

Development and metabolic rate of stage I spiny lobster (*Jasus edwardsii*) larvae under constant and fluctuating salinities

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Abstract The effects of fluctuating salinity on the development and metabolic rate of stage I *Jasus edwardsii* larvae were investigated. *J. edwardsii* larvae were reared from hatching through to stage II under continuous salinity regimes at 28, 31, 34, and 37 psu, and under repeated exposure to 28, 31, and 37 psu from a salinity of 34 psu. Continuous exposure to salinities between 28 and 37 psu did not affect the duration of larval development. In the repeated exposure treatments, only larvae subjected to 28 psu developed slower than those in the 34 psu continuous exposure group. Although post-moult growth to stage II was not suppressed by fluctuating salinity in the 31 and 37 psu groups, larvae repeatedly exposed to 28 psu and larvae under continuous salinities of 28, 31, and 37 psu were significantly smaller than those from the 34 psu continuous treatment. The effect of salinity acclimation on the respiratory response of mid-stage I larvae was

examined in a second experiment. The reduced oxygen consumption of acclimated larvae at subnormal salinities (i.e., 28 psu) was characteristic of stenohaline organisms, and overall there was little relationship between metabolic rate and larval growth performance. From the results obtained in this study we recommend the monitoring and control of environmental salinity for the propagation of *J. edwardsii* larvae to prevent prolonged exposure to suboptimal salinities.

Keywords *Jasus edwardsii*; larvae; salinity; growth; oxygen consumption

INTRODUCTION

The propagation of the southern rock lobster (*Jasus edwardsii* Hutton) is one of the current priorities of aquaculture research and development in Tasmania, Australia. Southern rock lobster post-larvae (i.e., pueruli) have been produced in small numbers (Kittaka 1994; Booth 1996). However, high mortalities experienced during larval development highlight the need for further research that addresses culture parameters and to extend our knowledge of the tolerance of phyllosomas to hatchery conditions. In the wild, the early life history of *J. edwardsii* is characterised by a pelagic larval phase occurring in oceanic waters (Booth 1994; Bruce et al. 2000). Keys to the success of the mass production of pueruli may lie either in our ability to mimic these oceanic conditions or in the capacity of phyllosomas to adapt to a hatchery environment. Although not a general rule, many marine hatcheries experience occasional and/or seasonal changes in the quality of the sea water they use to produce seed stock, and these shifts may be detrimental to larval development and survival. At this early stage of the research into the propagation of *J. edwardsii*, it is therefore essential to determine the tolerance of phyllosomas to shifts in water quality variables such as salinity, so as to design suitable culture systems that maximise larval survival. The study of salinity fluctuation tolerance

in *J. edwardsii* larvae was further prompted by sudden changes in the salinity of the water at the Marine Research Laboratories of the Tasmanian Aquaculture and Fisheries Research Institute (TAFI MRL), situated on the Derwent River estuary, where research in the propagation of *J. edwardsii* is currently underway. For instance, salinities ranging from 29.5 to 34.5 psu have been recorded (Chamchang 1997). Importantly, though, the estuarine water is drawn from the immediate vicinity of a large naturally-breeding population of *J. edwardsii*, which hatches larvae during a period of likely salinity fluctuations caused by spring rains.

The effects of salinity on marine invertebrates is well documented and ranges from sublethal to lethal according to the magnitude of the change in salinity and the tolerance of the species. Estuarine and coastal crustaceans are often euryhaline and can withstand large shifts in environmental salinity as opposed to their stenohaline oceanic counterparts that live in or actively select isohaline waters (Willmer et al. 2000). In a euryhaline species such as *Carcinus maenas* for instance, larval development is not affected by salinities ranging from 25 to 32 psu (Anger et al. 1998). In contrast, the stenohaline *Pandalus borealis*, known to have an optimal salinity c. 31 psu, does not complete larval development at 25 psu (Wienberg 1982). In culture, sublethal salinities may result in delayed development and reduced growth (Anger et al. 1998; Hereu & Calazans 2000; Pechenik et al. 2000; Kumlu et al. 2001).

The present work was carried out to examine the effect of salinity, either continuous or fluctuating, on the survival, growth, and oxygen consumption of the first stage of the spiny lobster, *J. edwardsii*. The experimental salinities cover the range of salinities occurring at TAFI MRL. Hypersaline conditions were also tested since they may occur in hatcheries working with a recirculation system.

MATERIAL AND METHODS

Origin of larvae

Larvae were sourced from ovigerous females caught off the east coast of Tasmania, Australia from June to October in 1999 and 2000, and brought to TAFI MRL. Newly-hatched larvae were collected from hatching tanks from September to December of both years. The ambient temperature and salinity at the time of hatching ranged from 12.0 to 17.1°C and from 31.2 to 35.3 psu, respectively.

Rearing under constant and fluctuating salinities

To examine the effect of frequent changes in salinity (28, 31, 34, and 37 psu) on the survival and growth of stage I larvae, newly-hatched animals were placed under two salinity regimes: continuous exposure and repeated exposure from a salinity level of 34 psu. In the repeated exposure group, larvae were exposed to their respective treatment salinity (28, 31, or 37 psu) at the start of the trial. After 24 h of exposure to these conditions, larvae were returned to 34 psu for 24 h before a further 24 h exposure to treatment salinity, and so on. The 34 psu continuous exposure treatment was used as a control for repeated exposure. In both continuous and repeated exposure groups, larvae were returned to 34 psu from day 9 after hatching and until moulting to stage II occurred, so that larvae in all treatments would moult under the same conditions. Throughout stage I, larvae in the repeated exposure group were placed 5 times for 24 h each under treatment salinities. Larvae were reared in 60 ml plastic jars with 50 ml water and 25 ppm oxytetracycline. There were 12 larvae per jar (four from each of three broods) and five replicated jars per treatment. Feeding of live *Artemia* (1–2 mm in length), complete water exchange (+25 ppm oxytetracycline), and removal of dead larvae were carried out daily. All larvae that moulted to stage II were measured for total body length (from the anterior of the cephalic shield to the end of the telson) on a Nikon Profile Projector Model 6C to the nearest 25 µm. Minimum (after water exchange) and maximum (before water exchange) salinities were recorded daily and averaged to obtain a salinity measurement for each day. Mean (\pm SD, n = duration of experiment in days) salinity levels were 28.05 ± 0.14 , 31.06 ± 0.08 , 34.09 ± 0.15 , and 37.08 ± 0.17 psu and are rounded down to the nearest integer in the text. The pH at each salinity was initially adjusted to 8.2 with a few drops of a sodium bicarbonate solution (pH 9). The mean (\pm SD, n = duration of experiment in days) pH in culture media from daily measurements of initial (after water exchange) and final pH (before water exchange) were 8.01 ± 0.04 , 8.02 ± 0.04 , 8.04 ± 0.05 , and 8.07 ± 0.05 at 28, 31, 34, and 37 psu, respectively. The pH was not significantly different between salinity treatments (ANOVA, $P > 0.05$). Mean (\pm SD; n = duration of experiment in days) rearing temperature was $18.5 \pm 0.2^\circ\text{C}$.

Routine metabolic rate in acclimated and non-acclimated larvae

The effect of acclimation to different salinities (28, 31, 34, and 37 psu) on the routine metabolic rate (oxygen consumption in unrestrained animals) of

stage I larvae was assessed from measurements of oxygen consumption (V_{O_2}) in acclimated and non-acclimated larvae. Acclimated phyllosomas were 4 days old and reared from hatching at constant salinities (mean \pm SD, $n = 3$ days) of 28.6 ± 0.5 , 31.7 ± 0.6 , and 37.6 ± 0.2 psu. Non-acclimated larvae of the same age had been cultured at the control salinity (mean \pm SD, $n = 3$ days) of 34.7 ± 0.4 psu. Larvae from three broods were reared, fed, and water was exchanged as described above. Food was withheld 18 h before the start of the experiment. Mean \pm SD ($n = 3$ days) temperature during acclimation was $17.9 \pm 0.2^\circ\text{C}$. There were 10 larvae per jar and animals from each jar were placed in a respirometer (12 ml plastic syringe). In each salinity treatment there were six replicate respirometers (two for each brood) and two control respirometers without larvae. The syringe respirometers were filled with UV-sterilised, $0.2 \mu\text{m}$ filtered sea water treated with oxytetracycline (25 ppm) to minimise background microbial respiration. Larvae were left to recover from handling stress while acclimating to the respirometers for 1–2 h before a first water sample (0.75 ml) was drawn to determine the initial oxygen saturation level. The oxygen content was left to decline in the respirometer for 4–6 h at an incubation temperature of 17.9°C , and a second water sample was obtained to determine final oxygen tension. Percentage oxygen saturation of initial and final samples was measured with a polarographic electrode connected to a digital controller (Rank Brothers Ltd, United Kingdom). The dry mass (DM) of test animals was determined from three samples of larvae rinsed in 0.9% ammonium formate and dried for 24 h at 60°C (Lovegrove 1962). The mass of each sample was measured to the nearest $10 \mu\text{g}$ on a precision balance (Mettler AT261 DeltaRange, Mettler-Toledo AG, Switzerland). Oxygen consumption was expressed in $\mu\text{l O}_2 \text{ mg DM}^{-1} \text{ h}^{-1}$ after background respiration obtained from the control respirometers was subtracted. Throughout these trials, oxygen saturation in the respirometers was kept above 80% as recommended by Ikeda et al. (2000).

Statistical analysis

All data were tested for normality (Shapiro-Wilk W test) and for homoscedasticity (Levene's test) or for the independence of standard deviation of the magnitude of the means (regression analysis). The effects of salinity on survival, duration of stage I, and growth under continuous and repeated exposure, and the effect of salinity on oxygen consumption in the acclimated and non-acclimated groups were tested

with analysis of variance (ANOVA). In the event of significant treatment effect among the repeated exposure groups or non-acclimated groups, comparisons with control (34 psu continuous exposure or 34 psu acclimated group, respectively) were carried out using the Dunnett's test. Tukey's honestly significant difference test (Tukey-HSD) was used for multiple means comparisons among continuous and acclimated groups. Differences in the patterns of response (survival, duration of stage I, growth, and oxygen consumption) between groups (continuous exposure versus repeated and acclimated versus non-acclimated) as well as interaction between the effects of salinity and exposure or acclimation were tested with two-way ANOVA excluding respective 34 psu groups. Linear regression and t -test analyses were used to further highlight specific trends and differences between groups or treatments. Survival data were arcsine square-root transformed before analysis. All computations were carried out with Microsoft Excel 2000 and JMP 3.1 statistical software.

RESULTS

Rearing under constant and fluctuating salinities

Overall mean (\pm SD) survival of stage I larvae to stage II was $75.0 \pm 15.6\%$. Survival was not affected by salinity in both continuous (ANOVA, $P = 0.665$) and repeated (ANOVA, $P = 0.243$) exposure groups (Table 1). Additionally, the form of exposure to salinity (continuous or repeated) did not influence survival (2-ANOVA, $P = 0.255$).

Overall, there was no significant difference in developmental time between larvae in the continuous and repeated exposure groups (2-ANOVA, $P = 0.690$). However, the pattern of response to salinity was different between the two exposure groups (2-ANOVA, $P < 0.01$). Although continuous exposure

Table 1 Percentage survival (mean \pm SD) to stage II in stage I *Jasus edwardsii* larvae reared either under continuous or repeated exposure to different salinities.

Salinity (psu)	Exposure	
	Continuous	Repeated
28	78.00 ± 10.95	68.00 ± 21.68
31	56.00 ± 26.08	80.00 ± 7.07
34	74.00 ± 15.17	–
37	70.00 ± 7.07	76.00 ± 5.48

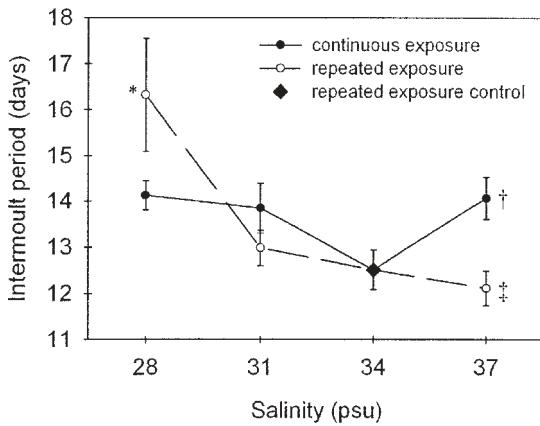


Fig. 1 Mean (\pm SE) duration of the intermolt period of stage I *Jasus edwardsii* larvae exposed continuously or repeatedly to different salinities. Treatments with different symbols differed significantly (t test, $P < 0.05$). (*, significantly different from control (Dunnett's, $P < 0.05$).

to salinities ranging from 28 to 37 psu had no significant effect on the duration of stage I (ANOVA, $P = 0.067$), repeated exposure within the same range of salinities significantly affected development (ANOVA, $P < 0.01$). Indeed, larval development was significantly slower in the 28 psu repeated exposure group than in the 34 psu continuous regime control (Dunnett's, $P < 0.05$; Fig. 1). The difference in response pattern between continuous and repeated exposure groups was particularly marked at 37 psu (t test, $P < 0.05$).

Salinity affected body length growth in both continuous exposure (ANOVA, $P < 0.01$) and repeated exposure (ANOVA, $P < 0.05$) groups (Fig. 2). Overall, the form of exposure to salinity (repeated and continuous) had no significant effect on the body growth of larvae throughout stage I (2-ANOVA, $P = 0.143$). However, the pattern of body growth response to salinity was significantly different between the two groups (2-ANOVA, $P < 0.05$). The continuous exposure to 28, 31, and 37 psu during stage I resulted in significantly reduced post-moult size compared with larvae cultured at 34 psu (Tukey-HSD, $P < 0.05$). In contrast, in the repeated exposure group, only larvae reared at 28 psu moulted to a significantly smaller size (Dunnett's, $P < 0.05$) than larvae in the control group (34 psu continuous exposure). The difference in growth response between repeated and continuous exposure groups was particularly marked at 37 psu (t test, $P < 0.05$; Fig. 2).

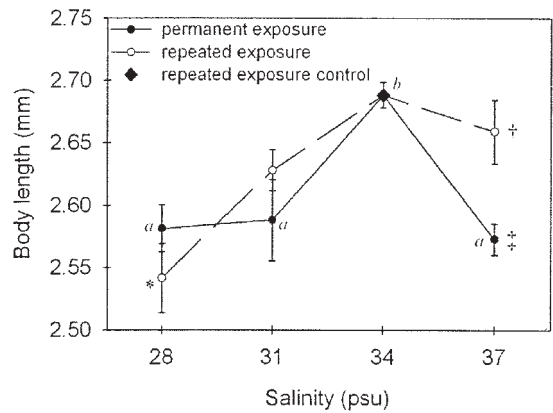


Fig. 2 Mean (\pm SE) body length at stage II in *Jasus edwardsii* larvae exposed continuously or repeatedly to different salinities during stage I. Treatments with different letters differed significantly (Tukey-HSD, $P < 0.05$). Treatments with different symbols differed significantly (t test, $P < 0.05$). (*, significantly different from control (Dunnett's, $P < 0.05$).

Routine metabolic rate in acclimated and non-acclimated larvae

A two-way ANOVA excluding the 34 psu acclimated group indicated a significant effect of salinity on the oxygen consumption of stage I larvae ($P < 0.05$; Fig. 3). Additionally, respiratory rates were higher overall in non-acclimated larvae than in acclimated animals (2-ANOVA, $P < 0.01$). Although the influence of salinity on VO_2 was significant in both acclimated (ANOVA, $P < 0.05$) and non-acclimated larvae (ANOVA, $P < 0.05$), the pattern of response to salinity was different between the two groups (2-ANOVA, $P < 0.01$). In acclimated larvae, oxygen consumption was significantly lower at 28 psu than at 31 and 34 psu, and uniform between 31 and 37 psu (Tukey-HSD, $P < 0.05$), whereas in non-acclimated larvae, VO_2 steadily declined from 28 to 37 psu (linear regression, $P < 0.01$). At 28 psu, this difference in response between the two groups was highlighted by a significantly lower VO_2 in non-acclimated phyllosomas than in acclimated larvae (t test, $P < 0.0001$).

DISCUSSION

During larval rearing, the survival of stage I *J. edwardsii* larvae was uniformly high over the range of salinities tested (i.e., 28–37 psu), whether in a continuous or repeated exposure environment.

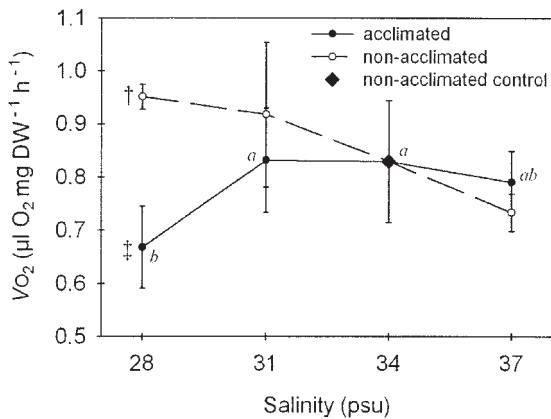


Fig. 3 Mean (\pm SD) oxygen consumption by stage I *Jasus edwardsii* larvae following a sudden change in salinity from the control condition (34 psu) and at different acclimation salinities. Treatments with different letters differed significantly (Tukey-HSD, $P < 0.05$). Treatments with different symbols differed significantly (t test, $P < 0.05$).

Larvae were exposed to sub and supranormal salinities for a maximum of 9 days, which is only a short period within a larval cycle that may last between 212 and 416 days in hatchery conditions (Kittaka 1994; Booth 1996). Therefore, the uniform survival across treatments observed in the present work does not preclude mortality under longer-term exposure to suboptimal salinities, as with *P. borealis* larvae (Wienberg 1982), *Metapenaeus monoceros* larvae (Kumlu et al. 2001), and *Farfantepenaeus californiensis* juveniles (Villarreal et al. 2003).

Continuous exposure to suboptimal salinities reduced post-moult larval size at 28, 31, and 37 psu. Similarly, slight shifts from optimal salinity (≤ 5 psu) were reported to impair growth in larval marine crustaceans such as *P. borealis* (Wienberg 1982) and in *M. monoceros* (Kumlu et al. 2001). Interestingly, the repeated exposure of *J. edwardsii* larvae to subnormal concentrations during stage I did not suppress development at 31 and 37 psu. However, larvae repeatedly subjected to wider shifts in salinity of larger amplitude (i.e., between 28 and 34 psu) were delayed in their development and moulted to a smaller size than larvae in the control group. This suggests that *J. edwardsii* larvae are not able to adapt to long-term changes in salinity but that they tolerate repeated shifts in salinities of 3 psu of magnitude, whereas exposure to shifts of larger magnitude (i.e., 6 psu) would affect their development. Additionally, growth data indicated that stage I *J. edwardsii* larvae

have a greater tolerance for short-term shifts in salinity than for continuous acclimation within the 31–37 psu range. However, this pattern did not hold true for larvae reared at 28 psu. In fact, at 28 psu, development was faster and larvae tended to be larger under continuous exposure than in a fluctuating salinity environment. Frequent shifts in salinity between 28 and 34 psu may have repeatedly altered the concentration of moulting hormones in the body fluid as was reported in fish (Woo et al. 1997) and interfered with the ecdysial processes of phyllosomas. Additionally, larvae repeatedly exposed to 28 psu may have faced significant energetic losses in the repeated accumulation and loss of endogenous proteins, known to occur in the regulation of cell volume in invertebrates during hyper- and hyposaline adjustments (Pierce 1971; Hawkins & Hilbish 1992; McAllen 2003).

The oxygen consumption response to declining salinity (i.e., a steady decline of VO_2 between 28 and 37 psu) observed in non-acclimated *J. edwardsii* larvae was similar to the increase in oxygen consumption at lower salinities reported in adult *Neomysis intermedia* (Simmons & Knight 1975) and adult *Trigriopus beruicornis* (McAllen & Taylor 2001), and to the salinity stress observed in *Cancer magister* megalopas (Brown & Terwilliger 1999). In contrast, the oxygen consumption of salinity acclimated *J. edwardsii* larvae increased above 28 psu before reaching a steady level between 31 and 37 psu. A decline in oxygen consumption at low salinity was also reported in acclimated *F. californiensis* juveniles (Villarreal et al. 2003). According to Kinne (1971), reduced oxygen consumption at subnormal acclimation salinities is characteristic of stenohaline organisms, which suffer from osmotic damage whenever the salinity deviates significantly from normal (i.e., from 34 to 28 psu in *J. edwardsii* larvae).

Previous investigations attempted to relate the metabolic rate of aquatic organisms with their growth performances under different salinities (Anger et al. 1998; Pechenik et al. 2000; Villarreal et al. 2003). In the present study, respiratory data in non-acclimated larvae indicated increased energy loss through metabolism under hyposmotic stress (i.e., 28 psu). This may explain the reduced growth recorded in the larvae repeatedly exposed to 28 psu. However, the respirometry results obtained for stage I larvae acclimated at different salinities did not provide convincing physiological evidence for the marked effect of suboptimal salinities on larval development in the continuous exposure group. For

instance, groups of larvae acclimated to 31 and 37 psu had similar oxygen consumption to larvae in the control group (34 psu), which contrast with the reduced growth observed at 31 and 37 psu under continuous exposure. Pechenik et al. (2000) also reported a poor relationship between growth rate and energy expenditure in the euryhaline polychaete (*Capitella* sp. I). Working on *Carcinus maenas* larvae, Anger et al. (1998) concluded that from measurements of oxygen consumption and food assimilation, only the decline in assimilation could provide a sensible explanation for the decrease in larval growth observed under reduced salinities. However, this cannot be confirmed for the present study because food assimilation was not determined.

As for most marine invertebrates, *J. edwardsii* larvae are stenohaline with a weak tolerance for hypo- and hypersaline conditions between 31 and 37 psu. Although we should stress that these findings apply to stage I only, the oceanic habitat of the more advanced larval stages suggest that they would also be stenohaline. Although the survival of stage I larvae was not affected by salinities ranging from 28 to 37 psu, their long-term tolerance may be diminished from the constant exposure to waters shifting only slightly from normal salinity (i.e., c. 34–35 psu) as larval growth was reduced at 31 and 37 psu. However, repeated shifts from normal salinity within the range of 31–37 psu did not appear to affect development during stage I. From these results, we therefore recommend the monitoring and control of ambient salinity during the larval rearing of *J. edwardsii* to avoid prolonged exposure to suboptimal salinities. Measurement of oxygen consumption alone was not sufficient to explain the effects of suboptimal salinities on larval growth. Therefore, a next step in understanding the long-term effects of changes in salinity in phyllosoma should be an integrated physiological approach including the studies of body fluid osmosis, respiration, excretion, feeding, and behaviour. Such detailed investigation would possibly provide the necessary data to assess the effects of constant and fluctuating salinities on a scale beyond the scope of the present study.

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