

Resistance to starvation in settling spiny lobsters in warming coastal waters

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ABSTRACT: Spiny lobsters have an extended larval development in offshore waters that ends with the non-feeding post-larvae swimming across the continental shelf and settling in shallow coastal waters. We tested the hypothesis that recent declines in the recruitment of a number of spiny lobster populations in different parts of the world are the result of increasing coastal water temperatures that deplete the metabolic reserves of recently settled post-larvae. We examined the resilience of recently settled juvenile lobsters to the depletion of their reserves in 2 species of spiny lobster from temperate waters. Juveniles of *Jasus edwardsii* survived on average (\pm SE) for 34.44 ± 3.44 d without feeding at 19–21°C, while *Sagmariasus verreauxi* survived for 39.96 ± 1.40 d, with no differences in survival among 3 temperature regimes (17, 20 and 23°C). The point of no return for recently-settled juveniles of *S. verreauxi* was estimated to be 30.40 ± 13.50 d, which was close to their average survival under starvation conditions. Starved juvenile lobsters close to the point of no return with extremely low remaining protein and lipid (6.52 % lipid and 27.18 % protein of dry body mass) were capable of surviving once food was supplied. These results indicate that newly settled juveniles of spiny lobsters from temperate waters can endure adverse nutritional conditions over a wide range of thermal conditions for prolonged periods, making them resilient to future increases in water temperatures as a result of climate change.

KEY WORDS: *Jasus edwardsii* · *Sagmariasus verreauxi* · Point of no return · PNR · Starvation · Spiny lobster juveniles · Thermal tolerance

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1. INTRODUCTION

Increasing ocean water temperatures, which are thought to be due to changes in global climate, have been hypothesised as the likely cause of a consistent pattern of recent declines in the recruitment of 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, 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,b, 2004, Fitzgibbon et al. 2014a). Protein may also be used, but to a much lesser extent (Jefferies 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. 2006). These changes include the development of hardened mouthparts and a fully functioning digestive tract that allows feeding, and therefore the subsequent resumption of a reliance on 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 (Jefferies et al. 2005, Wilkin & Jefferies 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 temperatures of the coastal surface waters inhabited by these lobsters, resulting in more energetic reserves being used by these lobster stages, as they are metabolic conformers (Fitzgibbon et al. 2014a). 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 & Fitchett 2010, Linnane et al. 2010a, Feng et al. 2011, Fitzgibbon et al. 2014a).

Starvation in crustacean larvae typically delays the progression of development, as digestive enzymes are downregulated and growth diminishes or is halted (Liddy et al. 2003, Calvo et al. 2012, 2013, Guerao et al. 2012). When starved, the larvae of spiny lobsters, known as phyllosomas, show an increase in protease activity and a 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 crustaceans, 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, mitochondria become swollen), and degeneration in epidermal and muscle

tissues also occurs (Loizzi 1971, Storch & Anger 1983, Al-Mohanna & 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 & Hempel 1963). The median PNR (PNR₅₀) 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 & 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 PNR₅₀ in *Panulirus cygnus* juveniles, a subtropical spiny lobster of Western Australia, is 22.5 d (Limbourn et al. 2008), and that of the tropical *P. argus* is only 9.5 d in the cold season and 12 d in the warm season on the Caribbean coast of Mexico (Espinosa-Magaña et al. 2017). *Jasus edwardsii* and *Sagmariasus verreauxi* are species of spiny lobsters that both occur in temperate waters with overlapping ranges across parts of Australasia (Jefferies 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 is 19–21°C for *J. edwardsii* juveniles (Thomas et al. 2000) and 21.5°C for *S. verreauxi* juveniles (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 2 spiny lobster species by determining their nutritional status and survival for a range of temperatures. In addition, the PNR₅₀ of *S. verreauxi* was experimentally determined to assess their capacity to recover from low nutritional status.

2. MATERIALS AND METHODS

2.1. Experimental animals

Pueruli of *Jasus 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). Live stage III pueruli were placed in individual containers within buckets filled with ambient seawater (19–22°C) aerated with an air-stone, and then successfully transported back to the Leigh Marine Laboratory.

Pueruli of *Sagmariasus verreauxi* (stages II and III sensu Booth 1979) were collected at 2 sites in shallow coastal waters of New South Wales, Australia, near Sydney (34° 4' 15" S, 151° 10' 56" E) and Ulladulla (35° 24' 41" S, 150° 27' 12" E). Seaweed-type pueruli collectors (Phillips & Booth 1994) were deployed by the New South Wales Department of Primary Industries, at both sites from September to November in 2015, and in October 2016. Pueruli collectors were cleared every month within the week after the new moon, weather permitting (Montgomery & Craig 2003). Seawater temperatures during collection ranged from 17–20°C. Live pueruli were placed in buckets filled with ambient seawater from the collection sites and aerated via an airstone, and successfully transported back to the aquaria facilities at the Sydney Institute of Marine Science.

2.2. Experimental setup

2.2.1. 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 food-withholding experiments, 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). Once at the laboratory, the containers holding individual pueruli were transferred to aquaria supplied with settled and filtered seawater (20 µm) held at 21 ± 0.5°C. The lobsters were held without food and observed every 12 h until

they died. When pueruli moulted to 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 closely inspected to ensure an absence of disease or malformation which provides an alternative cause of death to starvation, and then the lobster 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.

2.2.2. Food-withholding experiments in *S. verreauxi*

Stage II pueruli of *S. verreauxi* were frozen immediately upon collection for later determination of nutritional condition. We collected 32, 37 and 10 stage II pueruli 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, 9 collected in October 2015 were held at 20°C, and 11 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 2 d. Lobsters were housed in individual containers in aquaria in a darkened room supplied with filtered seawater (20 µm) at controlled water temperatures (±0.5°C) in a controlled temperature laboratory 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.

2.2.3. PNR in *S. verreauxi*

In October 2016, a total of 114 stage II and III pueruli of *S. verreauxi* were recovered from collectors, of which 55 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 6 starvation duration treatments, i.e. 0, 15, 20, 25, 30 and 35 d after moulting to FIJs. Following each period of star-

vation, 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).

2.3. Biochemical analyses

The carapace length (CL) and the wet weight of frozen lobsters were measured, and after being lyophilized, they were re-weighed to determine their dry weight (DW). Total lipid (TL) of each lobster was determined gravimetrically using a modified protocol of Jeffs et al. (1999). After extracting the lipids, the residual tissue was assayed for total protein (TP) using a Micro BCA protein assay (Thermo Scientific) based on the bicinchoninic acid determination method for protein quantification (Walker 1994, Jeffs et al. 1999). TL and TP are presented as absolute measures of nutritional resources within species, but the lipid and protein composition of lobsters as a percentage of DW were also calculated to facilitate comparison between the 2 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 at expiration from the mean TL of the pueruli at capture; the resulting difference provides an estimate of the lipid used 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 d for *J. edwardsii* (Kittaka 1990, Phillips & Booth 1994) and 12 d for *S. verreauxi* (Kittaka et al. 1997).

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*, 1-way ANOVAs were firstly used to compare mean CL, DW, TP and TL of the lobsters at capture 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 from the different collection events were pooled and 1-way ANOVAs were then used to compare the TL and TP in *S. verreauxi* sam-

pled at collection and after being held at 3 different temperatures without feeding. The durations of survival of lobsters held without feeding were compared for the 3 different temperatures using a Kruskal-Wallis test.

For the PNR experiment in *S. verreauxi*, a logistic regression model was used to determine the PNR₅₀ from the percentage of FIJs recovering from the different food-withholding treatments sufficiently to successfully moult to SIJs using R (R Core Team 2016).

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 was compared for the 3 temperature treatments using a 1-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, 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. ANOVAs, *t*-tests, normality and equality of variance were performed using GraphPad Prism version 7.00. Variation around means is presented as standard error throughout the results.

3. RESULTS

3.1. Food-withholding experiment in *Jasus edwardsii*

The 19 lobsters sampled immediately after removal from crevice collectors were highly variable in all morphological and biochemical measures (Table 1, Fig. 1). For example, their DW varied by more than 200%, ranging from 78.0 to 170.0 mg, and TL content by over 400%, ranging from 6.2 to 30.1 mg.

All lobsters held without food moulted to FIJs, but none transitioned to SIJs. The lobsters held without feeding survived from 25 to 48 d (mean = 34.44 ± 3.44 d) post-moult to FIJs and were less variable in their morphological and biochemical measures than the lobsters collected upon removal from collectors (Fig. 1). 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.

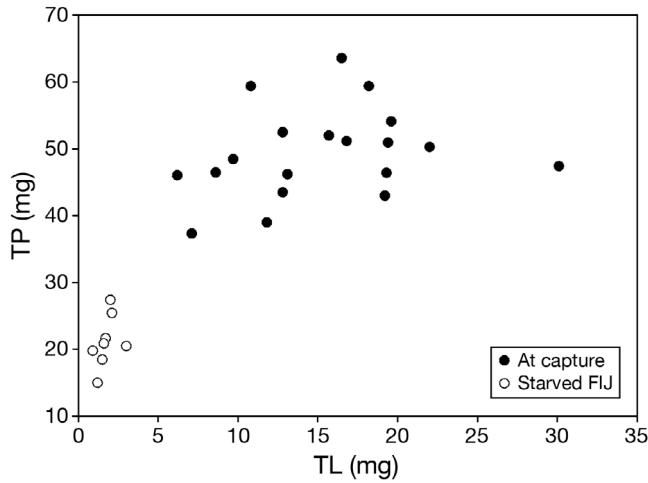


Fig. 1. Measured total lipid (TL) and total protein (TP) content for individual *Jasus edwardsii* at capture and after being held at $21.0 \pm 0.5^\circ\text{C}$ without feeding until expiration. FIJ: first-instar juvenile

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 $21.0 \pm 0.5^\circ\text{C}$ without feeding until expiration (Table 1). 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 1).

3.2. Food-withholding experiments at different temperatures in *Sagmariasus verreauxi*

The lipid and protein contents of lobsters at capture sampled from collectors were highly variable (Fig. 2),

Table 1. Mean (\pm SE) morphometric and biochemical measures of *Jasus edwardsii* at capture and after being held at $21.0 \pm 0.5^\circ\text{C}$ without feeding until expiration. Within-row significant differences of means are marked with asterisks (* $p \leq 0.05$, *** $p \leq 0.001$). NA: not applicable

Parameter	Treatment	
	At capture (n = 19)	Food withheld (n = 9)
Carapace length (mm)	11.1 \pm 0.1	11.5 \pm 0.2*
Dry weight (mg)	142.1 \pm 3.7	143.5 \pm 11.4
Total protein (TP) (mg)	50.70 \pm 1.50	22.00 \pm 1.11***
Total lipid (TL) (mg)	15.50 \pm 1.20	1.75 \pm 0.23***
DTP (mg d ⁻¹)	NA	0.59 \pm 0.05
DTL (mg d ⁻¹)	NA	0.28 \pm 0.02
Survival (d)	NA	34.44 \pm 3.4

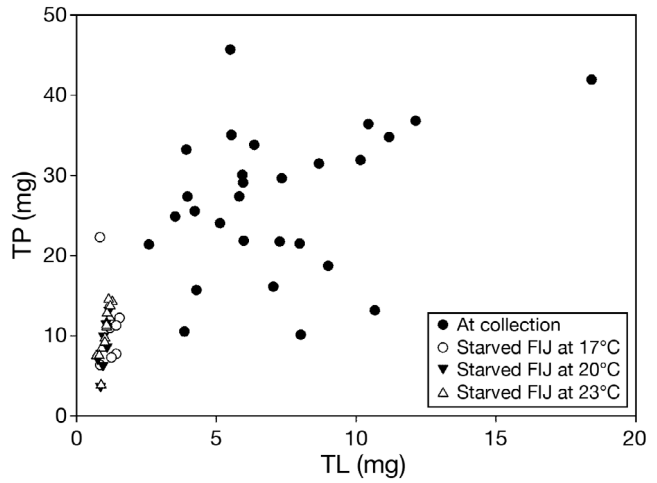


Fig. 2. Total lipid (TL) and total protein (TP) content of *Sagmariasus verreauxi* sampled at collection and after transitioning to first-instar juveniles (FIJs) under holding conditions at 17, 20 and 23°C without feeding until expiration

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 lobsters at capture 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 (Fig. 3).

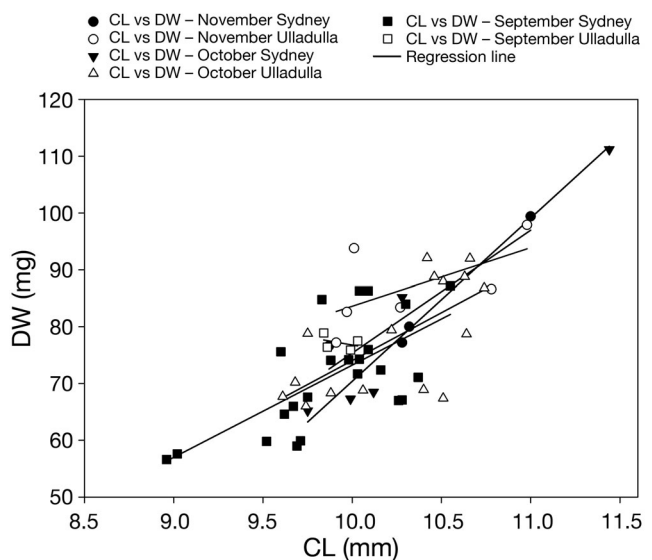


Fig. 3. Total dry weight (DW) and total carapace length (CL) of *Sagmariasus verreauxi* at collection, sampled from 2 collection sites and 3 collection months

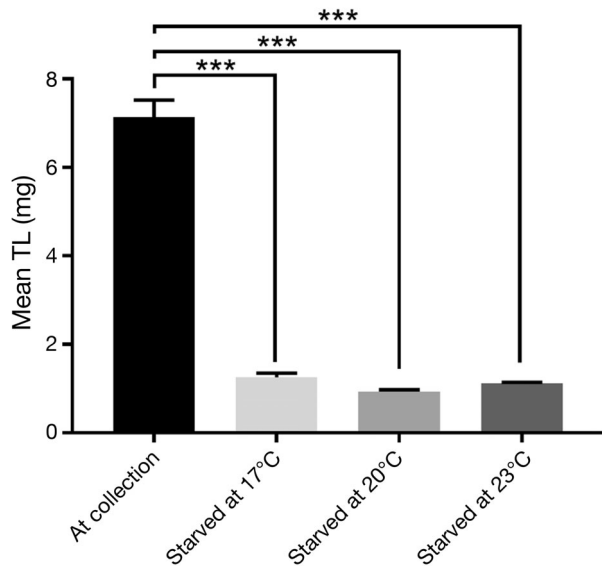


Fig. 4. Mean total lipid (TL, \pm SE) in *Sagmariasus verreauxi* at capture and held under food-withholding conditions at 17, 20 and 23°C. Asterisks represent statistical differences between means (***) $p \leq 0.001$

The TL content ranged from 2.59 to 18.42 mg in pueruli at capture and from 0.71 to 1.73 mg in starved FIJs across all temperature treatments. Overall, the TL was greater in pueruli at capture versus in starved lobsters ($F_{3,91} = 45.14$, $p < 0.0001$), and this difference was consistent at all temperatures ($p < 0.0001$ for each temperature), but there were no differences in TL of lobsters among the 3 temperature starvation treatments ($p > 0.99$, Fig. 4).

TP ranged from 10.13 to 45.69 mg in pueruli at capture and from 3.72 to 23.50 mg in starved FIJs across all 3 temperature treatments. Overall, TP was greater in the pueruli at capture versus in the starved lobsters from all 3 temperature treatments ($F_{3,59} = 30.43$, $p < 0.0001$; Fig. 5), but there were no differences in TP of lobsters among the 3 temperature starvation treatments ($p > 0.79$). For all temperature starvation treatments combined, the mean TP was 10.62 ± 0.63 mg.

Starved FIJs survived without food from 6 to 55 d across all temperature treatments, with an overall mean survival time of 39.96 ± 1.40 d. There was no difference in the length of survival of FIJs among the 3 temperature starvation treatments ($H = 1.81$, $p = 0.40$; Table 2).

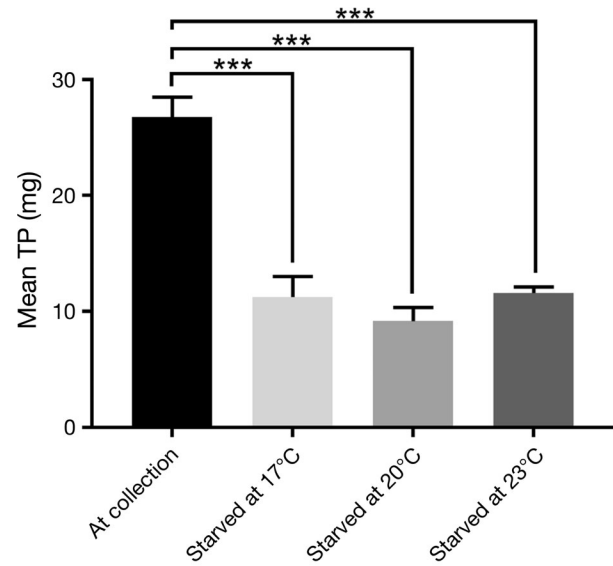


Fig. 5. Mean total protein (TP, \pm SE) in *Sagmariasus verreauxi* at capture and held under food-withholding conditions at 17, 20 and 23°C. Asterisks represent statistical differences between means (***) $p \leq 0.001$

The mean rate of catabolism of lipid and protein by starved FIJ lobsters was not different among the 3 temperature treatments ($F_{2,29} = 1.71$, $p = 0.20$; and $F_{2,24} = 1.83$, $p = 0.18$, respectively; Table 2). For all temperature starvation treatments combined, the rate of catabolism by starved lobsters was on average 0.110 ± 0.003 mg d^{-1} for lipid and 0.31 ± 0.01 mg d^{-1} for protein.

3.3. PNR in *S. verreauxi*

The 55 lobsters sampled immediately after removal from the collectors were highly variable in all mor-

Table 2. Mean (\pm SE) morphometric and biochemical measures of *Sagmariasus verreauxi* at capture and after transitioning to first-instar juveniles under holding conditions at 17, 20 and 23°C without feeding until expiration. Means with different alphabetical superscripts are significantly different within rows ($p \leq 0.05$). NA: not applicable

Parameter	At capture (n = 59)	Held at 17°C (n = 14)	Held at 20°C (n = 9)	Held at 23°C (n = 11)
Carapace length (mm)	10.2 \pm 0.1 ^a	10.7 \pm 0.1 ^a	10.4 \pm 0.1 ^a	10.9 \pm 0.1 ^a
Dry weight (mg)	76.6 \pm 1.5 ^a	76.6 \pm 3.3 ^a	76.8 \pm 3.5 ^a	91.2 \pm 4.3 ^a
Total protein (TP) (mg)	26.78 \pm 1.7 ^a	11.24 \pm 1.8 ^b	9.19 \pm 1.5 ^b	11.59 \pm 0.5 ^b
Total lipid (TL) (mg)	7.2 \pm 0.4 ^a	1.3 \pm 0.1 ^b	0.9 \pm 0.0 ^b	1.2 \pm 0.0 ^b
DTP (mg d^{-1})	NA	0.30 \pm 0.04 ^a	0.34 \pm 0.02 ^a	0.28 \pm 0.02 ^a
DTL (mg d^{-1})	NA	0.11 \pm 0.004 ^a	0.11 \pm 0.003 ^a	0.12 \pm 0.005 ^a
Survival (d)	NA	38.32 \pm 2.22 ^a	43.9 \pm 1.6 ^a	40.5 \pm 2.2 ^a

phological 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 d starvation) all moulted to SIJs within 25 to 32 d (28.67 ± 2.03 d). 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. 6). The PNR_{50} was calculated to be 30.4 ± 13.5 d (Fig. 6). The starved FIJs that failed to progress to SIJs survived from 35 to 65 d once feeding recommenced. Overall, mean survival time for FIJs exposed to different durations of food withholding prior to commencement of feeding were not different ($H = 6.84$, $df = 18$, $p = 0.14$). However, there was a trend for longer periods of survival associated with shorter durations of experimental food withholding (Fig. 7). 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 ($H = 15.37$, $df = 24$, $p = 0.01$). Comparisons among individual starvation treatment means showed consistently shorter periods for FIJs to transition to SIJs for lower durations of experimental food withholding, i.e. 15 versus 35 d ($p = 0.003$) (Fig. 8).

4. DISCUSSION

4.1. Effect of temperature on starvation survival

The length of survival of FIJ of *Sagmariasus verreauxi* without food did not vary among the 3 different temperatures tested (17, 20 and 23°C), unlike other larval marine invertebrates, where an increase in temperature has consistently resulted in a decrease in the duration of survival. For example, in larval crabs, a 5°C increase in temperature resulted in a reduction in survival of 1 to 2.5 d (depending on the species) whilst starved (Anger & Dawirs 1981). A 13°C increase in temperature in starved *Carcinus maenas* zoea 1 resulted in a decrease in survival of about 13 d (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 mo (Hardy et al. 2000). Nauplii of the barnacle *Balanus amphitrite* could recover from a maximum starvation duration of 204, 60 and

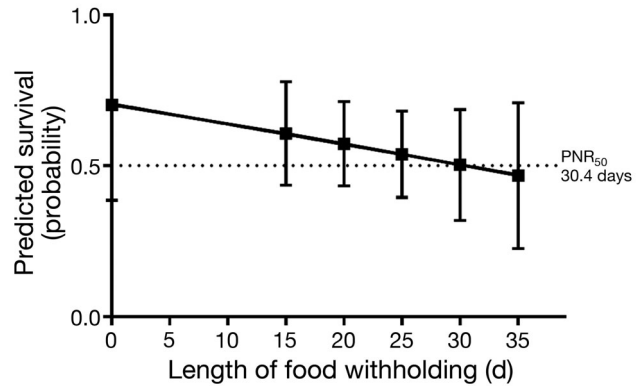


Fig. 6. Logistic regression model of survival of first-instar juveniles of *Sagmariasus verreauxi* following experimental periods of 0, 15, 20, 25, 30 and 35 d of starvation prior to the commencement of feeding. The median point of no return (PNR_{50}) was calculated at 30.4 d

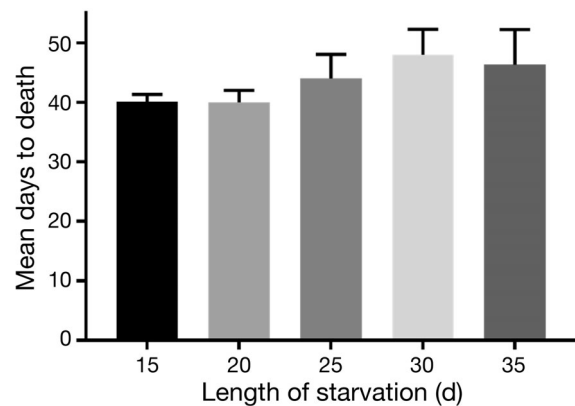


Fig. 7. Mean (\pm SE) number of days after moulting into first-instar juveniles until lobsters died after being subjected to 15, 20, 25, 30 and 35 d of food withholding prior to feeding commencing in *Sagmariasus verreauxi*

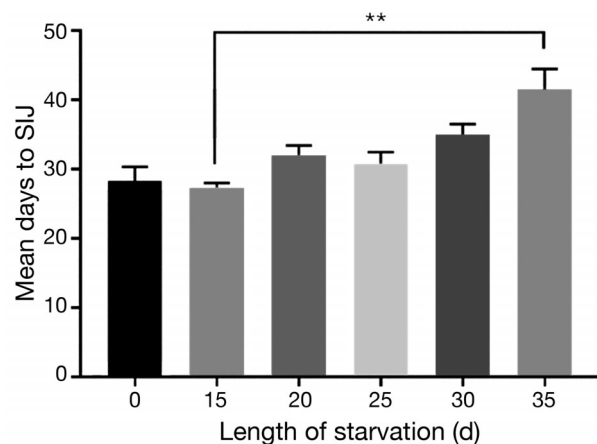


Fig. 8. Mean (\pm SE) number of days taken for first-instar juveniles of *Sagmariasus verreauxi* to moult to second-instar juveniles (SIJs) once food was provided after 0, 15, 20, 25, 30 and 35 d of prior starvation. Asterisks represent statistical differences (** $p \leq 0.01$)

24 h at 5, 15 and 25°C, respectively. Likewise, in ephyrae of the moon jellyfish *Aurelia aurita*, the PNR₅₀ was at 58.6, 38.4 and 33.8 d at 9, 12 and 15°C, respectively (Fu et al. 2014).

It appears that FIJs of *S. verreauxi* have a wide thermal tolerance, enabling extended resistance to starvation conditions. Although nutritionally replete pueruli and early juveniles of *S. verreauxi* increase their standard and routine 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 FIJs of *S. verreauxi* maintain their nutritional reserves across a range of temperatures, and can greatly extend their survival during starvation.

4.2. Interspecies comparison of starvation in spiny lobster

None of the starved FIJs for either lobster species had sufficient nutritional reserves to moult to SIJs, like other spiny lobsters held under similar experimental conditions (Limbourn et al. 2008, Espinosa-Magaña et al. 2017). FIJs of *Jasus edwardsii* survived an average of 34.44 d from starvation, and *S. verreauxi* survived on average 39.96 d; this 5 d difference could be due to the higher metabolic rate in *J. edwardsii*, since the 2 species 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 *Panulirus cygnus* (Phillips et al. 2006) and 16.5% of pueruli of *J. edwardsii* caught offshore have 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* FIJs starved at 17°C (1.67% lipid and 14.95% protein; $p = 0.74$ and 0.92 , respectively). These low levels are close to

those of other starved invertebrates, such as the krill *Euphasia superba*, that with 1–3% lipid have a 100% mortality rate within 3 d, even with evidence of recent feeding (Virtue et al. 1996), and *Pimpla turionellae* wasps starved to death, with 3.14–4.21% of lipid and 13.89–16.77% 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 suggested as a structural cellular requirement for other crustaceans of this size, (e.g. *E. superba*; Hagen et al. 2001), and suggests that structural lipid was being catabolised before expiration, thereby providing them with further resilience to nutritional adversity (Storch & Anger 1983, Hagen et al. 2001).

The proportions of remaining protein at expiration were similar in the 2 spiny lobster species when comparing between *S. verreauxi* kept at 17°C and *J. edwardsii* at 19–20°C, suggesting that FIJs of both species respond to starvation in a similar way, by firstly catabolising available lipid and protein reserves, and once depleted, autolysing remaining tissues until their essential function is lost. It would seem that 1.7% lipid and 15% remaining protein are the minimum levels of composition in these lobster stages, which are past the PNR.

Although starved lobsters were depleting their lipid and protein reserves, their DW remained unchanged, which could be due to assimilation of dissolved calcium for strengthening the carapace (Graf 1978).

4.3. Rate of energy used

FIJs of *J. edwardsii* were estimated to be using on average 0.28 ± 0.02 mg d⁻¹ of lipid and 0.59 ± 0.05 mg d⁻¹ of protein, while in *S. verreauxi* the estimated lipid use was 0.110 ± 0.003 mg d⁻¹ and protein use was 0.31 ± 0.01 mg d⁻¹. The rate of lipid being consumed during starvation in FIJs of *S. verreauxi* over a 6°C temperature range was the same, unlike in larval stages of the crayfish *Cherax quadricarinatus* and the fairy shrimp *Artemia franciscana*, where lipid was used faster at higher temperatures (Evjemo et al. 2001, García-Guerrero et al. 2003).

During starvation, the rate of lipid and protein used by FIJs of *J. edwardsii* was twice that of *S. verreauxi*, but the body mass of *J. edwardsii* was also double that of *S. verreauxi* (142.1 ± 3.7 mg versus 76.6 ± 1.5 mg DW, respectively). FIJs of *S. verreauxi* used an estimated 1.40 mg g DW⁻¹ of lipid and 3.06 mg g DW⁻¹ of protein per day, while FIJs 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 1994), which is much greater than the rates for juveniles enduring starvation in the current study.

Swimming pueruli of *J. edwardsii* use 1.61 mg of lipid per day (García-Echaury & 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 d⁻¹ in *J. edwardsii*. However, these juveniles were inactive and not feeding.

Adding the lipid and protein being used, the estimated energy used is around 25.01 J d⁻¹ for *J. edwardsii* and 11.68 J d⁻¹ for *S. verreauxi*, based on Winberg's (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 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 d⁻¹ and *S. verreauxi* 10.75 J d⁻¹.

The rates of lipid and protein use by juveniles during starvation could be lower than measured, since the increased energetic cost for moulting from puerulus to juvenile is not accounted for, and after death necrosis could decrease the lipid concentration by up to 1% (Kunapareddy et al. 2008).

4.4. PNR

The time it took for *S. verreauxi* to recover from periods of starvation increased as the experimental period of starvation increased, as has been found in other decapod species (Anger & Dawirs 1981, Limbourn et al. 2008, Gebauer et al. 2010, Calvo et al. 2012, Espinosa-Magaña et al. 2017). Continuously fed FIJs of *P. argus* took 18 to 24 d to transition to SIJs (Espinosa-Magaña et al. 2017), and *P. cygnus* moulted to the second instar between Days 19 and 26 (Limbourn et al. 2008). In our study, it took from 25 to 32 d for continuously fed *S. verreauxi* 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 & 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 during which the PNR₅₀ experiments were performed) compared to DW of 24.7 and 55.1 mg for *P. argus* and *P. cygnus*, respectively (Phillips et al. 2006, 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 PNR₅₀.

PNR₅₀ was calculated at 30.4 d in the temperate *S. verreauxi* FIJs, while PNR₅₀ in the subtropical spiny lobster *P. cygnus* is 28.5 d (Limbourn et al. 2008), and in the tropical spiny lobster *P. argus*, PNR₅₀ is 9.5–12 d, depending on the season (Espinosa-Magaña et al. 2017). In the crab *Petrolisthes laevigatus*, PNR₅₀ was calculated at 32.8–42.5% of the developmental time in continually fed larvae (Gebauer et al. 2010), but in the case of our study, PNR₅₀ was close to the time continually fed juveniles moulted into SIJs (28.7 ± 2.0 d).

Using the lipid and protein utilization rate from the starvation experiments, at the PNR₅₀ *S. verreauxi* had 6.52% lipid and 27.18% 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 PNR₅₀ in 30 d, but their protein content would reach this limit in 21 d. The length of time for lobsters to reach their PNR₅₀ would depend on their initial biochemical condition, which may vary among years (Caputi & Brown 1993, Griffin et al. 2001, de Lestang et al. 2015, García-Echaury & Jeffs 2018). There is evidence that 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 appears that FIJs of *J. edwardsii* and *S. verreauxi* are highly resistant to starvation, through their ability to reduce their rate of energy expenditure. The 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 PNR₅₀. Furthermore, the FIJs of *S. verreauxi* whilst under starva-

tion conditions appear able to maintain the same rate of lipid use over a 6°C 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 result in smaller larvae (Tong et al. 2000b); increased temperature can also increase mortality during larval development and cause smaller increments in size between larval stages (Tong et al. 2000a, Bermudes & Ritar 2008, Fitzgibbon & Battaglene 2012). Late-stage lobster larvae of *P. cygnus* retained in ocean eddies with cooler water temperatures accumulate greater amounts of protein and lipid compared to those living in eddies with warmer water despite consuming similar diets (O'Rorke et al. 2015, Wang et al. 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 with sufficient remaining reserves to recommence feeding and successfully recruit into the population.

Coastal environments are warming faster than oceanic environments (Lima & Wetthey 2012), and hence could be expected to be of most impact on FIJs attempting to become 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 could constrain potential effects of climate change on this phase of the lifecycle in temperate spiny lobster populations.

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