	At capture (n = 19)	**Food withholding (n = 9)**
CL (mm)	11.1 ± 0.1	11.5 ± 0.2 *
DW (mg)	142.1 ± 3.7	143.5 ± 11.4
TP (mg)	50.70 ± 1.50	22.00 ± 1.11 ****
TL (mg)	15.50 ± 1.20	1.75 ± 0.23 ****
ΔTP as mg day^{-1}	N/A	0.59 ± 0.05
ΔTL as mg day^{-1}	N/A	0.28 ± 0.02
Survival (days)	N/A	34.44 ± 3.4

4.3.2 *Food withholding experiments at different temperatures in S. verreauxi*

The lipid and protein content of control lobsters sampled from collectors were highly variable (Fig. 18) but there were no differences for any of their morphological and biochemical measures among different collection sites and dates of collection: CL ($F_{(2, 53)} = 0.02$, P = 0.98), DW ($F_{(2, 53)} = 0.45$, P = 0.64), TL ($F_{(2, 53)} = 1.59$, P = 0.21), and TP ($F_{(2, 40)} = 2.62$, P = 0.08). This homogeneity of the morphological and biochemical measures among the control lobsters sampled over different sites and dates provided confidence for direct comparisons among the pooled results for the lobsters used for the subsequent experimental food withholding treatments.

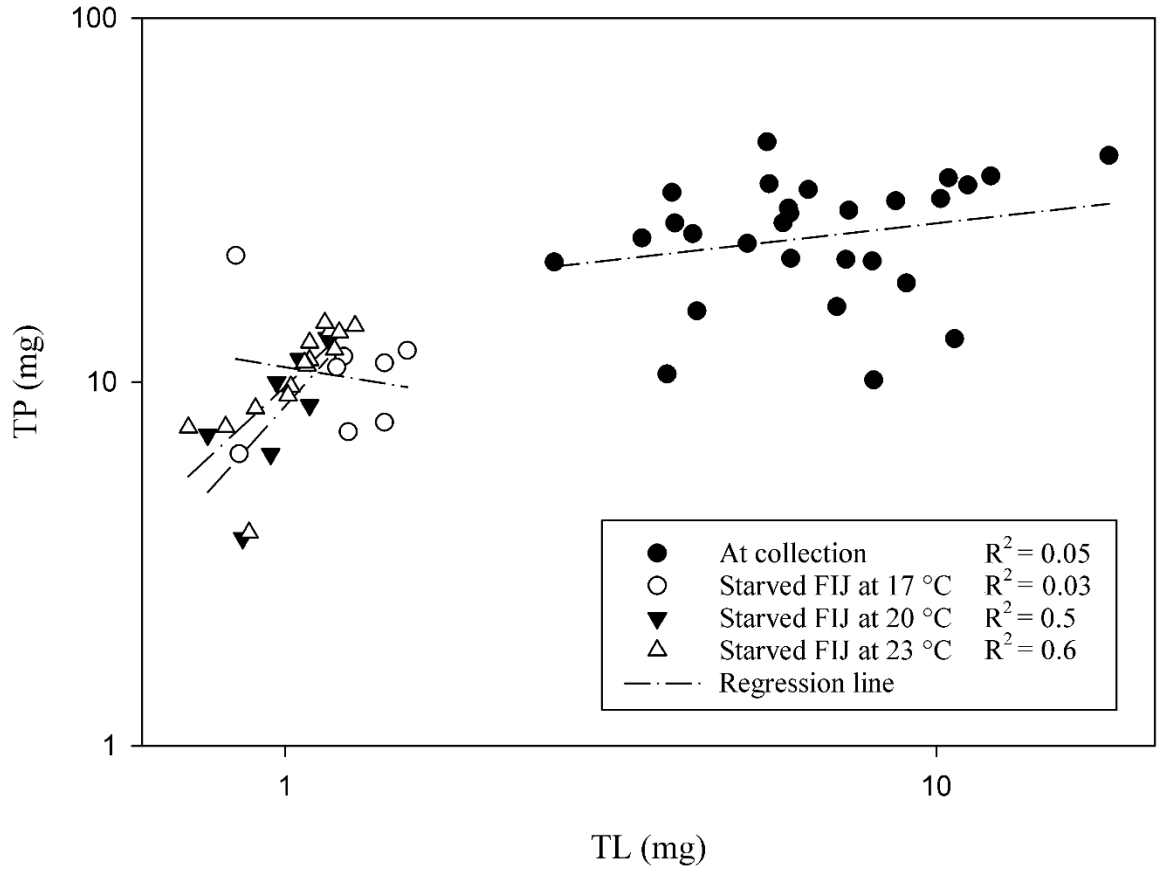

Figure 18. Total lipid and total protein content of *S. verreauxi* sampled at collection and after transitioning to FIJs under holding conditions at 17, 20 and 23 °C without feeding until expiration. Axes are in the logarithmic scale.

The TL content of control pueruli ranged from 2.59 to 18.42 mg and in starved FIJs ranged from 0.7 to 1.7 mg across all temperature treatments. Overall the TL was greater in control pueruli versus starved lobsters ($F_{(3, 91)} = 45.14$, $P < 0.0001$), and this difference between control pueruli and starved lobsters was consistent at all temperatures ($P < 0.0001$ for each temperature), but there were no differences in TL of lobsters among the three temperature treatments ($P > 0.99$, Fig. 19).

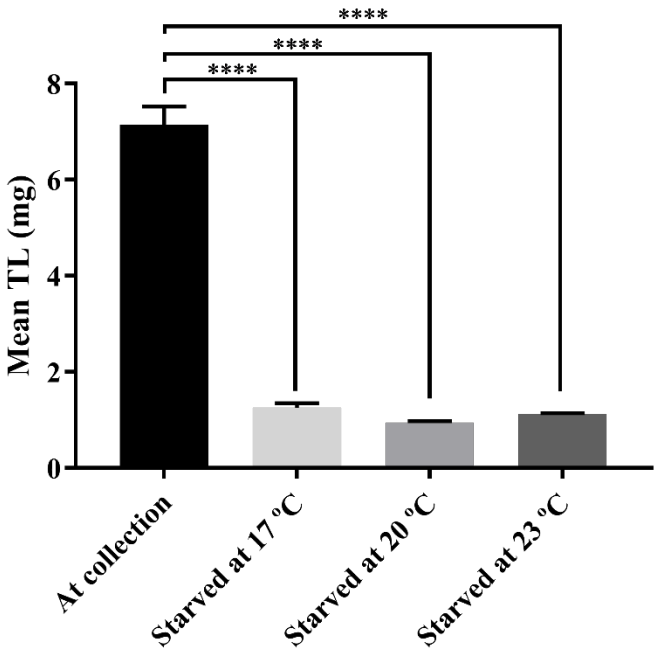

Figure 19. Mean total lipid (±SE) in *S. verreauxi* at capture and held under food withholding conditions at 17, 20 and 23 °C. Asterisks represent statistical differences between means (**** $P \leq 0.0001$).

The TP of control pueruli ranged from 10.13 to 45.69 mg and in starved FIJs it ranged from 3.7 to 23.5 mg across all three temperature treatments. Overall the TP was greater in the control pueruli versus the starved lobsters from all three temperature treatments ($F_{(3, 59)} = 30.43$, $P < 0.0001$) (Fig. 20), but there were no differences in TP of lobsters among the three temperature treatments ($P>0.79$). For all temperature treatments combined the mean TP was 10.62 ± 0.63 mg.

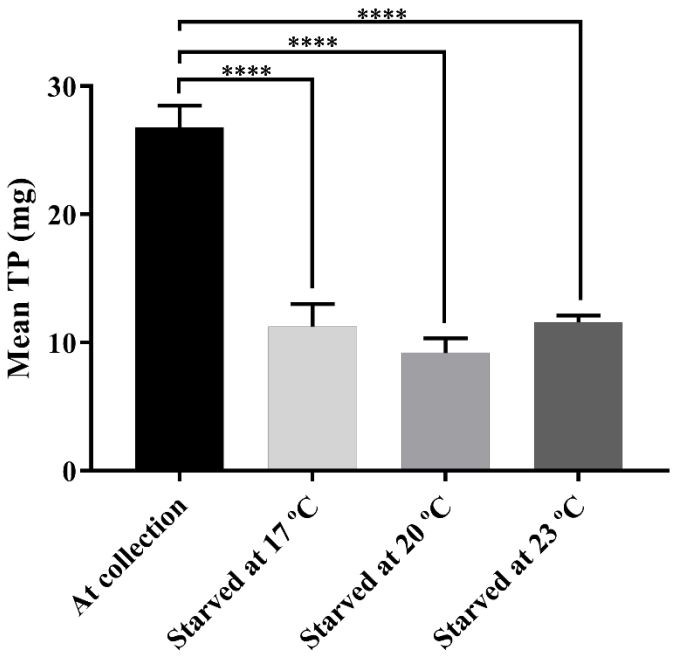

Figure 20. Mean total protein (±SE) in *S. verreauxi* at capture and held under food withholding conditions at 17, 20 and 23 °C. Asterisks represent statistical differences between means (**** $P \leq 0.0001$).

Starved FIJs survived without food from 6 to 55 days across all temperature treatments with an overall mean survival of 39.96 days ± 1.40 days. There was no difference in the length of survival of FIJs among the three temperature treatments ($H = 1.81$, $P = 0.40$) (Table 6).

4.4.2.1 Catabolism of nutritional reserves

The mean rate of catabolism of lipid and protein by starved FIJ lobsters was not different among the three temperature treatments, i.e., ($F_{(2, 29)} = 1.71$, $P = 0.20$) and ($F_{(2, 24)} = 1.83$, $P = 0.18$), respectively (Table 6). For all temperature treatments combined, the rate of catabolism of lipid by starved lobsters was on average 0.110 ± 0.003 mg day^{-1} and for protein 0.31 ± 0.01 mg day^{-1}.

Table 6. Mean (±SE) morphometric and biochemical measures of *S. verreauxi* at capture and after transitioning to FIJs under holding conditions at 17, 20 and 23 °C without feeding until expiration. Means with different alphabetical superscripts are significantly different within rows ($P \leq 0.05$).

Mean measure	At capture (n = 59)	Held at 17 °C (FIJs, n=14)	Held at 20 °C (FIJs, n = 9)	Held at 23 °C (FIJs, n =11)
CL (mm)	10.2 ± 0.1 [a]	10.7 ± 0.1 [a]	10.4 ± 0.1 [a]	10.9 ± 0.1 [a]
DW (mg)	76.6 ± 1.5 [a]	76.6 ± 3.3 [a]	76.8 ± 3.5 [a]	91.2 ± 4.3 [a]
TP (mg)	26.78 ± 1.7 [a]	11.24 ± 1.8 [b]	9.19 ± 1.5 [b]	11.59 ± 0.5 [b]
TL (mg)	7.2 ± 0.4 [a]	1.3 ± 0.1 [b]	0.9 ± 0.0 [b]	1.2 ± 0.0 [b]
ΔTP as mg day^{-1}	N/A	0.30 ± 0.04 [a]	0.34 ±0.02 [a]	0.28 ± 0.02 [a]
ΔTL as mg day^{-1}	N/A	0.11 ± 0.004 [a]	0.11 ± 0.003 [a]	0.12 ± 0.005 [a]
Survival (days)	N/A	38.32 ± 2.22 [a]	43.9 ± 1.6 [a]	40.5 ± 2.2 [a]

4.3.3 *Point of no return in S. verreauxi*

The 55 lobsters sampled immediately after removal from the collectors were highly variable in all morphological and biochemical measures. Their CL ranged from 9.15 to 11.56 mm with a mean of 10.27 ± 0.06 mm, while DW ranged from 56.55 to 119.9 mg, with a mean of 84.46 ± 1.87 mg, TL ranged from 3.17 to 23.12 mg, with a mean of 10.17 ± 0.62 mg, and TP ranged from 16.74 to 59.66 mg with a mean of 36.10 ± 1.19 mg.

The lobsters that were fed continuously after moulting to FIJs (i.e., 0 days starvation) all moulted to second instar juveniles (SIJs) within 25 to 32 days (28.67 ± 2.03 days). The proportion of the starved FIJs that successfully progressed to SIJs following the commencement of feeding was variable for each of the different periods of starvation, but showed an overall downward trend with increasing duration of starvation (Fig. 21) The PNR_{50} was calculated to be 30.4 ± 13.5 SE days (Fig. 21). The starved FIJs that failed to

progress to SIJs survived from 35 to 65 days once feeding recommenced. Overall, mean survival time for FIJs exposed to different durations of food withholding prior to commencement of feeding were not different (P = 0.21). However, there was a trend for longer periods of survival associated with shorter durations of experimental food withholding (Fig. 22). Overall, the mean time taken by FIJs to successfully progress to SIJs after feeding had commenced increased in proportion to the duration of the prior starvation (P = 0.01). Comparisons among individual treatment means showed consistently shorter periods for FIJs to transition to SIJs for lower durations of experimental food withholding, i.e., 15 versus 35 days (P = 0.01), and 25 versus 35 days (P = 0.02) (Fig. 23).

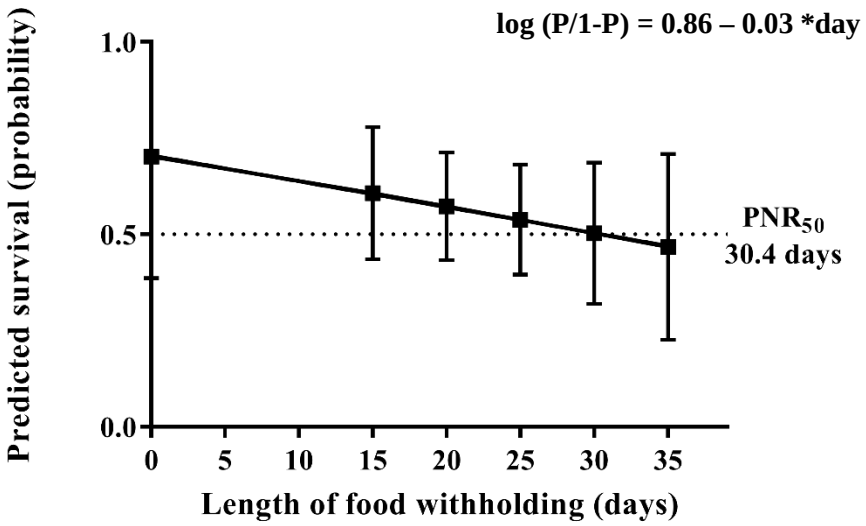

Figure 21. Logistic regression model of survival of FIJ of *S. verreauxi* following experimental periods of 0, 15, 20, 25, 30 and 35 days of starvation prior to the commencement of feeding. PNR_{50} was calculated at 30.4 days. P = probability

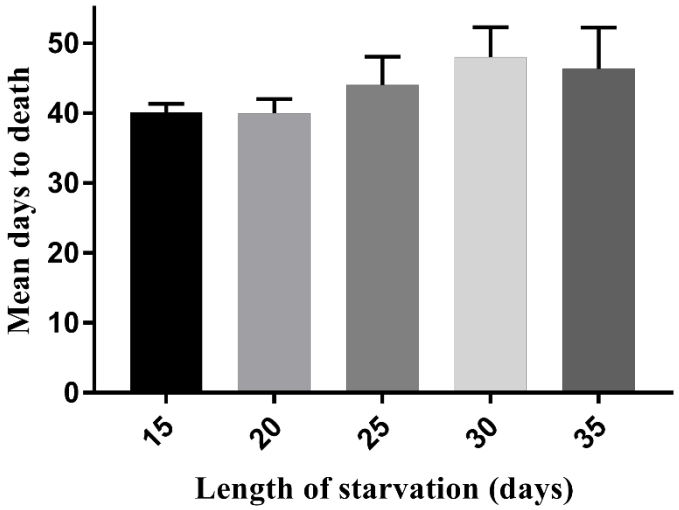

Figure 22. Mean number (±SE) of days after moulting into FIJ until lobsters died after being subjected to 15, 20, 25, 30 and 35 days of food withholding prior to feeding commencing in *S. verreauxi*.

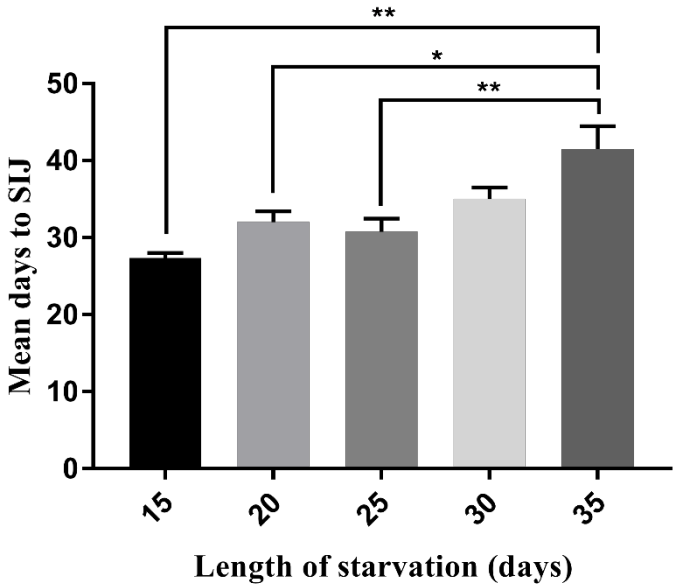

Figure 23. Mean number (±SE) of days taken for FIJs of *S. verreauxi* to moult to SIJs once feeding was provided after 15, 20, 25, 30 and 35 days of prior starvation. Asterisks represent statistical differences (** $P \leq 0.01$, * $P \leq 0.05$).

4.4. Discussion

4.4.1 *Effect of temperature on starvation survival*

The length of survival of FIJ of *S. verreauxi* without food did not vary among the three different temperatures tested (17, 20, and 23º C), unlike what has been reported for other larval marine invertebrates, where an increase in temperature has consistently resulted in a decrease in their length of survival. For example, in larval crabs a 5 °C increase in temperature was found to result in 1 to 2.5 days (depending on the species) reduction in survival from starvation (Anger and Dawirs 1981). A 13 °C increase in temperature in *Carcinus maenas* zoea 1 resulted in a decrease in survival from starvation of about 13 days (Dawirs 1984). In adult crabs at 1, 5 and 10 °C mortality was 7.1, 12.9 and 20.7% respectively after fasting for 5 months (Hardy et al. 2000). For *Balanus amphitrite* nauplii could recover from a maximum starvation duration of 204, 60 and 24 hours when at 5, 15 and 25 °C respectively. Likewise, in ephyrae of *Aurelia aurita* the PNR_{50} was at 58.6, 38.4 and 33.8 days at 9, 12 and 15 °C, respectively (Fu et al. 2014).

It would appear that the FIJ of *S. verreauxi* has a wide thermal tolerance, enabling extended resistance to starvation conditions. Although nutritionally replete pueruli and early juveniles of *S. verreauxi* increase their standard and resting metabolic rate, as well as their aerobic scope as temperature increases from 15 to 27 °C (Fitzgibbon et al. 2014b, 2017), under nutritional duress the juveniles will downregulate their standard metabolic rate by up to 52% (Simon et al. 2015). Such flexibility in managing metabolic activity would explain how FIJ of *S. verreauxi* maintain their nutritional reserves across a range of temperatures, and can greatly extend their survival during starvation.

4.4.2 *Interspecies comparison of starvation in spiny lobster*

None of the starved FIJ for either lobster species had sufficient nutritional reserves to moult to second instar juvenile, as has been previously reported in other spiny lobsters held under similar experimental conditions (Limbourn et al. 2008, Espinosa-Magaña et al. 2017). FIJ of *J. edwardsii* survived an average of 34.44 days from starvation, and *S. verreauxi* survived on

average 39.96 days, this 5 day difference could be due to the higher metabolic rate in *J. edwardsii*, since they started with similar levels of lipid and protein (10.90% and 9.39% lipid and 35.67% and 34.96% protein in *J. edwardsii* and *S. verreauxi*, respectively).

Other studies of the biochemical status of captured migrating pelagic pueruli have made estimations that 9.4% of *P. cygnus* (Phillips et al. 2006b) and 16.5% of pueruli of *J. edwardsii* caught offshore as having insufficient energy reserves to complete their migration to the coast (Jeffs et al. 2001a) based on a theoretical estimate of 5% of remaining lipid to accommodate structural requirements (Hagen et al. 2001). However, the present findings indicate that pueruli could make it to the coast with lower lipid content (i.e., less than 5% lipid content), but without possibilities to subsequently recruit to the population. The mean quantity of remaining lipid and protein as a percentage of dry body mass in FIJ of *J. edwardsii* (1.75 % lipid and 15.24 % protein) after perishing from the food withholding experiments was similar to that in *S. verreauxi* FIJ starved at 17 °C (1.67% lipid and 14.95% protein) ($P = 0.74$ and $P = 0.92$ respectively). These low levels are close to what has been found in other starved invertebrates, such as the krill *Euphasia superba*, that with 1-3% lipid have a 100% mortality rate within 3 days, even with evidence of recent feeding (Virtue et al. 1996), and *Pimpla turionellae* wasps starved to death with 3.14 to 4.21% of lipid and 13.89 to 16.77% of protein remaining, depending on their age at initial starvation (Ortel 1991).

The lipid estimates for spiny lobster juveniles starved to death in this study are below the 5% lipid that has been suggested as a structural cellular requirement for other crustaceans of this size, (i.e., *E. superba*, Hagen et al 2001), and suggests that structural lipid was being catabolized before expiration, and thereby providing them with further resilience to nutritional adversity (Storch and Anger 1983, Hagen et al. 2001).

The proportions of remaining protein at expiration were similar in the two species when comparing between *S. verreauxi* kept at 17 ºC and *J. edwardsii* at 19-20 ºC. These results suggest that the FIJ of both species of temperate spiny lobster respond to starvation in a similar way, by firstly catabolising available lipid and protein reserves, and once depleted then autolysing remaining tissues until their essential function is lost. It would seem 1.7% lipid and 15% remaining protein are the minimum levels of composition in these lobster stages.

Although starved lobsters were depleting their lipid and protein reserves, their dry weight remained unchanged, this could be due to continuing mineralisation of the carapace. During the starvation period the FIJ carapace was strengthened with minerals assimilated from the environment (Graf 1978), and when they perished the carapaces were much harder than for pueruli.

4.4.3 *Rate of energy used*

FIJ of *J. edwardsii* are estimated to be using on average 0.28 ± 0.02 mg day^{-1} of lipid and 0.59 ± 0.05 mg day^{-1} of protein, while in *S. verreauxi* the estimated lipid use was 0.110 mg ± 0.003 mg day^{-1} and protein use was 0.31 mg ± 0.01 mg day^{-1}. The rate of lipid being consumed during starvation in FIJ of *S. verreauxi* over a 6 °C temperature range was the same, unlike what has been observed in larval stages of *Cherax quadricarinatus* and *Artemia franciscana* (Evjemo et al. 2001, García-Guerrero et al. 2003), where lipid was utilized faster at higher temperatures.

During starvation, the rate of lipid and protein used by FIJ of *J. edwardsii* was twice that of *S. verreauxi*, but the body mass of *J. edwardsii* was also double that of *S. verreauxi* (i.e., 142.1 ± 3.7 mg versus 76.6 ± 1.5 mg, respectively). The FIJ of *S. verreauxi* utilized an estimated 1.40 mg gDW^{-1} of lipid per day and 3.06 mg gDW^{-1} of protein per day, while FIJ of *J. edwardsii* used 1.83 mg gDW^{-1} of lipid and 4.01 mg gDW^{-1} of protein per day. In comparison, the basal metabolic rate of post-settlement pueruli of *P. cygnus* is 5.32 mg gDW^{-1} of lipid (Lemmens 1994b), much greater than the rates of juveniles during starvation observed in the current study.

Swimming pueruli of *J. edwardsii* use 1.61 mg of lipid per day (García-Echauri and Jeffs 2018), while the rate of energy use found in juveniles in this study is much lower, at 0.10 - 0.12 mg of lipid per day in *S. verreauxi*, and 0.26 mg per day in *J. edwardsii*. However, these juveniles were inactive and not feeding.

Adding the lipid and protein being used, the estimated energy used by *J. edwardsii* is around 25.01 J per day and *S. verreauxi* 11.68 J per day based on Winberg (1971) estimates for the conversion of calorific value of protein and lipid. The starved spiny lobster juveniles were not engaging in foraging or physical activity and their development was halted, so that these energy utilization rates would represent the basal rates necessary for biological maintenance. If they

are down-regulating their metabolism by 52% during starvation, as occurs in larger juveniles (Simon et al. 2015), *J. edwardsii* could be sparing as much as 23.09 J day^{-1} and *S. verreauxi* 10.75 J day^{-1}.

4.4.4 *PNR*

The time it took for *S. verreauxi* lobsters to recover from periods of starvation increased as the experimental period of starvation increased, as has been previously reported in other decapod species (Anger and Dawirs 1981, Limbourn et al. 2008, Calvo et al. 2012, Espinosa-Magaña et al. 2017). Continuously fed first instar juveniles of *P. argus* took 18 to 24 days to transition to a second instar juvenile (Espinosa-Magaña et al. 2017), and *P. cygnus* moulted to second instar between days 19 and 26 (Limbourn et al. 2008). In this study it took from 25 to 32 days for continuously fed *S. verreauxi* lobster to moult from FIJs to SIJs.

The ability for temperate spiny lobster species, compared to their counterparts from warmer regions, to survive for longer under starvation conditions while taking longer to develop through to the next stage is consistent with their slower metabolic rate (Scholander et al. 1953, Clarke and Johnston 1999). It could also be explained by their difference in size and capacity to store energetic reserves. Recently-settled stage I pueruli of *S. verreauxi* had a mean DW of 81.59 mg (in the season the present PNR_{50} experiments were performed) compared to DW of 24.7 and 55.1 mg for *P. argus and P. cygnus* respectively (Phillips et al. 2006b, Espinosa-Magaña et al. 2018). The species of spiny lobsters with pueruli possessing larger body sizes appear to take longer to gather sufficient energy reserves to continue development, but also take longer to consume their energetic reserves before reaching their PNR_{50}.

PNR_{50} was calculated at 30.4 days in the temperate *S. verreauxi* FIJ, while PNR_{50} in the subtropical spiny lobster *P. cygnus* has been calculated to be 28.5 days (Limbourn et al. 2008), and in the tropical spiny lobster *P. argus* PNR_{50} has been found at 9.5-12 days, depending on the season (Espinosa-Magaña et al. 2017).

Using the lipid and protein utilization rate from the starvation experiments, at the PNR_{50} *S. verreauxi* had 6.52 % lipid and 27.18 % of protein, close to the 5% lipid suggested as the minimum structural cellular requirements for *E. superba* (Hagen et al. 2001). FIJs can continue

to survive below this nutritional level, but the structural damage increasingly makes it difficult for the lobsters to recover and continue development should feeding resume. Assuming 5% lipid and 27% protein as minimum structural requirements, and rates of lipid and protein utilization as calculated in the previous section, *J. edwardsii* lipid content would reach the PNR_{50} in 30 days, but their protein content would reach this limit in 21 days. The length of time for lobsters to reach their PNR_{50} would depend on their initial biochemical condition, which may vary among years (Caputi and Brown 1993, Griffin et al. 2001, Lestang et al. 2015, García-Echauri and Jeffs 2018). There is evidence that a 5% lipid is necessary for structural integrity (Hagen et al. 2001), but if lipid reserves are plentiful it is unclear if they could recover from protein levels below 27%.

Overall it would appear that first instar juveniles of *J. edwardsii* and *S. verreauxi* are highly resistant to starvation, through their ability to reduce their rate of energy expenditure. Capacity to endure adverse environmental conditions was demonstrated in the current study when recently moulted wild FIJs had a month to recommence feeding before passing their PNR_{50}. Furthermore, the FIJs of *S. verreauxi* whilst under starvation conditions appear able to maintain the same rate of lipid use over a 6 ºC range of temperatures. Collectively, these results indicate that juveniles of these temperate spiny lobster species have a high degree of metabolic resilience to future increases in sea temperatures associated with climate change. Provided that the resilience exhibited by *J. edwardsii* and *S. verreauxi* is representative of spiny lobster species generally, this suggests that recently observed declines in recruitment among several spiny lobster populations in different parts of the world (Fitzgibbon et al. 2014a) are unlikely to be the result of increasing energetic demands during the settling phase of spiny lobster recruitment. However, warming oceans may restrict the ability of the preceding larvae to accumulate sufficient nutritional reserves to fuel the subsequent settlement phase. Elevated metabolic rates due to greater temperatures, acting over the lengthy larval period, may hinder the accumulation of energy reserves or erode existing reserves. Increases in temperature during the embryonic phase results in smaller larvae (Tong et al. 2000b), it can also increase mortality during larval development and cause smaller increments in size between larval stages (Tong et al. 2000a, Bermudes and Ritar 2008, Fitzgibbon and Battaglene 2012b). Late stage lobster larvae of *P. cygnus* retained in ocean eddies with cooler water temperatures were found to have

accumulated greater amounts of protein and lipid compared to those living in eddies with warmer water despite consuming similar diets (O'Rorke et al 2012, 2015, Wang et al. 2014, 2015). The greater mortality and reduced capacity to store energetic reserves during the larval phase in warmer waters could potentially reduce the resulting number of pueruli that reach the coast, that have sufficient remaining reserves to recommence feeding and successfully recruit into the population.

Coastal environments are warming faster than oceanic environments (Lima and Wethey 2012), and hence could be expected to be of most impact on FIJs attempting to established under elevated temperature conditions. However, the resilience of FIJs to elevated temperatures and depletion of nutritional reserves demonstrated in this study indicates that it will constrain any potential effects of climate change on this phase of the lifecycle in temperate spiny lobster populations.

Chapter 5. General discussion

The landings of some economically important spiny lobsters have been declining and this has been associated with an unexplained decline in numbers of settling pueruli in a number of fisheries where settlement is monitored (Caputi et al. 2010a, Ehrhardt and Fitchett 2010, Linnane et al. 2010a). One of the possible reasons that have been proposed for these changes in lobster populations is higher mortality of pueruli due to changes in ocean temperature and currents associated with climate change processes (Fitzgibbon et al. 2014a).

Spiny lobsters have a long larval phase, the phyllosoma phase, followed by a secondary lecithotrophic nektonic phase, the puerulus. Phyllosoma metamorphose to pueruli in offshore ocean waters and have to make their way into shallow coastal waters where they settle. This migration can cover hundreds of kilometres (Dennis et al. 2001, Jeffs et al. 2001a), and during this time the pueruli rely entirely on the energetic reserves they gathered during the larval phase. The onshore migration of pueruli is active, with well-developed swimming appendages under the abdomen providing impressive swimming speeds for a relatively small pelagic organism (Calinski and Lyons 1983, Jeffs and Holland 2000). The swimming behaviour of pueruli is poorly described, with only short term observations made of swimming pueruli while attached to tethers or in aquaria (Kittaka 1988, Kittaka and Ikegami 1988, Kittaka and Kimura 1988, Jeffs and Holland 2000, Kough et al. 2014). The energetics of swimming in pueruli is also poorly understood. There have been indirect estimations of the energetic cost of migration in pueruli derived from differences in lipid content with distance to the coast (Jeffs et al. 2001a) and measurements of metabolic rate of active and inactive pueruli (Fitzgibbon 2010).

Since pueruli are not feeding it is important to better understand their energy utilization and behaviour during this nektonic phase. The energy a puerulus gathers during the larval stage has to be sufficient to fuel the migration to the coast, as well as locating a settlement site, then moulting to a juvenile, before the pueruli can begin to feed. However, the amount of energy required to complete this phase of the lifecycle and how this requirement changes with water temperature is uncertain, despite suggestions that restricted energy reserves that are depleted

more quickly in warmer waters is responsible for observed declines in pueruli settlement in some spiny lobster fisheries (Fitzgibbon et al. 2014b).

The research conducted for this thesis has determined the energetic reserves required during the pueruli phase, how much more energy is required with temperature increments, how pueruli change their behaviour due to changes in temperature, and the threshold amount of lipid needed for survival.

Increments in temperatures affect the onset of spawning and the production of embryos in spiny lobsters (Tong et al. 2000b, Moss et al. 2004, Lestang et al. 2015), and the development of phyllosoma (Bermudes and Ritar 2008, Fitzgibbon and Battaglene 2012b) accelerating their development at the cost of energy storage capacity (smaller pueruli). In this work we found that the negative effects of temperature in terms of conserving stored energy continues during the puerulus phase.

In this work it was found that pueruli use markedly more energy swimming when temperature increases from 17 to 23 °C, while also spending considerably less time swimming. Even with the reduction of the swimming behaviour the energetic cost is not reduced to the levels of lower temperatures.

In contrast to pueruli, this study found that first instar juveniles did not modify their rates of energy utilization with increasing temperatures under starvation conditions. It appears the developmental changes that occur from pueruli to first instar juvenile (Guerao et al. 2006) allow the juveniles to tolerate higher temperatures in a more energetically efficient way. Phyllosoma have poor oxygen regulation, mostly relying on a thin epithelium for gas exchange (Haond et al. 2001), whereas in pueruli the respiratory system depends on ventilated gills, but is still underdeveloped compared to juveniles (Nishida et al. 1990, Fitzgibbon et al. 2015). The greatly increased ventilation capacity of juveniles could help compensate for the increased oxygen demand at higher temperatures (Pörtner 2010). In addition, during starvation juveniles can downregulate metabolic rate by 52% (Simon et al. 2015), this is evidenced in the lengthy survival from starvation found in this current research.

Pueruli of *J. edwardsii* and *S. verreauxi* have comparable rates of energy utilization when swimming as pueruli, and during starvation as juveniles, if their differences in body size is taken into account. It could be expected that the pueruli of both species would react to temperature increases within their thermal range in a similar way, i.e., reducing the time pueruli spend swimming and requiring more energy expenditure to maintain active swimming, and as juveniles, downregulating their metabolism during starvation.

5.1. General conclusions

Overall the research presented in this thesis provides insight in the swimming energetics of the pueruli of two spiny lobster species, which can be extrapolated to other spiny lobster species, by relating the energy spent to their body size. It quantifies the negative effect of increasing temperature on the swimming energetics and swimming behaviour of pueruli, demonstrating the compounding negative effect on pueruli migration by the behavioural compensations to increased energetic demand. In light of climate change scenarios these results support previously hypothesised increased mortality of pueruli resulting from elevated water temperatures.

The minimum lipid levels to maintain viable pueruli and juveniles were determined through this research, with the results indicating a high resilience of juveniles to starvation and to temperature stress, despite the comparative vulnerability of prior puerulus to elevated temperature conditions.

Secondary lecithotrophy in the pueruli spiny lobsters has been seen as an effective means to facilitate long distance onshore migration of larvae (Anger 2001). However, indications from this research are that warming ocean increases the energetic strain during an already challenging phase in the lifecycle of spiny lobsters. In contrast, primary lecithotrophy, as seen in many other marine invertebrates, provides for fast development to forms with mature respiratory systems that are likely to be less affected by warming oceans.

5.2. Future research directions

The new evidence provided with the research presented in this thesis highlights knowledge gaps that should be addressed to better understand the energetics of spiny lobsters.

We know that lipid levels below 6% are in their lower limit for structural viability, but we do not know how much energy is used during the moult from pueruli to first instar juvenile. This could be another bottleneck in their survival, since juveniles must have 6% lipid or more before re-commencing feeding if they are to continue their development. Knowing at what energetic levels pueruli are unable to moult and die as pueruli, and the energy levels at which pueruli do moult but die soon after can improve the estimations of recruitment.

This current research found that pueruli actively swimming used the same amount of energy as pueruli held inactive for the same duration at the same temperature. Casual observations suggested that swimming pueruli delayed their progression toward moulting to a first instar juvenile, compared to inactive pueruli held in tanks. This would suggest that the morphological changes from pueruli to first instar juvenile are also highly energy demanding, on what appear to be limited energy reserves. To confirm this, swimming and holding experiments could be repeated, tracking the subsequent development of pueruli to determine if swum pueruli delay their development to juveniles.

There are contradictory observations of the circadian patterns of swimming in pueruli, with some reporting they only swim at night (Calinski and Lyons 1983, Phillips and Booth 1994, Acosta and Butler 1999), and others reporting them swimming day and night (Kough et al. 2014). In this current research it was observed that pueruli of *S. verreauxi* swam both during the day and during the night, but since the experiments were conducted in darkness it could be that in the wild they behave differently. Resolving this would provide more precise information on the swimming distance pueruli can achieve in a day.

The experiments in this study were performed on pueruli that were collected on the coast that had already completed their onshore migration. Therefore, it would be of great value to determine if newly metamorphosed pueruli behave in a similar manner. It is likely that their swimming behaviour changes with age and following contact with settlement substrate in

coastal waters. Observing the swimming behaviour of recently metamorphosed pueruli can be done by swimming pueruli obtained from larviculture.

A further question to resolve is the impact of elevated sea temperatures on the ability of phyllosomas to accumulate sufficient energetic reserves to fuel the puerulus phase. Pueruli are faced with a high energetic demand to reach the coast, and if phyllosoma cannot build sufficient energetic reserves it will exacerbate the energetic limitations of the pueruli. Although feed intake and feed conversion rates have been studied at different temperatures in phyllosoma (Tong et al. 2000a, Fitzgibbon and Battaglene 2012b), there are no observations about the activity of phyllosomas that could explain if they are slower to react to prey or stimulus, and if they also reduce the time spent active. If increments in temperature affect the vertical migration of phyllosomas it could increase predation and decrease food encounters. Studying for how long or how much phyllosoma ingest to build enough reserves for the subsequent puerulus to successfully cross the continental shelf depending on temperature can also help predict settlement numbers.

The energetic demand of the pueruli phase is high, and its success can be determined by the abiotic conditions it encounters while migrating or during previous developmental phases. The research in this thesis provides quantifiable information of the energetic requirements of the pueruli phase through to the early juvenile stage.

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