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Marine and Freshwater Research
https://doi.org/10.1071/MF17145

Nutritional vulnerability in zoeal stages of the yellowline arrow crab *Stenorhynchus seticornis* (Brachyura: Majoidea)

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Abstract. Knowledge of the critical points in larval stages is essential to evaluate the physiological state of the larvae in their natural environment. This study investigated the nutritional vulnerability index (NVI) of the first (ZI) and second (ZII) zoeal stages of *Stenorhynchus seticornis*. Zoeae were assigned to two experiments: (1) point of no return (PNR), consisting of treatments with increasing days of starvation and subsequent days of feeding; and (2) point of reserve saturation (PRS), consisting of treatments with increasing days of feeding and subsequent days of starvation. There were two control groups: continuous starvation (CS) and continuous feeding (CF). Mortality was used to estimate the time when 50% of initially starved larvae (PNR_{50}) lost the ability to moult to the next stage and when 50% of initially fed larvae (PRS_{50}) were capable of moulting to the next stage. The mean (±s.d.) development time of ZI and ZII under CF was 4.4 ± 1.2 and 5.1 ± 1.8 days respectively. Mortality in the CF groups was 30 and 52% for ZI and ZII respectively. For ZI, PNR_{50} and PRS_{50} were 1.0 ± 0.0 and 2.1 ± 1.0 days respectively. The estimated NVI for ZI was 2.2, which indicates that *S. seticornis* depends on exogenous food and is considered planktotrophic during the first larval stage.

Additional keywords: Decapoda, experimental zoology, Inachidae, nutrition, plankton.

Received 23 May 2017, accepted 18 December 2017, published online 16 March 2018

Introduction

The majoid crab *Stenorhynchus seticornis* (Herbst, 1788), commonly known as the yellowline arrow crab, lives in a variety of sediments, such as rock, coral, sand or a combination of gravel and sand, from the intertidal zone to a depth of ~200 m (Williams 1984). The marine ornamental species trade exploits *S. seticornis* because of their hardiness in captivity and unique colouration and morphology (Calado *et al.* 2003). Aside from their exploitation, only a few aspects are known about the larval development of *S. seticornis*, such as their complete description (Yang 1976) and how temperature and salinity affect said development (Hernández *et al.* 2012).

One of the key factors affecting the survival of planktotrophic larvae is food availability (Yin and Blaxter 1987; Boidron-Métairon 1995; Morgan 1995; Pechenik *et al.* 1996). Tolerance over extended periods of starvation is paramount for the survival of decapod larval stages, especially in nutritionally unstable habitats (Anger 2001).

As described by Bryant and Hartnoll (1995), majoid crabs have large eggs compared with other brachyurans. Larger eggs may indicate that females provide endogenous food resources to their larvae, which help them survive (Choy 1988) in oligotrophic waters, such as the warm currents off the Brazilian coast (Valentin and Monteiro-Ribas 1993; De Léo and Pires-Vanin

2006). As food availability fluctuates spatially or seasonally, some species depend on endogenous reserves in their egg yolk. The reserves persist in varying quantity and quality throughout the hatching process into the early larval stages of development (Rainuzzo *et al.* 1997; Anger 2001). Thus, in the absence of exogenous food, larvae metabolise these reserves, which prevents the hardships of starvation and allows larval development to go on during periods of low planktonic productivity (Paschke *et al.* 2004; Guerao *et al.* 2012).

The literature describes at least two indices to assess the effects of food deprivation. Anger and Dawirs (1981) first demonstrated the existence of a 'point of no return' (PNR) in decapod larvae that corresponds to the limit from which the larvae can no longer recover from nutritional stress caused by previous starvation even though they are subsequently fed. A 'point of reserve saturation' (PRS) was also defined, beyond which food uptake is no longer essential for the subsequent moult to the next larval stage. The PRS represents the earliest time of food access in which the accumulated food resources are enough to successfully complete the current larval phase (Anger 2001; Gebauer *et al.* 2010).

PNR and PRS are useful for comparisons of nutritional vulnerability among different species and developmental life stages (Anger 1995; Figueiredo *et al.* 2008; Stumpf *et al.* 2010; Calvo *et al.* 2012; Guerao *et al.* 2012; Pantaleão *et al.* 2015) or broods from different seasons (Gebauer *et al.* 2010). Knowledge of the critical points in early larval stages is key when evaluating the physiological state of the larvae in their natural environment (Paschke *et al.* 2004; Rotllant *et al.* 2010). It is also helpful for minimising mortality in early zoeal stages and increasing survival during metamorphosis in aquaculture target species (Calado *et al.* 2007). These studies are even more relevant when the PNR can be quantified experimentally, because it helps reveal the nutritional flexibility of planktotrophic decapod larvae (Sulkin and van Heukelem 1980; Sulkin *et al.* 1998; Giménez and Anger 2005; Calado *et al.* 2007).

Females of *S. seticornis* produce large eggs with a yolk reserve that provides endogenous food for their larvae (Okamori and Cobo 2003). With that in mind, the aim of the present study was to determine whether this reserve is enough for the initial development, when the larvae are exposed to different periods of starvation or feeding. We investigated the early larval stages (first and second zoea, ZI and ZII) of the yellowline arrow crab *S. seticornis*, in particular its nutritional vulnerability, using PNR, PRS and the nutritional vulnerability index (NVI), defined as PRS_{50}/PNR_{50}, where PNR_{50} is the time when 50% of initially starved larvae lost the ability to moult to the next stage and PRS_{50} is the time when 50% of initially fed larvae were capable of moulting to the next stage. If the nutrients transferred by the parental female to the egg sufficed to sustain the development of ZI or ZII larvae, we expected that: (1) larvae exposed to different starvation periods would have similar development time (in days) and mortality; (2) larvae would tolerate a long period of starvation, being able to moult to the next stage (i.e. high PNR value and lower PRS value); and (3) there would be a low NVI value (i.e. NVI $\leq$0.5). This evaluation is important because this species is marketed, usually for ornamental purposes. The results of the present study could inform how feasible rearing of *S. seticornis* is and whether exogenous nutrition during the early phases of its life cycle is needed.

Material and methods

Sampling of ovigerous females

Samples were collected in accordance with Brazilian State and Federal laws concerning wild invertebrates. Ovigerous females of *S. seticornis* were captured manually from March through May 2012 by scuba diving during the day in the rocky sublittoral zone of the Couves Island (23°25′S, 44°51′W) in Ubatuba, on the northern coast of the State of São Paulo, Brazil. Before transportation, crabs were examined macroscopically; only females carrying embryos in the final stage (i.e. embryos bearing well-developed pigmented eyes; Wehrtmann 1990) were isolated in small plastic bowls with small holes for water flow. Subsequently, all bowls were placed inside a thermal box filled with seawater from the sampling site and aerated continuously. The boxes were transported to the laboratory.

Broodstock maintenance and rearing of larvae

Seven ovigerous *S. seticornis* females were maintained in individual rectangular aquaria (450 × 200 × 300 mm; 27-L capacity) containing calcareous rock fragments for refuge. The seven aquaria were interconnected in a water-recirculation system adapted from Calado *et al.* (2007) and Gregati *et al.* (2010). The water-recirculation system was equipped with an additional container for chemical and biological filtering, containing a protein skimmer (Bubble Magus – Nac 7, Jiyang Aquarium Equipment Co., Ltd, Jiangmen, Guangdong, China), two heaters (300 W), a digital thermostat (±0.1°C accuracy), an ultraviolet (UV) lamp (15 W) and activated carbon (500 g; replaced monthly).

Newly hatched larvae were attracted using a light-emitting diode (LED) light trap installed in each aquarium (for more details, see Gregati *et al.* 2010), leading the larvae into rearing net tanks with a 125-μm mesh size net. Then, larvae were captured using a Pasteur pipette and transferred to experimental containers. These traps have been used by others (see Calado *et al.* 2010; Gregati *et al.* 2010), and their effectiveness has been well demonstrated. For experiments, only the most active newly hatched larvae were used (i.e. those exhibiting pronounced positive phototactic responses by swimming towards a light), following the same criteria using in previous studies on other decapod larvae (Calado *et al.* 2008, 2010; Gregati *et al.* 2010).

The dissolved oxygen concentration was maintained at 5–8 mg L^{-1} and the crabs were kept under a 12-h light–dark photoperiod. Mean (±s.d.) temperature and salinity were maintained at 25 ± 1°C and 30 ± 1. Ovigerous females were fed daily with fragments of shrimp and cuttlefish and commercial balanced food (TetraColor – Tropical granules; TETRA, Spectrum Brands, Inc., Blacksburg, VA, USA) available *ad libitum*. This diet was tested before the experiments started and its adequacy for this species was verified because the females fed this way produced viable larvae. Thus, to reduce possible effects of feeding in captivity, we only used larvae from females collected with embryos in the final stage of development. The bottoms of the aquaria containing the ovigerous females were siphoned twice a week to remove faeces and non-ingested food particles.

The larvae used in the experiments were either in the first (ZI) or the second (ZII) zoeal stages. Every morning the rearing tanks (LED light trap) were checked for newly hatched larvae, capturing some of them for the experiments with ZI. Newly

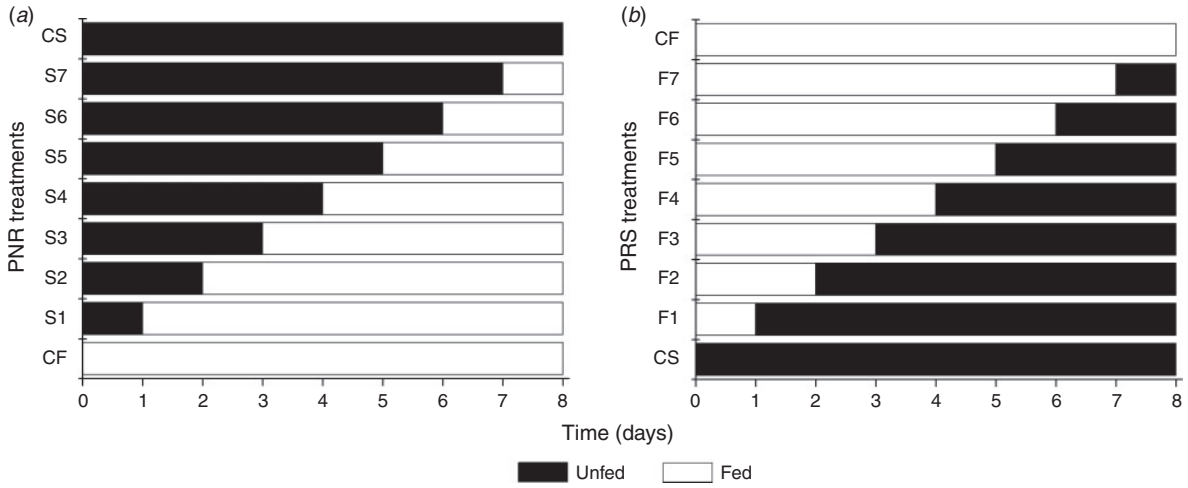

Fig. 1. Protocol used during the restriction period in the larval stages (zoea (Z) I and II) of *Stenorhynchus seticornis*. (*a*) Starvation treatments to determine the point of no return (PNR). Larvae were initially starved (S) for the specified number of days and then fed for the remainder of the experiment. (*b*) Feeding treatments to determine the point of reserve saturation (PRS). Larvae were initially fed (F) for the specified number of days and then fed for the remainder of the experiment. The experiments with ZI and ZII larvae lasted for 7 and 8 days respectively. CF, continuously fed control; CS, continuously starved control. (Experimental design is based on Paschke *et al.* (2004).)

hatched larvae from one of the females were mixed in a plastic container, and five to six of these larvae were distributed randomly among the treatment groups. Whenever larvae from another female hatched, the above procedure was repeated until 40 replicates (larvae) were completed for each treatment to ensure that all treatments contained the same number of larvae from each female. In the experiment with ZII larvae, larvae were used from the same group of ovigerous females used in the experiment with ZI larvae. In this case, newly hatched larvae were kept in a larviculture tank. The larvae were fed newly hatched *Artemia* nauplii (density: 2 prey mL^{-1}) daily and were inspected for survival and signs of activity until they reached the ZII stage (indicated by the presence of exuviae). Once identified, the ZII larvae were removed from the larviculture tank, and isolated in plastic containers (80 mL) with 50 mL of seawater. Then, they were pooled in a group of 40 individuals, mixed in a plastic container and randomly distributed among treatment groups.

Experiment 1: PNR

In Experiment 1 we tested the effects of starvation on survival and moulting of ZI and ZII larvae. ZI larvae were assigned to the starvation treatments (Fig. 1*a*), consisting of six different periods of initial starvation and subsequent days of feeding, as follows: S1, 1 day starved/6 days fed; S2, 2 days starved/5 days fed; S3, 3 days starved/4 days fed; S4, 4 days starved/3 days fed; S5, 5 days starved/2 days fed; and S6, 6 days starved/1 day fed. In addition, there were two control groups: continuous feeding (CF) and continuous starvation (CS; Fig. 1*a*). ZII larvae were assigned to the same groups with different periods of initial starvation and subsequent days of feeding as the ZI larvae, with an additional S7 (7 days starved/1 day fed) treatment, as well as the two CF and CS control groups (Fig. 1*a*). Each treatment consisted of 40 replicates, totalling 320 and 360 replicates for ZI and ZII larvae respectively.

Experiment 2: PRS

In Experiment 2 we evaluated the effect of feeding on survival and moulting of ZI and ZII larvae. ZI were assigned to the following feeding treatments, consisting of six different periods of initial feeding and subsequent days of starvation, as follows (Fig. 1*b*): F1, 1 day fed/6 days starved; F2, 2 days fed/5 days starved; F3, 3 days fed/4 days starved; F4, 4 days fed/3 days starved; F5, 5 days fed/2 days starved; and F6, 6 days fed/1 day starved. ZII larvae were assigned to the same groups with different periods of initial feeding and subsequent days of starvation as the ZI larvae, with an additional F7 (7 days fed/1 day starved) treatment. As for the PNR experiments, there were two control groups: CF and CS. Each treatment consisted seven different periods of 40 replicates, totalling 320 and 360 replicates for ZI and ZII larvae respectively.

Experimental conditions and end points

During all experiments, larvae were reared individually in plastic containers (80 mL) with 50 mL seawater from the collection site, which was sterilised using UV light (5 W) to prevent microbial growth. Thus, each larva was considered as a replicate, in line with the procedures reported by Gebauer *et al.* (2010), Guerao *et al.* (2012), Pantaleão *et al.* (2015) and Espinoza *et al.* (2016). The containers were arranged inside a plastic tray (530 × 400 × 120 mm) filled with fresh water and maintained at 25 ± 1°C using thermostats (AT 180–100 W, ATMAN, Chuangxing Electrical Appliances Co., Ltd, Zhongshan, P.R. China). The plastic containers were cleaned daily and the existing water was replaced with filtered water in both the ZI and ZII experiments. Temperature and salinity were checked daily. In the experiments with feeding (ZI and ZII larvae), newly hatched *Artemia* nauplii were provided as food at a density of 2 prey mL^{-1}, and any remaining food was removed the next morning. Larvae were checked twice a day (morning and late afternoon) to register deaths or moults (evidenced by the presence of exuviae). The

experiments with ZI and ZII larvae lasted for 7 and 8 days at most respectively. During this time, the larvae moulted, died or were killed. This procedure took into account the fact that, in larvae of decapod crustaceans, the effects of stress (e.g. temperature, salinity or nutritional) have been observed in delayed development, reduced survival and in extended or suspended moulting cycles (i.e. the larvae remain in the same stage and eventually die; Gore 1985; Anger 2001).

'Moulting' was determined as the percentage of individuals from each treatment that moulted before the end of the experiment (Day 7 for ZI larvae and Day 8 for ZII larvae); 'no moulting' refers to the percentage of individuals in a certain treatment that did not die and never moulted. 'Mortality' was calculated the percentage of individuals from a certain treatment that died before the end of the experiment, as per the methods of Calvo *et al.* (2012). Development time in days (mean ± s.d.) from one stage to the next (ZI to ZII; ZII to megalopa) was calculated for all larvae per treatment.

The PNR_{50} and PRS_{50} indices were calculated from the mortality data of each treatment. As noted above, mortality was calculated as the percentage of individuals in a given treatment that died before the end of the experiment; and 'mortality before moulting' was calculated as the percentage of individuals in a given treatment that died and never moulted. PNR_{50} and PRS_{50} values were calculated by adjusting the sigmoidal Boltzmann model, described in the following equation:

$$M = \frac{A_1 - A_2}{1 + e^{(x - x_o)/d_x}} + A_2$$

where M is the percentage of 'mortality' + 'no moulting' individuals, A_1 is the minimum value of M, A_2 is the maximum value of M, x_0 is the time (days) in which the M reaches 50%, d_x is the time constant and x is the time (days) of initial starvation (PNR) or feeding (PRS); Paschke *et al.* 2004; Bas *et al.* 2008; Gebauer *et al.* 2010). Values of PNR_{50} and PRS_{50} were estimated considering the development time of ZI to ZII, and of ZII to the megalopa stage. The PRS_{50}/PNR_{50} ratio constitutes the NVI (for details, see Gebauer *et al.* 2010).

Statistical analysis

When data met the model assumptions, parametric tests were used; otherwise, equivalent non-parametric tests were used. The model assumptions of homoscedasticity (Levene's test) and normality (Shapiro–Wilk's test) were initially tested (Zar 2010). The effects of each treatment on development time were compared with the effects of the CF control using the Mann–Whitney (non-parametric) test. The effects of each treatment on larval 'mortality' + 'no moulting' were compared with the effects of the CF control using the Mann–Whitney (non-parametric) test. The level of significance was set at 5% for two-sided P-values (Zar 2010). Statistical tests were conducted using Statistica (ver. 7.0, StatSoft, Tulsa, OK, USA).

Results

Experiment 1: PNR

Considering all the ZI larvae subjected to the initial starvation period, only larvae in the CF and S1 treatment moulted to the next stage. Mean (±s.d.) development time was lower for larvae in the CF than S1 group (4.4 ± 1.2 and 6.3 ± 0.5 days respectively; Mann–Whitney, $U = 12.00$, $P = 0.001$; Fig. 2*a*). In the CF group, 30% of ZI larvae died and 67% of larvae moulted to the next stage. The percentage mortality was >80% in the S1–S6 and CS treatments (Mann–Whitney, $P < 0.001$; Fig. 2*b*).

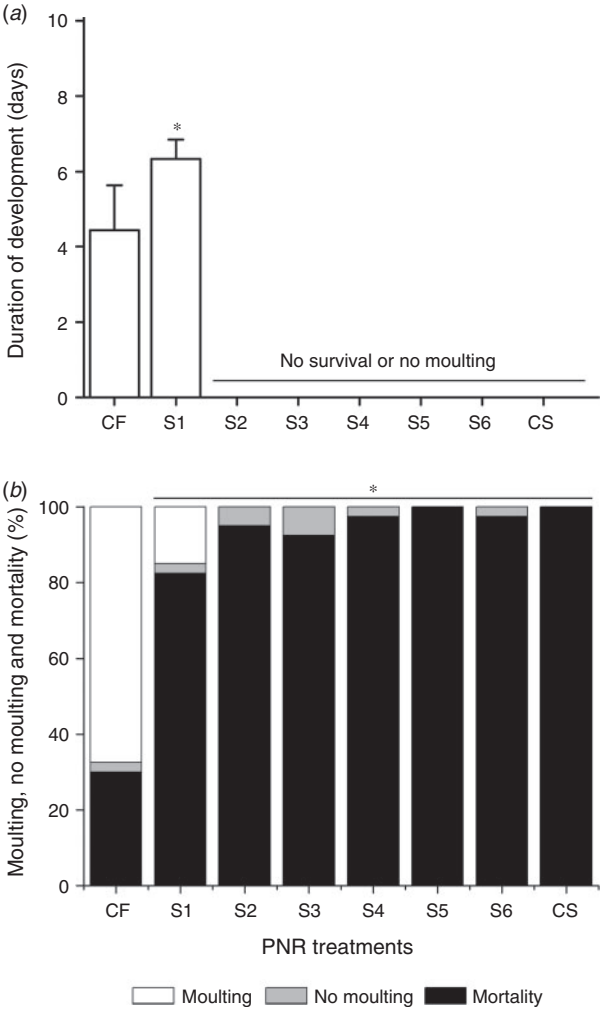

Fig. 2. (*a*) Mean (±s.d.) development time and (*b*) percentage of moulting, no moulting and mortality in zoea I larvae of *Stenorhynchus seticornis* in the different point of no return (PNR) treatment groups. Larvae were initially starved (S) for the specified number of days and then fed for the remainder of the experiment (see text for details). CF, continuously fed control; CS, continuously starved control. *, $P < 0.0001$ compared with the CF control.

Of all the ZII larvae subjected to the initial starvation period, only larvae in CS and S5 groups did not reach the next stage. Mean (±s.d.) development time was lower for larvae in the CF than S3 group (5.1 ± 1.8 and 7.3 ± 1.2 days respectively; Mann–Whitney, $U = 6.00$, $P = 0.046$; Fig. 3*a*). In the S4, S6 and S7 groups, only one larva reached the next developmental stage (Fig. 3). In the CF group, 52% of ZII larvae died and only 40% of larvae moulted to the next stage (megalopa). The 'mortality' + 'no moulting' for the ZII larvae in all treatment groups differed significantly from those in the CF group

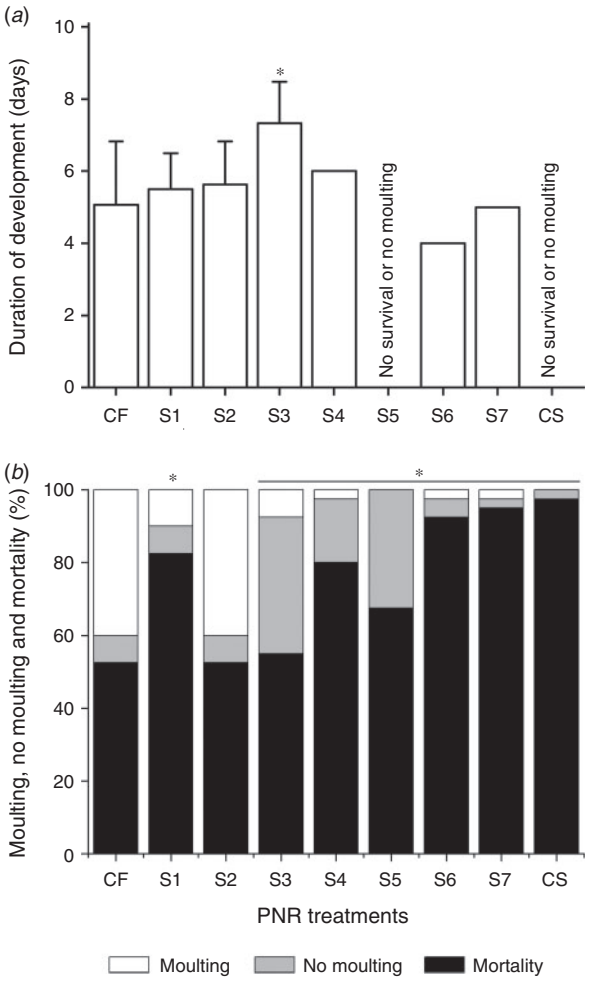

Fig. 3. (*a*) Mean (±s.d.) development time and (*b*) percentage of moulting, no moulting and mortality in zoea II larvae of *Stenorhynchus seticornis* in the different point of no return (PNR) treatment groups. Larvae were initially starved (S) for the specified number of days and then fed for the remainder of the experiment (see text for details). CF, continuously fed control; CS, continuously starved control. *, $P < 0.0001$ compared with the CF control group.

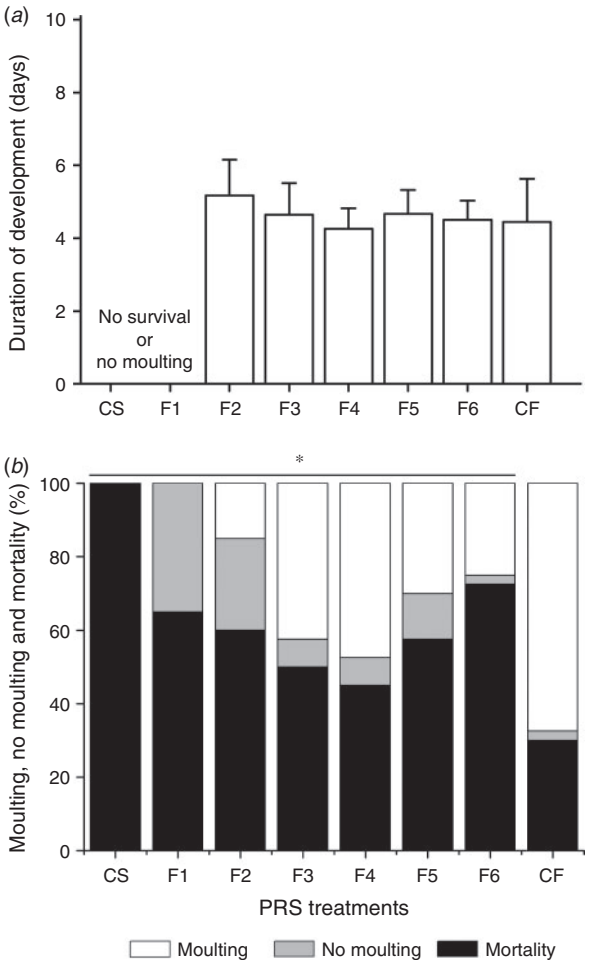

Fig. 4. (*a*) Mean (±s.d.) development time and (*b*) percentage of moulting, no moulting and mortality of zoea I larvae of *Stenorhynchus seticornis* in the different point of reserve saturation (PRS) treatment groups. Larvae were initially fed (F) for the specified number of days and then fed for the remainder of the experiment. CF, continuously fed control; CS, continuously starved control. *, $P < 0.0001$ compared with the CF control group.

($P < 0.001$), with the exception of the S2 group (Mann–Whitney, $U = 720.00$, $P = 0.351$; Fig. 3*b*). In treatments S1, S3–S7 and CS, mortality was >80% (Fig. 3*b*).

Experiment 2: PRS

The ZI larvae in the CF treatment took 4.4 ± 1.2 days to moult, which did not differ significantly compared with the other treatment groups (Mann–Whitney, $P > 0.05$), except for the CS and F1 treatments, in which the larvae did not moult to the next stage (Fig. 4). In the CF group, 30% of ZI larvae died and 67% of larvae moulted to the next stage. The percentage of ZI larvae that died and did not moult in the CF group was significantly different from that in all other treatment groups, in which the percentage of 'mortality' + 'no moulting' was >50% (Mann–Whitney, $P < 0.05$; Fig. 4*b*).

In ZII larvae subjected to an initial feeding period, mean (±s.d.) development time for larvae in the CF treatment was 5.1 ± 1.8 days, which was not significantly different from that in the other treatment groups (Mann–Whitney, $P > 0.05$; Fig. 5*a*). Only larvae in the CS treatment did not reach the next developmental stage (Fig. 5*b*). In the CF group, 52% of ZII larvae died and only 40% of larvae moulted to the next stage (megalopa). The percentage of ZII larvae in the CF group that died and did not moult differed significantly from that in all other treatment groups (Mann–Whitney, $P < 0.01$), except for the F3 group (Mann–Whitney, $U = 640.00$, $P = 0.0524$). In all the treatments except the CF group, 'mortality' + 'no moulting' was >75% and only 20% of larvae reached the megalopa stage (Fig. 5*b*).

Nutritional vulnerability

The PNR_{50} and PRS_{50} mean (±s.d.) values of for ZI larvae, obtained from sigmoid curves were 1.0 ± 0.0 and 2.1 ± 1.0 days respectively (Fig. 6). From these values, NVI was estimated to be 2.2. The PNR_{50} and PRS_{50} values were not calculated for ZII larvae because mortality was >50% in all treatment groups.

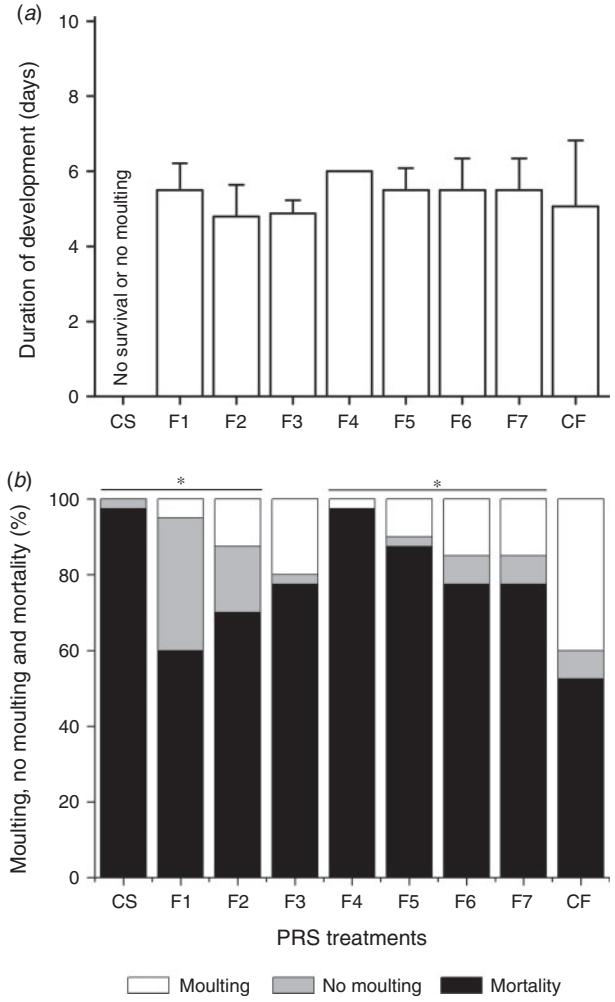

Fig. 5. (*a*) Mean (±s.d.) development time and (*b*) percentage of moulting, no moulting and mortality of zoea II larvae of *Stenorhynchus seticornis* in the different point of reserve saturation (PRS) treatment groups. Larvae were initially fed (F) for the specified number of days and then fed for the remainder of the experiment. CF, continuously fed control; CS, continuously starved control. *, $P < 0.0001$ compared with the CF control group.

Discussion

As is the case for all majoid crabs, female *S. seticornis* produce large amounts of yolk reserve (Okamori and Cobo 2003) and, until the present study, it was unclear whether this yolk reserve was enough for the development of the larval stages (ZI and ZII) of *S. seticornis*. The results of the present study indicate that ZI and ZII larvae of *S. seticornis* are highly dependent on exogenous food to complete their larval development.

First, we observed that only 1 day of initial starvation after hatching (PNR experiment) caused irreversible damage, with significant effects on both survival and the duration of ZI development. Similar results were found for ZII larvae of *S. seticornis*. However, a small tolerance to initial starvation was verified in ZII larvae (i.e. similar values for 'mortality' + 'no moulting' between the CF and S2 treatments, and similar development time in the CF *v.* S1 and CF *v.* S2 treatments). These results indicate that feeding during the first larval stage (ZI) may provide a higher tolerance to starvation in the second larval stage (ZII). Similar results have been reported for other decapods, such as *Neohelice granulata* (Dana, 1851) (Bas *et al.* 2008), *Hyas araneus* (Linnaeus, 1758) (Anger and Dawirs 1981) and *Crangon crangon* (Linnaeus, 1758) (Paschke *et al.* 2004).

High values for 'mortality' and 'no moulting' were also observed in all treatments in which larvae were exposed to periods of initial feeding (PRS). For example, no ZI larvae moulted to the ZII stage with only 1 day of initial feeding (followed by continuous starvation). Moreover, mortality of ZI larvae in the CF control treatment was significantly lower than that recorded in the other treatment groups (F1–F6 and CS). In this experiment, we noted that periods of initial feeding had less of an effect on development time in ZI larvae (i.e. larvae that had at least 2 days of initial feeding (F2–F6 groups) moulted at a similar time to those in the CF control treatment). Thus, we can suggest that short periods of initial starvation followed by continuous feeding caused more damage to *S. seticornis* ZI larvae than did feeding immediately after hatching followed by continuous starvation (see Anger *et al.* 1981). This has also been observed for other decapod crustaceans, like the spider crab *Maja brachydactyla* Balss, 1922 (Guerao *et al.* 2012). In PRS experiments, ZII larvae also exhibited a lower tolerance to starvation than that recorded in the PNR experiments, with similar values for 'mortality' + 'no moulting' in the F3 and CF groups and in development time in the F1–F3 and F5–F7 *v.* CF group.

The second piece of evidence that *S. seticornis* is not able to moult without exogenous food was provided by the PNR_{50} and PRS_{50} values of ZI larvae. In accordance with the PNR_{50} value after hatching, at least 50% of larvae exposed to 1 day of starvation died, even if they were fed after the initial period of starvation. Conversely, if larvae were fed for more than 2 days, at least 50% of the larvae tended to moult to the ZII stage, even after being exposed to periods of starvation (see Results, Experiment 2: PRS). Thus, the results of the present study show that *S. seticornis* is less resistant to starvation than larval stages of other decapod crustaceans studied previously (Table 1).

Owing to high mortality for ZII larvae in the PNR and PRS experiments, the values for PNR_{50} and PRS_{50} could not be calculated. Correspondingly, high mortality values are common when the transition from one larval phase to another is associated with metamorphosis in decapod crustaceans (Gebauer *et al.* 1999, 2003). Thus, during the transition from pelagic to benthic habitats, major changes (e.g. morphological, physiological and behavioural) lead to increased larval mortality (Gebauer *et al.* 1999, 2003; Simith *et al.* 2010). The high and variable mortality in zoeae observed in the present study (>40% under the test conditions) were expected during the culture of *S. seticornis* because of several factors, including the rearing system. Similar results have been reported for other crustacean decapods (see Rhyne and Lin 2004; Calado *et al.* 2005), considering that not all factors can be controlled under laboratory conditions and the stress imposed there may also affect results (Dittel *et al.* 1995). Under natural conditions, food resources are more available and diverse (Felder *et al.* 1985; Rodriguez *et al.* 1990; Sheen 2000).

The results of the present study indicate that the ZI larvae of *S. seticornis* are obligatory planktotrophic larvae, given their dependence on exogenous food is corroborated by a high NVI

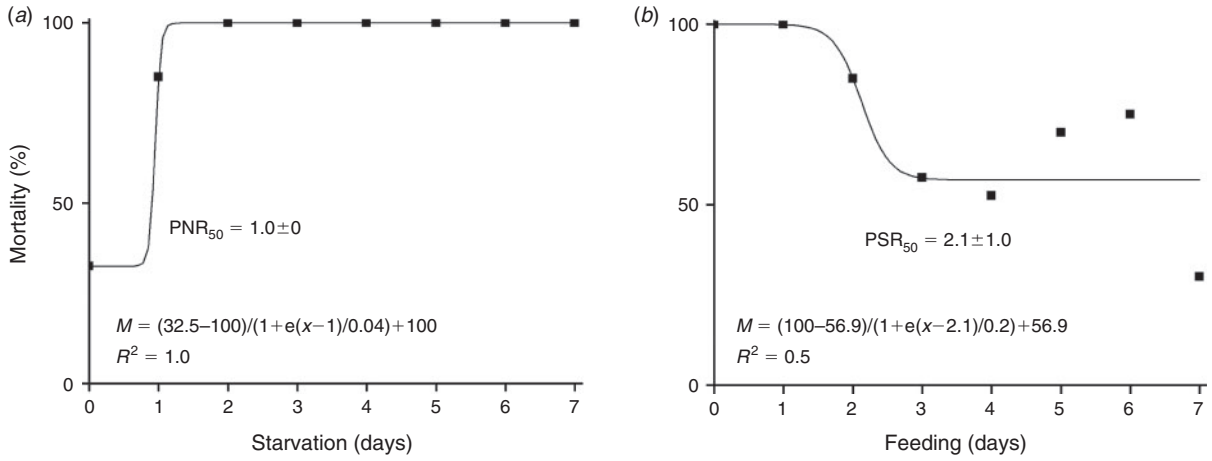

Fig. 6. Adjustment to the sigmoid curve for estimation of mean (±s.d.) time when (*a*) 50% of initially starved larvae (PNR_{50}) lost the ability to moult to the next stage and (*b*) 50% of initially fed larvae (PRS_{50}) were capable of moulting to the next stage for zoea I larvae of *Stenorhynchus seticornis* in the different treatment groups. *M*, percentage of 'mortality' + 'no moulting' individuals; *x* is the time (days) of initial starvation (PNR) or feeding (PRS).

Table 1. Time when 50% of initially starved larvae (PNR_{50}) lost the ability to moult to the next stage and when 50% of initially fed larvae (PRS_{50}) were capable of moulting to the next stage, as well as Nutritional Vulnerability Index (NVI) values for some larval decapods

Where appropriate, data are given as the mean ± s.d. or range. NIV was calculated as PRS_{50}/PNR_{50}

Species	Stage	PNR_{50}	PRS_{50}	NVI	Reference
Hyas araneus	Zoea I	8.0	3.0	0.4	Anger and Dawirs (1981)
Hyas araneus	Zoea II	4.0	5.0	1.2	Anger and Dawirs (1981)
Sesarma cinereum	Zoea I	1.8	1.9	1.0	Staton and Sulkin (1991)
Crangon crangon (summer)	Zoea I	3.5	1.6	0.5	Paschke *et al.* (2004)
Neohelice granulata	Zoea I	0.6	3.4	0.2	Bas *et al.* (2008)
Petrolisthes laevigatus	Zoea I	3.7 ± 1.5–7.2 ± 0.7	6.5 ± 0.3–15.7 ± 0.2	1.7–2.2	Gebauer *et al.* (2010)
Cherax quadricarinatus	Juvenile III	4.3	3.5	0.8	Stumpf *et al.* (2010)
Maja brachydactyla	Zoea I	2.8	1.9	0.7	Guerao *et al.* (2012)
Neocaridina davidi	Juvenile I	16.2 ± 0.3	0.0	0.0	Pantaleão *et al.* (2015)
Neocaridina davidi	Juvenile III	9.4 ± 0.3	0.0	0.0	Pantaleão *et al.* (2015)
Pleuroncodes monodon	Zoea I	3.0	1.9	0.6	Espinoza *et al.* (2016)
Panulirus argus (warm season)	Juvenile I	12.1 ± 1.2	13.1 ± 0.7	1.1	Espinosa-Magaña *et al.* (2017)
Stenorhynchus seticornis	Zoea I	1.0 ± 0.0	2.1 ± 1.0	2.2	Present study

(2.2). According to Gebauer *et al.* (2010), NVI values >1.0 represent a high dependence on exogenous food. Nevertheless, other decapod crustaceans previously studied had lower PRS than PNR values (Anger and Dawirs 1981; Mikami *et al.* 1995; Giménez 2002; Liddy *et al.* 2003; Gebauer *et al.* 2010; Espinoza *et al.* 2016). A high dependence on exogenous food (NVI >1) has been documented primarily for marine species (Table 1). In contrast, NVI ≥1 has been reported for ZI larvae of other decapod species, such as *Armases cinereum* Bosc, 1802 (NVI = 1.0; Staton and Sulkin 1991) and *Petrolisthes laevigatus* (Guérin, 1835) (NVI = 1.7–2.2; Gebauer *et al.* 2010), as well as for ZII larvae, such as *H. araneus* (Linnaeus, 1758) (NVI = 1.2; Anger and Dawirs 1981; Table 1).

Conclusions

Based on our experiments, by imposing gradual periods of initial starvation or feeding (PNR and PRS experiments; Anger and Dawirs 1981), we found that both larval stages of *S. seticornis* (ZI and ZII) need exogenous food to reach the next stage and that their survival was similar to the one recorded for the CF control treatment. Thus, larvae of *S. seticornis* in the ZI stage are obligatorily planktotrophic. However, despite NVI values not being calculated for ZII larvae of *S. seticornis*, the high mortality in all treatments evidence that these larvae are also planktotrophic. This pattern has been reported for the majoid species *H. araneus* (Anger and Dawirs 1981, 1982; Anger *et al.* 1989) and *Maja squinado* var. *brachydactyla* (Herbst, 1788) (Andrés *et al.* 2008; Rotllant *et al.* 2010; Guerao *et al.* 2012). However, a greater number of species should be studied to determine patterns in groups of species that occupy different environments, different habitats and have different lifestyles.

The importance of the present study remains in the establishment of culture protocols, primarily focusing on the feeding decapod larvae, such as ZI and ZII larvae of *S. seticornis*, in a rearing system. To this end, we provide information as to how

many days of exogenous food are needed for high survival of the larvae while maintaining low costs and efficient management of food. Based on this information, we expect the larviculture of this ornamental crab to become efficient and economically viable.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors are grateful to Coordernação de Aperfeiçoamento de Pessoal de Nível Superior/Ministerio de Ciencia, Tecnología e Innovación Productiva (CAPES-MINCYT) (number 217/2012) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (number 481435/2011-5) for their financial support of this research. Maria Lucia Negreiros-Fransozo thanks CNPq for a research grant (number 302691/2015-4).

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