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TEMPERATURE AND SALINITY TOLERANCES OF THE LARVAE OF THE NORTHERN PACIFIC SEASTAR, *ASTERIAS AMURENSIS*

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SUMMARY

The salinity and temperature tolerances of larvae of the introduced northern Pacific seastar, *Asterias amurensis*, were investigated under laboratory conditions in order to determine the relative risks of successful introduction of the species to broad areas of mainland Australia. The primary emphasis of the work was targeted towards ballast water mediated introductions as this is considered to be the most likely vector for initial introduction of the species to southern Tasmania and the most likely means of spread from that area to mainland Australia.

The optimal temperature range for *A. amurensis* larvae from the current Tasmanian population is <8.0–16.5°C. Larvae are most likely able to survive and develop normally in temperatures up to 20°C. However, the corresponding decrease in rate of development and hence increase in larval duration may reduce larval survival within the 16.5–20°C range. Larvae appear unable to develop normally above 20°C and this temperature may thus limit successful colonisation via larval inoculation.

Development of early stage larvae at 32‰ suggests estuarine conditions may be optimal for larval survival. However, growth of bipinnaria was slightly retarded at 32‰ relative to 35‰ suggesting some level of stage dependent tolerance. The interaction between salinity and temperature effects suggests that tolerance to temperatures above 16.5°C is reduced under conditions of both high (≥ 35‰) and low salinity (≤ 28‰).

Short term exposure to salinities ≤ 8.75‰ were effective in destroying *A. amurensis* bipinnaria.

Given these parameters, the likelihood of successful colonisation by *A. amurensis* larvae in broad regions of southern Australia based on salinity and temperature tolerances can be classified as follows:

High Risk:	All Tasmanian, Victorian and South Australian ports including Albany and east coast ports as far north as Eden.
Medium Risk:	East coast Ports between Eden and Newcastle; west coast ports between Albany and Fremantle.
Low risk:	East coast ports north of Newcastle and west coast ports north of Fremantle.

It is important to note that although tolerances of larval stages may play a significant role in recruitment success and thus establishment and persistence of populations, a complete analysis of the risks of further successful introductions cannot proceed without considering a range of other parameters. These include: survival rates of larvae within ballast tanks, settlement requirements (including cues and specificity of substrates), factors influencing

post-settlement mortality (including the susceptibility of the host environment to invasion), spawning cues and the environmental tolerances of other life history stages.

It is also noteworthy that many of these issues are not specific to *A. amurensis* but are relevant to marine introductions in general and thus multispecies approach to resolving some of these issues may be more appropriate.

1. INTRODUCTION

Asterias amurensis, is a boreal seastar, inhabiting cold-temperate, sub-littoral and shallow waters of the north and north-east areas of the Pacific Ocean. It is native to the coasts of Japan, Korea and eastern Russia (D'Yakanov 1968, Onguru & Okutani 1991) and although also found in Alaska and Canada, it is uncertain whether these latter populations are native or introduced (Mcloughlin & Bax 1993, Fukuyama 1994).

In its native environment, *A. amurensis* is the dominant inshore predator (McLoughlin & Bax 1993) and, in Japan, it sometimes undergoes massive, sporadic, population outbreaks which cause significant damage to both natural and cultured shellfish beds (Nojima *et al.* 1986).

A. amurensis was first recorded from the Derwent River estuary (southern Tasmania) in 1986 and since then has proliferated to become a dominant member of the benthic community (Buttermore *et al.* 1994). The most likely vector for its introduction to southern Tasmania is considered to be via eggs or larval stages in ballast water (Davenport & McLoughlin 1993).

The discovery of *A. amurensis* in southern Tasmania has raised considerable concern amongst biologists, fisheries managers and the fishing industry regarding the potentially serious impact such a species may have on native ecosystems and the aquaculture industry. Its presence has also raised concerns regarding the potential for the species to spread throughout remaining Tasmanian waters and northwards to mainland Australia.

Dispersal of *A. amurensis* northwards of the current, primarily SE Tasmanian distribution is most likely to be via its pelagic larval stage. The protracted nature of this stage (60–90 days under local conditions; Bruce *et al.* 1995, CSIRO unpublished data) suggests that *A. amurensis* larvae may have the potential for extensive dispersal. However, the most rapid and unpredictable method by which *A. amurensis* is likely to spread in southern Australia is via translocation of larvae in ballast water. Abundances of *A. amurensis* larvae in excess of $1500/m^3$ have been recorded in the vicinity of port facilities within the Derwent River (CSIRO unpublished data). Vessels taking on ballast during periods of high larval abundance in the Derwent may thus operate as potential vectors for further larval dispersal.

The uptake of *A. amurensis* larvae from Hobart and subsequent discharge into other Australian (or international) ports, however, does not necessarily mean that *A. amurensis* larvae will survive, settle and establish populations in these localities. Successful colonisation depends on survival of larvae within ballast tanks, settlement requirements, factors influencing post settlement mortality (including the susceptibility of the host environment to invasion), and the environmental tolerances of both larvae and other life history stages.

This report describes the temperature and salinity tolerances of *A. amurensis* larvae. The primary objective was to identify broad regions within mainland Australia that are potentially at risk of successful introduction via larval stages.

2. TEMPERATURE AND SALINITY EXPERIMENTS

2.1 SCOPE AND RATIONALE

Three experiments were conducted to assess the temperature and salinity tolerance of *A. amurensis* larvae: effects of temperature and salinity on early development; effects of temperature and salinity on bipinnaria and effects of short term exposure of larvae to low salinities.

The specific rationale was to focus on the survival of larvae after inoculation into a new host environment. Clearly this process may occur either naturally, by way of ocean currents, or by anthropogenic means (e.g., in ballast water). We considered ballast water mediated transport (where source water is dumped directly to the host environment) to be the most likely transport scenario and thus our experiments, by not providing an acclimatisation period for adults or larvae prior to treatment, reflect this. Similarly, we chose to conduct experiments on the early developmental stages (eggs, gastrula and bipinnaria) as these are the most commonly occurring larval stages within source ports and thus the most likely to be transported.

Due to interest in the ability of larvae to survive in areas north of their current Australian distribution, the selection of treatment temperatures reflect ranges for the Australian mainland and do not cover the lower temperature tolerance of the species. Salinity ranges tested similarly cover those commonly found in Australian ports.

2.2. EXPERIMENT 1: EFFECTS OF TEMPERATURE AND SALINITY ON EARLY DEVELOPMENT

2.2.1 METHODS

Mature *A. amurensis* were collected in early June (6/6/95) from the Derwent River. Ovaries were removed from 10 female in the laboratory and each bathed in a seawater solution of $1x10^{-5}$ M 1-methyladenine for 90 minutes. Eggs released were graded as normal and ready to fertilise if they were spherical and had an indistinct nucleor region. Abnormal eggs and those not yet ready to be fertilised were either non spherical and/or had a distinct nucleor region. Two females with the highest percentage of normal eggs (70%) were used for the experiment. Eggs from both females were mixed and washed repeatedly by sieving through a 60 µm filter and then replaced into fresh seawater in a 2 litre beaker. A sperm solution was obtained from a small piece of mature, excised testes diluted in seawater. Eggs were fertilised by adding drops of sperm solution into the beaker until 90% fertilisation was achieved. Eggs were then washed and distributed among twenty-seven beakers each containing 250 ml of filtered seawater (35‰) at a concentration of 50 eggs per ml (stock solutions).

The experimental design was orthogonal with larvae exposed to three salinities (28‰, 32‰ and 35‰) at three temperatures (15°C, 19°C and 24°C) with three replicates per treatment. Salinities were obtained by diluting a known salinity (35‰) with distilled water. Two litres of each treatment salinity were placed in three controlled temperature cabinets the day before the experiment to allow the water to reach treatment temperatures.

Fertilised eggs from the stock solutions were transferred by washing through a 60 µm filter and were then placed into 250 ml of treatment water. Each beaker was covered with aluminium foil to prevent evaporation and placed into the controlled temperature cabinets. Eggs were kept in motion by placing beakers on suspended shaking platforms.

A sub-sample was taken from each beaker every 4 hours for the first 24 hours then twice daily for the next 4 days. Each sub-sample was preserved in 10% Steadmans solution and scored for percentage abnormality after the experiment was completed.

Data analyses

Linear regression of percent abnormality vs time (96 hours) was performed on each beaker. Due to heterogeneity of variances, an arcsin transformation was performed on percent abnormality data and regressed against log time. The 27 slopes (corresponding to each beaker) were compared using a factorial ANOVA. The variance estimates for the slopes were similar to the variance of the observations obtained from the ANOVA ensuring that the estimation and analysis of the slopes was reasonable. A Fisher's PLSD post hoc test was used to identify significant differences between the treatments at $P \leq 0.05$.

The 24°C treatments were not further assessed because by the end of the experiment all larvae were abnormal (see results). Treatments at 15°C and 19°C were similar in the levels of abnormality recorded and further analyses were conducted to assess differences. The total length (TL) (see Bruce *et al.* 1995 for details) of remaining normal larvae was measured under a compound microscope using NIH Image (Version 1.25). An orthogonal factorial ANOVA was used with factors temperature and salinity at two (15°C and 19°C) and three (28‰, 32‰ and 35‰) levels respectively. A Fisher's PLSD post hoc test was used to identify significant differences between treatments.

2.2.2. RESULTS

Percentage abnormality increased over the first day (24.5 h) then levelled off or increased more slowly in all treatments. In general, both the level of abnormality and the initial rate at which it progressed (over the first 24 h), increased with temperature. The level of abnormality tended to be lower at 32‰ relative to 28‰ and 35‰, except at 24°C where all three salinities eventually reached 100% abnormality. The 15°C/28‰ and 15°C/35‰ treatments levelled off between 35–50% abnormality at 69 h, whereas 15°C/32‰ levelled

off earlier (42.5 h) and at a lower level (15–35%) (Figure 1a–c). Note also that there was a tendency in some treatments for percent abnormality to decrease and then to increase again (Figure 1a, d, e and g). We believe that this was due to the death of abnormal larvae and thus "loss" from the experiment rather than recovery by larvae after initial abnormal development. Because the percentage abnormality subsequently increased, this phenomenon did not effect the regression analysis and no attempt was made to include it in our model.

In general, the 19°C treatments reached a higher level of abnormality than the 15°C treatments and tended to increase slowly after the first day rather than level off. Percent abnormality in the 19°C/35‰ treatment levelled off after 24.5 h before increasing again after 70 h and finally reaching 50–70% by the end of the experiment. Both the 19°C/28‰ and 19°C/32‰ decreased slightly after 24.5 h before increasing again after 42.5 h; the 19°C/28‰ treatment reaching a slightly higher level of abnormality (40–50% and 35–45% respectively; Figure 1d–f).

All larvae within the 24°C treatments reached 100% abnormality, the 24°C/28‰ and 24°C/32‰ by the end of the experiment and the 24°C/35‰ at 18.5 h (Figure 1g-i). There was a drop in percent abnormality in the 24°C/28‰ treatment after 24.5 h followed by a rapid rise after 69 h. Percentage abnormality was more stable for larvae at 24°C/32‰ after 18.5 h followed by a similar rapid rise after 69 h.

Comparing rate of abnormality

There was a significant difference in rate of abnormality between treatments (Figure 2). The effect of both temperature and salinity were significant (F^2_{18}=254.27, p<.0001 and F^2_{18} F^2_{18}=16.81, p<0.0001 respectively). However, there was also a significant interaction (F^4_{18} 15.06, p<0.001) where the effect of salinity became more prominent with increasing temperature. At 15°C there was no salinity effect, at 19°C there was an increase in rate of abnormality at 28‰ and 35‰ and at 24°C rate of abnormality increased with salinity (Figure 2).

Length comparison between 15°C & 19°C treatments

Total length of larvae varied between the treatments and there was a significant temperature and temperature/salinity interaction (F^1_{84}=11.19, p=.0012 and F^2_{84}=3.6, p=.03 respectively) but no salinity effect (F^2_{84}=0.48, p=0.62). Larvae reared at 15°C were significantly larger than larvae reared at 19°C but only at salinities 28‰ and 35‰. There was no difference in total length between larvae reared at 15°C or 19°C in the 32‰ treatment. Within temperature treatments, larvae reared at 19°C/32‰ were larger than those reared at 19°C/28‰ or 19°C/35‰ and larvae reared at 15°C/32‰ were smaller than at 15°C/28‰ or 15°C/35‰. However, these differences were not significant (Figure 3).

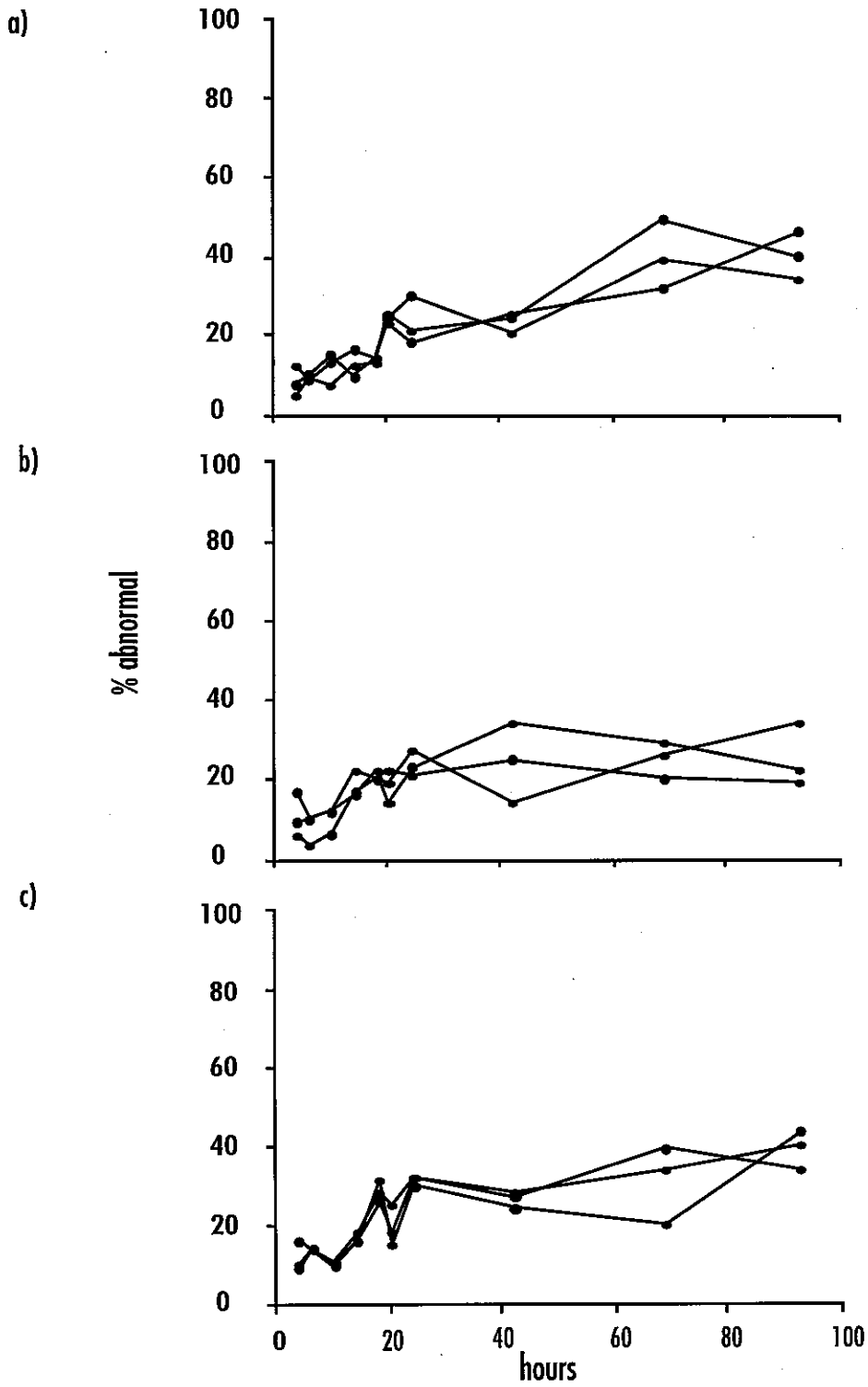

Figure 1a, b, c. Change in percent abnormality of *A. amurensis* larvae over time for the nine treatments. Lines indicate data from individual replicate beakers for each treatment: a) 15°C/28‰, b) 15°C/32‰, c) 15°C/35‰.

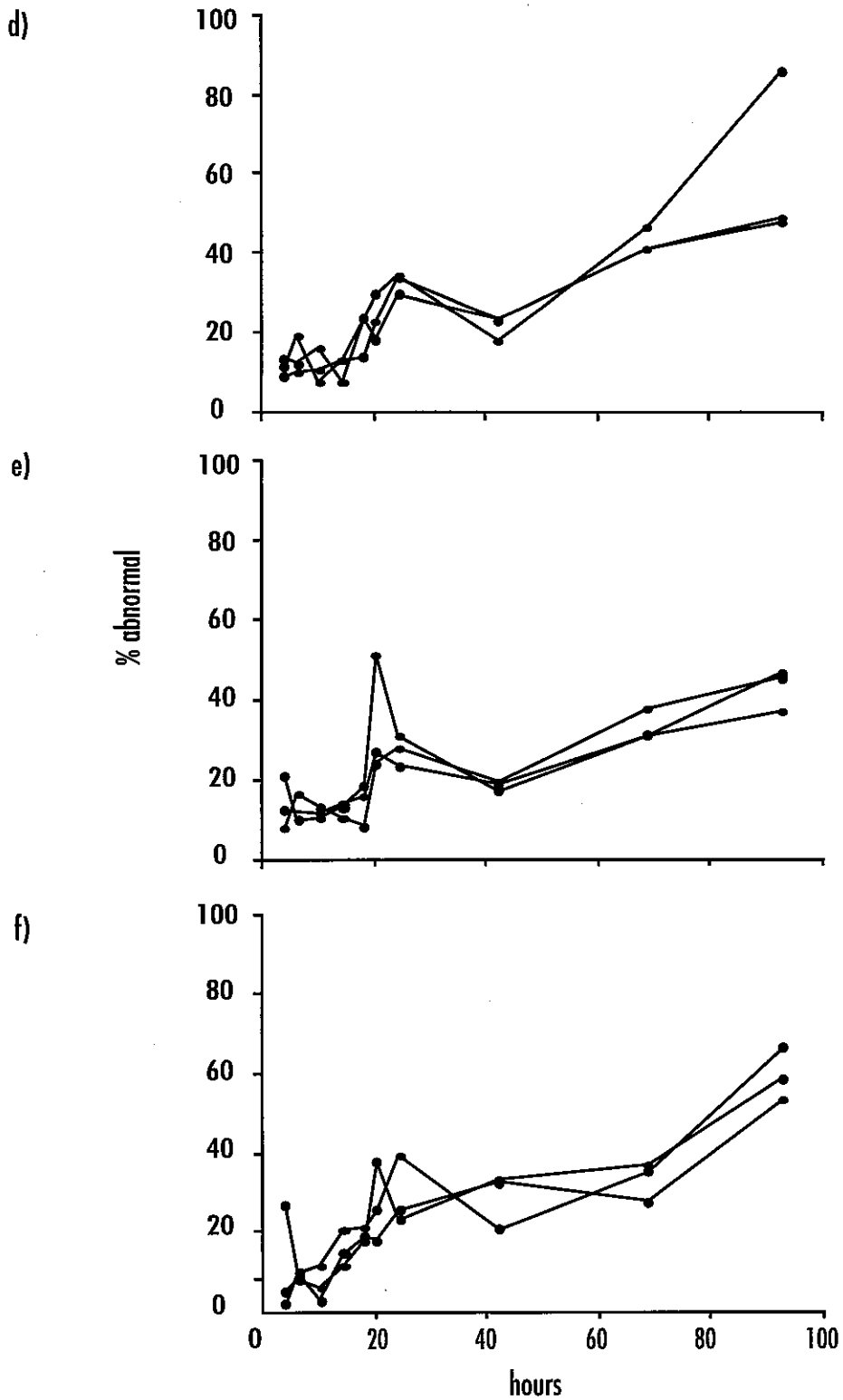

Figure 1 d, e, f. See figure caption page 5: d) 19°C/28‰, e) 19°C/32‰, f) 19°C/35°C

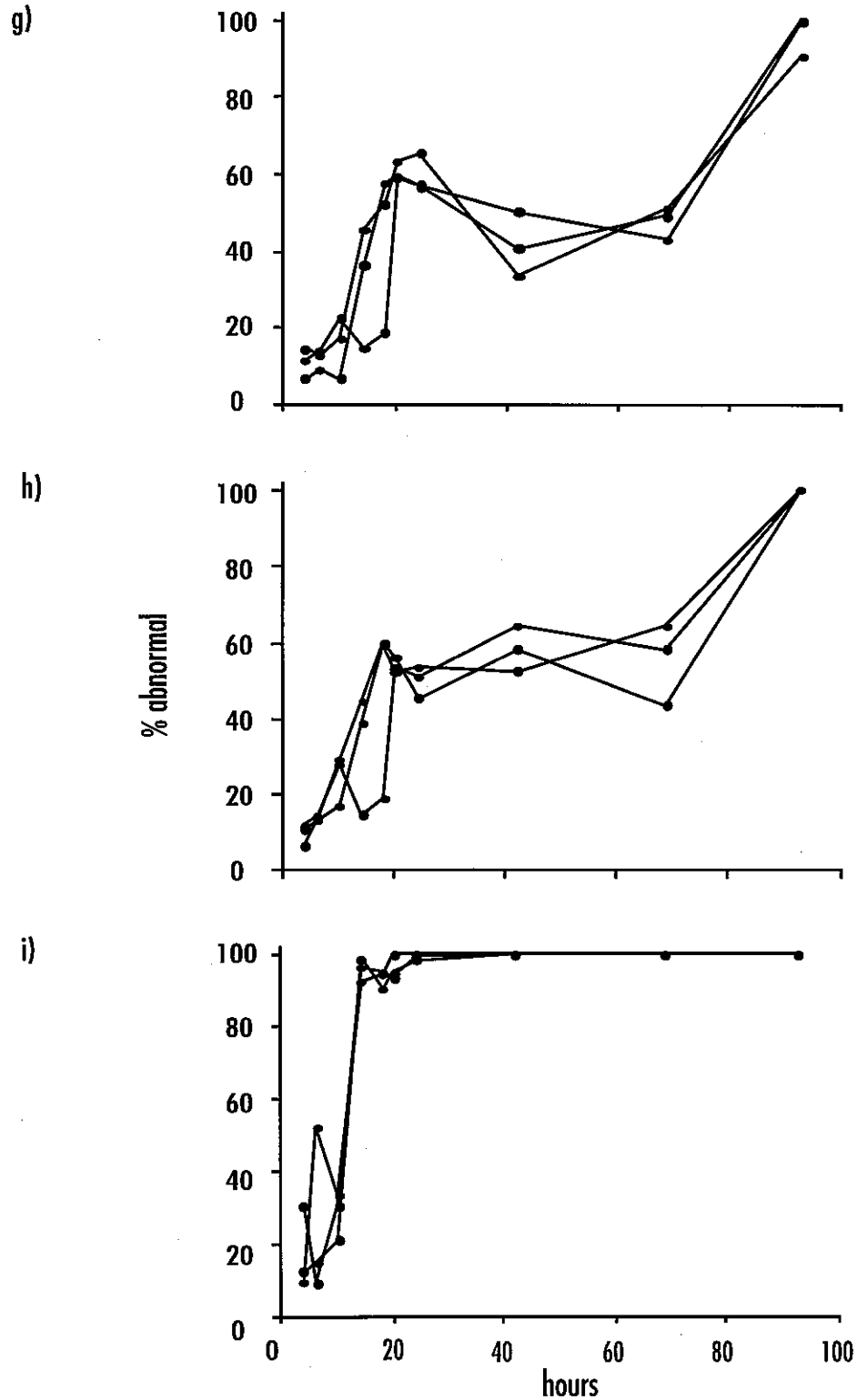

Figure 1 g, h, i. See figure caption page 5: g) 24°C/28‰, h) 24°C/32‰, i) 24°C/35‰

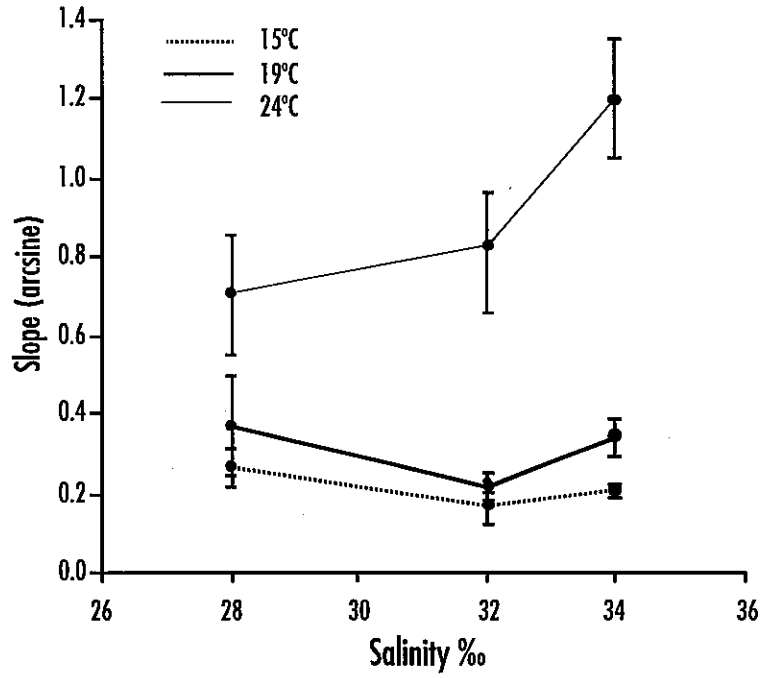

Figure 2. Larval mortality rate expressed as slope (arcsine $\sqrt{p}$) for different treatment temperatures and salinities. Vertical bars indicate standard errors.

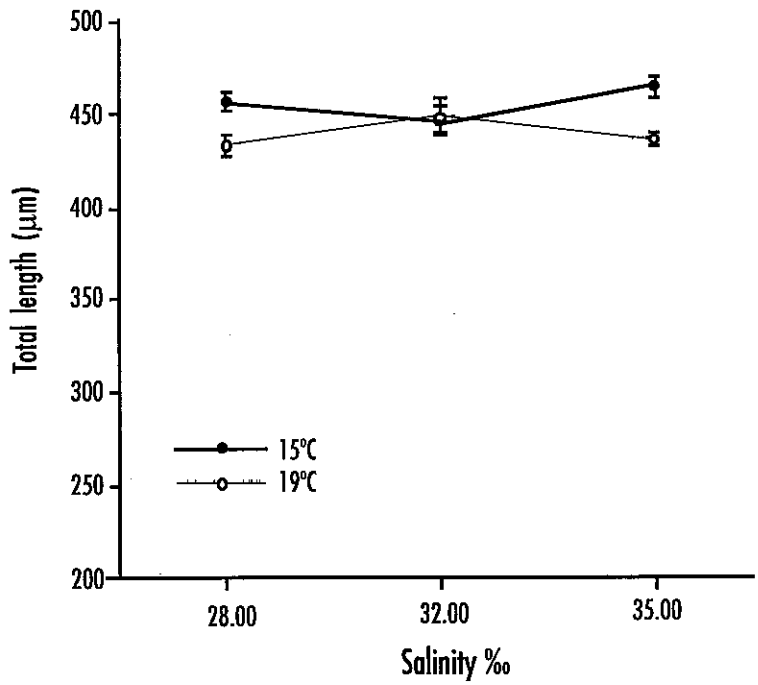

Figure 3. Comparison of the effect of various tempertaure/salinity combinations on the total length (TL) of *A. amurensis* bipinnaria. Vertical bars indicate standard errors.

2.3. EXPERIMENT 2: EFFECT OF TEMPERATURE AND SALINITY ON BIPINNARIA

2.3.1. METHODS

Mature *A. amurensis* were collected in late July (25/7/95). Fertilised eggs were obtained by the procedures mentioned above, transferred to 500 ml beakers and reared in a constant temperature growth cabinet at 15°C for a period of one week prior to the experiment. Larvae were fed *Dunaliella tertiolecta* at a concentration of 20,000 cells per ml when four days old.

The experiment design was orthogonal, with twelve temperatures (8°C, 10°C, 12°C, 14.5°C, 16.5°C, 18.5°C, 20°C, 22°C, 24°C, 26°C, 28°C and 30°C) and two salinities (35‰ and 32‰) with three replicates for each treatment. A temperature gradient block was used to generate treatment temperatures. The unit consisted of a solid piece of aluminium within which 72 holes (12 rows of 6) had been drilled each accommodating a 60 ml treatment vial. Water channels at either end of the block were connected to a heating and cooling unit respectively, resulting in a separate temperature for each row.

Salinities were prepared one day prior to the experiment and allowed to equilibrate within the temperature block. One hundred, one week old bipinnaria were transferred to each 60 ml transparent treatment container and placed in the temperature gradient block. Treatment containers were fitted with a screw top lid to minimise evaporation. After seven days, the number of surviving larvae were counted and examined in each container and then preserved in 10% Steadmans solution.

Data analysis

Morphometric measurements (total length (TL), anterior dorsal process (AD) and mid dorsal curve (MDC) (see Bruce *et al.* 1995 for details) were made on 10 larvae fixed prior to the experiment (defined herein as "baseline larvae") and from all treatments after the experiment using NIH Image (Version 1.25). The ratio AD/MDC was used to describe the complexity of the ciliated band for each larva measured (Bruce *et al.* 1995). The data were analysed in two ways. First, all the treatments were compared to each other and then all the treatments were compared to the baseline larvae.

Between treatment comparison

Mean values of five larvae from each container (to avoid pseudo-replication) were used to compare treatment effects. Comparisons of percent survival, TL and AD/MDC were made with a factorial ANOVA (Statview 4.02). A Fisher's PLSD post hoc test was used to identify significant differences between treatments at $P \leq 0.05$. A Bonferroni adjustment was performed on the p values to reduce the possibility of a Type II error, therefore significance at 5% was accepted if $p < 0.001$ (0.05/45 tests).

Comparison of treatments to baseline

The ten baseline larvae were measured and compared to the means of the larvae from each treatment container using multiple two-sample t-tests, assuming unequal variances. No salinity effect on morphology was detected (F^1_{40}= 9.5x10^{-3}, P=0.92, F^1_{40}=,542, p=0.4658, TL and AD/MDC respectively) so data from both treatment salinities were combined to produce six replicate beakers for each treatment temperature. A Bonferroni adjustment was performed on the p-values (5% was accepted if p<.005, 0.05/10 tests) to avoid TYPE II errors resulting from multiple t-tests.

2.3.2. RESULTS

Survival

Mortality of bipinnaria was unaffected by treatment temperatures below 26°C (F^9_{40}=0.73, p=0.67). There was 100% mortality at the two highest temperatures (28°C and 30°C) (Figure 4). There was also a significant salinity effect on survival (F^1_{40}=5.7, p=0.02) (Figure 5) but this was not apparent in morphology. Percentage survival at 35‰ was slightly higher than at 32‰ (88%±6, 83%±10 respectively).

Despite there being no significant temperature effect on survival, the condition of bipinnaria varied with temperature. Larvae exposed to temperatures higher than 14.0°C regressed in both size and development. In temperatures above 20°C the ciliated band lost complexity and the mid dorsal curve (MDC) disappeared (Figure 6).

Morphometric Analysis

Total length:

The length of bipinnaria was significantly different between treatment temperatures (F^9_{40}= 33.88, P<.001). Total length decreased with increasing temperature (Figure 7). However, length was not significantly different within the following ranges: 8-16.5°C, 18.5-22°C and 24-26°C. The average total length of larvae from 8–16.5 °C treatments was not significantly different to baseline larvae. Larvae from treatment temperatures above 16.5°C were significantly smaller than baseline larvae (Figure 7).

AD/MDC

The AD/MDC ratio was significantly affected by treatment temperature (F^9_{40}=40.12, p<.0001). The ratio decreased with increased temperature (Figure 8). Larvae within the treatment ranges of 8–14.5°C, 16.5–22.0°C and 24–26°C ranked together. However, these three groups were not as clearly defined as those for total length. The AD/MDC ratio for bipinnaria in treatment temperatures below 14.5°C was not significantly different from baseline bipinnaria.

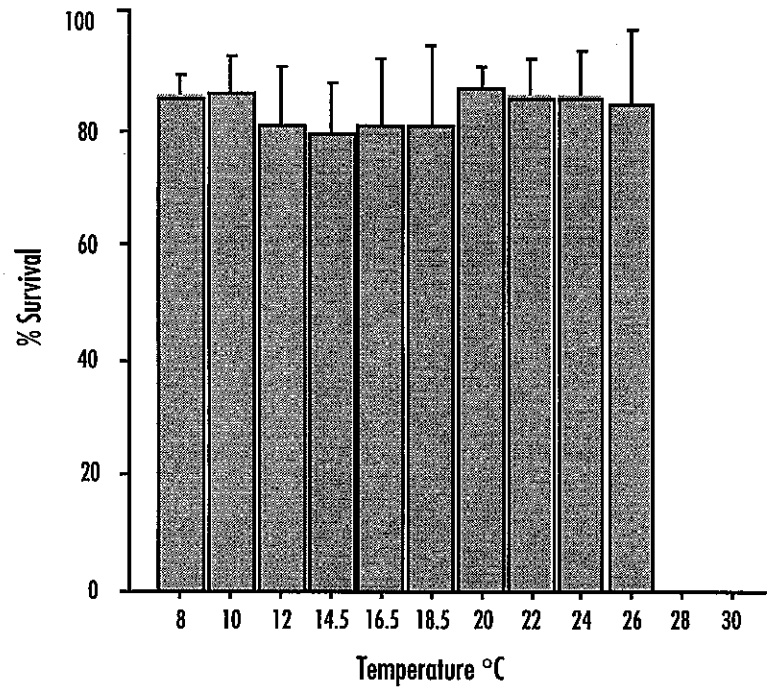

Figure 4. Percent survival of *A. amurensis* bipinnaria following exposure to different tempertures for one week.

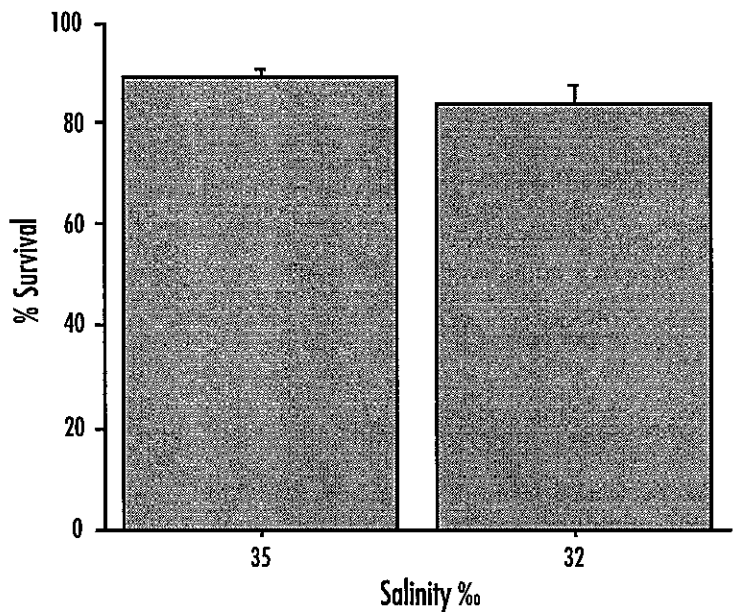

Figure 5. Percent survival of *A. amurensis* bipinnaria following exposure to salinities of 32‰ and 35‰ for one week.

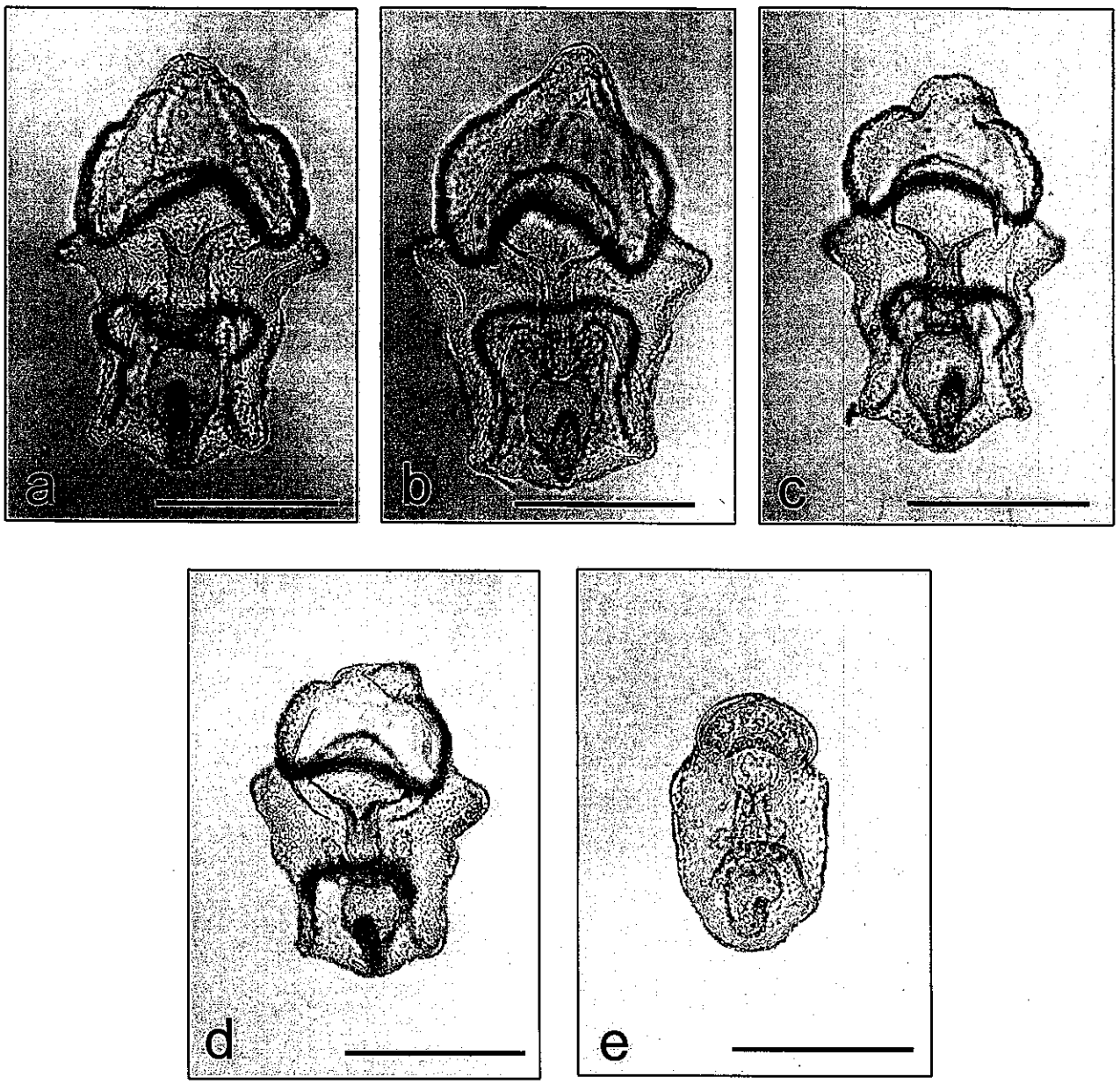

Figure 6. Photomicrographs of *A. amurensis* bipinnaria showing the effects on the physical appearence of larvae that had been held for one week at different temperatures: a) baseline, b) 8°C, c) 14.5°C, d) 20.5°C and e) 26°C. Scale bar = 200 µm.

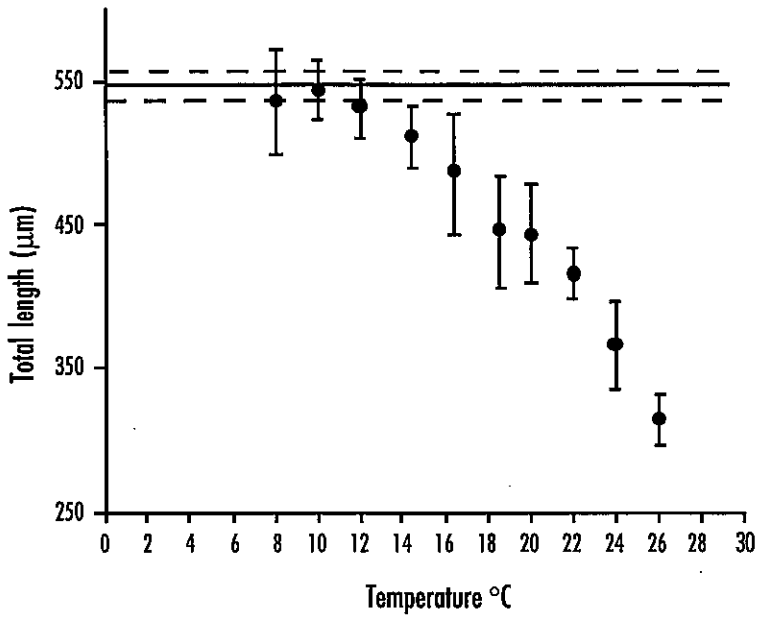

Figure 7. Mean values for total length of *A. amurensis* larvae held at different treatment temperatures; vertical bars indicate standard errors. Mean values for "baseline" bipinnaria shown by solid line; standard error shown by dotted lines.

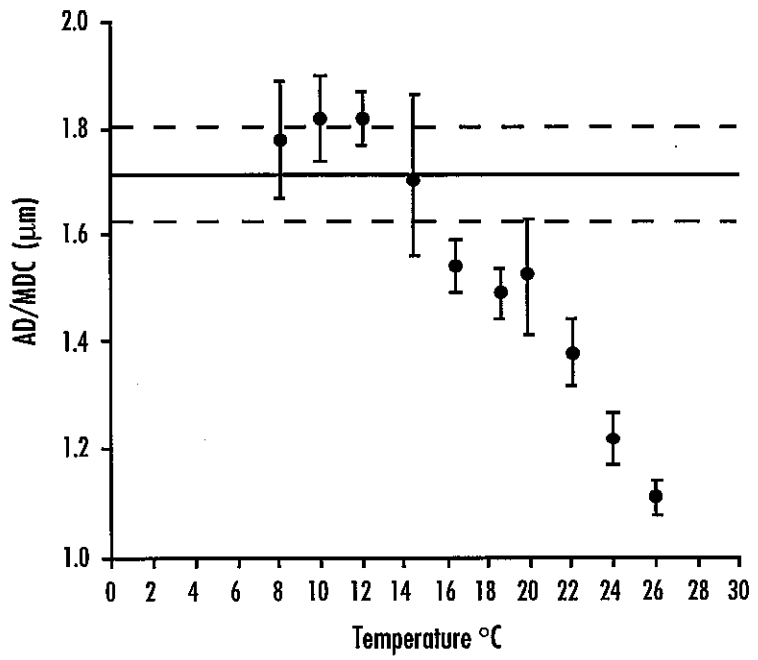

Figure 8. Mean values for AD/MDC of *A. amurensis* larvae held at different treatment temperatures; vertical bars indicate standard errors. Mean values for "baseline" bipinnaria shown by solid line; standard error shown by dotted lines.

2.4. EXPERIMENT 3: EFFECTS OF SHORT TERM EXPOSURE TO LOW SALINITIES ON BIPINNARIA

2.4.1. METHODS

Five treatment salinities were prepared prior to the experiment: 35‰ (control), 26.2‰, 17.5‰, 8.75‰ and freshwater. Treatments were then randomly labelled A–E.

Ten, 10 d old bipinnaria were transferred to a small petrie dishes of treatment and control water and observed under both dissection and compound microscopes. The observer was unaware of the treatment salinity and dictated observations. Larvae were observed for 10 min then transferred back into control water and observed again for a further 5 min. The same procedure was repeated for each treatment and replicated twice.

3.4.2. RESULTS

Bipinnaria were significantly affected by all treatments except for the control salinity (35‰) (Table 1). The majority of bipinnaria (90%) exposed to 26.2‰ resumed normal swimming after transfer back to control water. In the 17.7‰ treatment, transfer back to control water resulted in considerable cellular damage and loss of body wall integrity in 40% of larvae whilst 60% resumed very slow swimming. However 50% of those that resumed swimming retained morphological irregularities after 5 min.

All bipinnaria exposed to both 8.75‰ and freshwater suffered extensive cell lysing and body wall rupture, None survived transfer back to the control water.

3. DISCUSSION

3.1. EFFECTS OF TEMPERATURE AND SALINITY ON EARLY DEVELOPMENT

Low tolerance to temperature salinity stress during gastrulation has been recorded for a variety of taxa (Baldwin 1948, Blaxter 1969, Holliday 1969, Riley 1974). Our data similarly suggests that gastrulation is also a critical period in the development of *A. amurensis*. The initial peak in abnormality levels at 20–24 h after fertilisation (in all treatments), corresponded to the early-mid gastrula stage. The reason why this stage may be more susceptible is unclear. Gastrulation represents the initial change from a mono-layered to multi-layered embryo and involves considerable cellular differentiation and migration (Kuraishi & Osanai 1992). Interruption of these processes induced by temperature or salinity stress may well be a source of mortality. We observed several abnormalities during gastrulation including bent archenterons, excessive development of mesenchyme cells and deformed body profiles.

The rate of abnormality in developing eggs and early stage *A. amurensis* larvae increased with temperature in all salinity treatments. Eggs and early stage larvae reached 100% abnormality in all 24°C treatments indicating this temperature exceeds the thermal tolerance of the species. Overall abnormality rates stabilised at 15°C after 40–80 h and thus we conclude that a considerable

Table 1. Summary of observations on the appearance and behaviour of *A. amurensis* bipinnaria subjected to short term exposure to salinities ranging from 35 ppt (parts per thousand) to fresh water.

Exposure	35 ppt	26.2 ppt	17.5 ppt	8.75 ppt	fresh water
Initial response	normal behaviour: • swimming in a spiral fashion, covering the entire depth of the petri dish • cilia movement in oesophagus and ciliated band • occasional gut contractions and dorsal flexure.	• 90% normal behaviour • 10% slowed swimming	• slow swimming, otherwise normal behaviour	• slow swimming • rapid gut contractions and dorsal flexure • loss of transparency due to cell lysing	• no swimming • rapid gut contractions • rapid dorsal flexure • loss of transparency due to cell lysing
1–2 minutes	• larvae demonstrate a positively phototactic response (migration to the edges of the petri dish towards the light source)	• slowed swimming and reduced vertical movement, staying on bottom of petri dish • otherwise normal behaviour	• stationary • cilia movement in the oesophagus and ciliated band • occasional gut contractions	• stopped swimming and settled on bottom of petri dish • rapid gut contractions • loss of transparency due to cell lysing	• ciliated band appears crenulated due to extensive cell damage and lysing • very slow gradual backwards dorsal flexion
5-10 minutes	• staying close to edges of petri dish but continued spiral swimming and the vertical movement.	• 50% stationary • 50% slow swimming, no positive phototactic response (not moving towards light source) • slight lysing of cells along ciliated band (band appears slightly 'fuzzy')	• no movement • loss of transparency due to cell lysing	• no movement, gut contractions ceased • ciliated band has a crenulated appearance due to cell lysing • extensive cell lysing around oesophagus and mouth • 50% have body wall lesions	• extensive cell lysing in oesophagus and entire gut • massive lesions in body wall resulting in loss of body fluid and 'peeling' off of body wall membrane
Transfer back to control salinity: initial response	• normal behaviour as above	• no movement; larvae floating at surface	• no movement; larvae floating on surface	• rupturing of entire body wall and loss of body fluid • no movement • internal organs intact	• disintegration upon transfer
2–5 minutes after transfer	• normal swimming	• 90% swimming normally • 10% remain floating; body wall appears ruptured	• 40% body lesions and loss of fluid; larvae floating on surface • 60% very slow swimming, resumption of cilia movement, gut contractions and flexion • 50% have collapsed anterior dorsal processes		

percentage of larvae are able to develop normally at this temperature. Abnormality rates continued to rise at 19°C particularly at 28‰ and 35‰ during the period of exposure, suggesting this temperature is approaching the upper lethal limit for development.

The magnitude of temperature effects on development were dependent on treatment salinity. However, the relationship between these two variables was complex. Abnormality rates increased as a function of salinity at 24°C. At 15°C and 19°C, rates of abnormality peaked at low (28‰) and high (35‰) salinities and were lowest at the intermediate (32‰) salinity. This trend was also apparent in the size of larvae reared at 15°C and 19°C (Figure 3). These data suggest a preference towards estuarine environments. This may thus increase the chances of *A. amurensis* larvae surviving in estuarine ports compared to those with limited freshwater input, particularly at temperatures above 15°C.

3.2. EFFECTS OF TEMPERATURE AND SALINITY ON BIPINNARIA

The survival of bipinnaria was not significantly different between 8°C and 26°C. No larvae survived exposure to water temperatures above 26°C. Larvae from 22°C, 24°C and 26°C treatments, however, developed morphological abnormalities and a reduction in the complexity of the ciliated band. Both these conditions have previously been associated with eventual larval mortality in echinoderms (Roller & Stickle 1985). Growth rates were also retarded in temperatures between 16.5 and 20°C with the size of larvae being inversely proportional to temperature. However, apart from being smaller, larvae were morphologically normal and retained the complexity of their ciliated band, suggesting that they may be capable of surviving over this temperature range. This potential upper temperature limit (20°C) thus approximately corresponds with data from the early development trial (19°C).

A. amurensis bipinnaria were substantially better developed at temperatures below 16.5°C and in particular, at temperatures between 8°C and 12°C indicating these to be the optimum temperature conditions for this stage.

Sagara and Ino (1954) reported that the optimum temperature range for *A. amurensis* bipinnaria was 5–20°C. However, their use of the term optimum is misleading as they used it to describe the range over which larvae survived their experimental period rather than the range over which larvae grew substantially better. These authors did not provide data on size differences, growth rates or variations in levels of abnormality between treatment temperatures and thus their data is difficult to interpret further. Despite this, the similarity in upper tolerance limits between our data and Sagara and Ino (1954) suggests that *A. amurensis* larvae may be able to develop normally at temperatures up to 20°C.

A contributing factor to the observed reduction in larval size with increasing temperature in bipinnaria (experiment 2) may have been that larvae were not fed during the experiment. Morphological plasticity has been demonstrated in

larvae of several echinoderms (notably echinoid plutei) under food limiting conditions. Morphological changes have included lengthening of ciliated bands associated with feeding (Strathman 1971, Boidron-Metairon 1988) and decreasing larval size (Chen & Chen 1992, Allison 1994). High temperatures or non-optimal salinities may also lead to a reduction in growth rates or regression in larval size even when food is not limiting (Hart & Scheibling 1988). Roller and Stickle (1985) attributed this to variations in either the rate of energy acquisition or metabolic maintenance costs under conditions of stress.

At least some asteroid larvae appear to tolerate lengthy periods of food deprivation and may temporarily suspend development (Allison 1994). In these cases, even if larvae had regressed, they retained their morphological integrity and the complexity of their ciliated bands (Roller & Stickle 1985, Allison 1994). We used these criteria to evaluate the results of our experiments. Larvae reared at 16.5–20°C, although having regressed in size relative to baseline larvae, maintained morphological integrity and ciliated band complexity. Larvae reared in temperatures above 20°C developed morphological abnormalities consistent with those considered by Roller and Stickle (1985) to precede mortality. Thus we believe that although not feeding larvae may have influenced their rate of regression, this had little effect on the interpretation of tolerance ranges.

The observed reduction in developmental rates, particularly above 15–16.5°C may, however, significantly increase larval duration at these temperatures. Prolonging the duration of the larval phase, and in particular (as in this case) the period prior to larvae attaining settlement competency, increases the probability of larval mortality (Thorson 1950, Roller & Stickle 1985, 1993). In such cases temperature and salinity induced variations in developmental rates may increase mortality indirectly by reducing the ability of larvae to compete effectively for food and successfully avoid predators (Strathmann 1978).

3.3. SHORT TERM EXPOSURE TO SALINITY EXTREMES

A. amurensis larvae were adversely affected by short term exposure to salinities ≤ 17.5‰ and did not survive exposure to salinities ≤ 8.75‰. This suggests that low salinity treatment may be an effective means of destroying *A. amurensis* larvae.

3.4. IMPLICATIONS FOR DISTRIBUTION

Observations on a number of echinoderm species suggest that larval stages are the least tolerant to temperature and salinity stress (Watts *et al.* 1982, Roller & Stickle 1985, 1989) and thus recruitment and subsequent distribution may be controlled by larval tolerances. Roller and Stickle (1985) demonstrated concordance between larval tolerances and adult distribution along a salinity gradient in Friday Harbour, Washington. The discontinuous distribution of the sea urchin *Lytechinus variegatus* in the Gulf of Mexico has similarly been attributed to salinity tolerances limiting recruitment to low salinity areas (Roller & Stickle 1993). Lucas (1973) hypothesised that the southern limit of

distribution of crown of thorns starfish, *Acanthaster planci*, off eastern Australia was defined by temperature tolerance of larvae. However, Johnson and Babcock (1994) have since demonstrated a far more complex relationship between temperature, survival and distribution for *A. planci* than suggested by Lucas's data.

Interpreting the effects temperature and salinity on recruitment and population distribution can be confounded by geographical variability in ranges tolerated. Geographic differences in tolerances to temperature and salinity for the same species have been reported for a number of echinoderms (Johnson & Babcock 1994) and may be due to local selection, physiological acclimatisation or a combination of these (Tester 1985). The tolerance of larval stages may also be influenced by the recent conditions to which adults have been exposed. However, this appears to hold primarily with respect to temperature rather than salinity (Roller & Stickle 1993). Johnson and Babcock (1994) noted that *A. planci* offspring from adults collected in November (ambient temperature 27°C) were able to develop normally at higher temperatures than those collected at the same site in October (21–25°C).

3.4.1. NATIVE ENVIRONMENT

The normal range of *A. amurensis* is from the northern coast of China, around Japan, Korea and along the Russian coast to the Bering Straits. It also occurs in Alaskan and Canadian waters but this is suspected to be the result of introduction to these areas (Mcloughlin & Bax 1993). Distributional records are complicated by the presence of at least one separate species (*A. amurensis versicolor*) – a warm water form probably restricted to southern Japan and overlapping with *A. amurensis* in some areas (Nojima *et al.* 1986).

The broad scale distribution of *A. amurensis* appears, at least on the east coast of Japan to be restricted by water temperature (Kim 1968). However interpreting the relationship is difficult due to the broad depth range over which the species occurs (down to 200 m). *A. amurensis* appears not to be able to sustain significant shallow water populations south of central Honshu where summer surface water temperatures exceed 25 °C. Several authors report the movement of *A. amurensis* into and out of shallow water in response to temperature although the specific details are rarely recorded. Indeed, our own observations of *A. amurensis* in the Derwent River support that at least some adults move into shallow water during winter period. Whether this is a direct response to temperature or one mediated by spawning cues/behaviour is yet to be determined.

The appearance of large numbers of *A. amurensis* in shallow waters of central Japan appears to be associated with periods of below average water temperatures during the pelagic larval stage (e.g., the 1954 Tokyo Bay outbreak – see Ino *et al.* 1955). This suggests that the temperature tolerance of larvae plays a significant role in regional recruitment.

Larval supply and subsequent persistence of populations may also be influenced by temperature controls of spawning. The timing of spawning in A. amurensis varies geographically, however with the exception of Russian populations in Peter the Great Bay (Novikova 1979), spawning occurs over a similar temperature range (6–12 °C) in all localities (Hayashi 1954, Ino *et al.* 1955, Hatanaka & Kosaka 1959, Kim 1968). Spawning is most common immediately after the lowest water temperature for the region when temperatures begin to rise. This also matches both the temperature range and seasonal timing of spawning of *A. amurensis* in southern Tasmania (Morrice 1995, CSIRO unpublished data). The extent to which exposure to temperatures within the 6–12°C range or the rise in temperature after the seasonal minimum induces spawning is unknown. However, if spawning is not initiated in temperatures above 12°C then this may represent an important control for the geographic maintenance of populations.

3.4.2. POTENTIAL DISTRIBUTION IN AUSTRALIA

Our data suggests that *A. amurensis* larvae are most likely to survive in waters less than 16.5°C and that local distribution (i.e., within estuaries) may be further mediated by salinity. Survival in waters up to 20°C is possible but less likely. Larvae are present in the Derwent River from July to November with peak abundances in August–September (CSIRO unpublished data) estuary. The northern most position of the 16.5°C isotherm in Australian coastal waters (Eden on the east coast and Albany in Western Australia) occurs during July–August and thus roughly coincides with the period of peak larval availability. In subsequent months, temperatures begin to rise and the 16.5°C isotherm moves progressively further south.

Inshore areas such as ports, bays and estuaries are subject to local variations in water temperature and salinity that might either enhance or reduce opportunities for larval survival. Data to asses the extent of local variations is scattered and unavailable for many ports. Uncertainties are highest for Great Australian Bight and south west Western Australian ports. Furthermore, given the depth range over which *A. amurensis* has been recorded, it is possible that populations may survive in deeper waters further north of what surface water temperatures predict.

Given the above, the following relative risks of colonisation by *A. amurensis* can be assigned to broad regions of mainland Australia (Figure 9).

High Risk:	All Tasmanian, Victorian and South Australian ports including Albany and east coast ports as far north as Eden.
Medium Risk:	East coast Ports between Eden and Newcastle; west coast ports between Albany and Fremantle.
Low risk:	East coast ports north of Newcastle and west coast ports north of Fremantle.

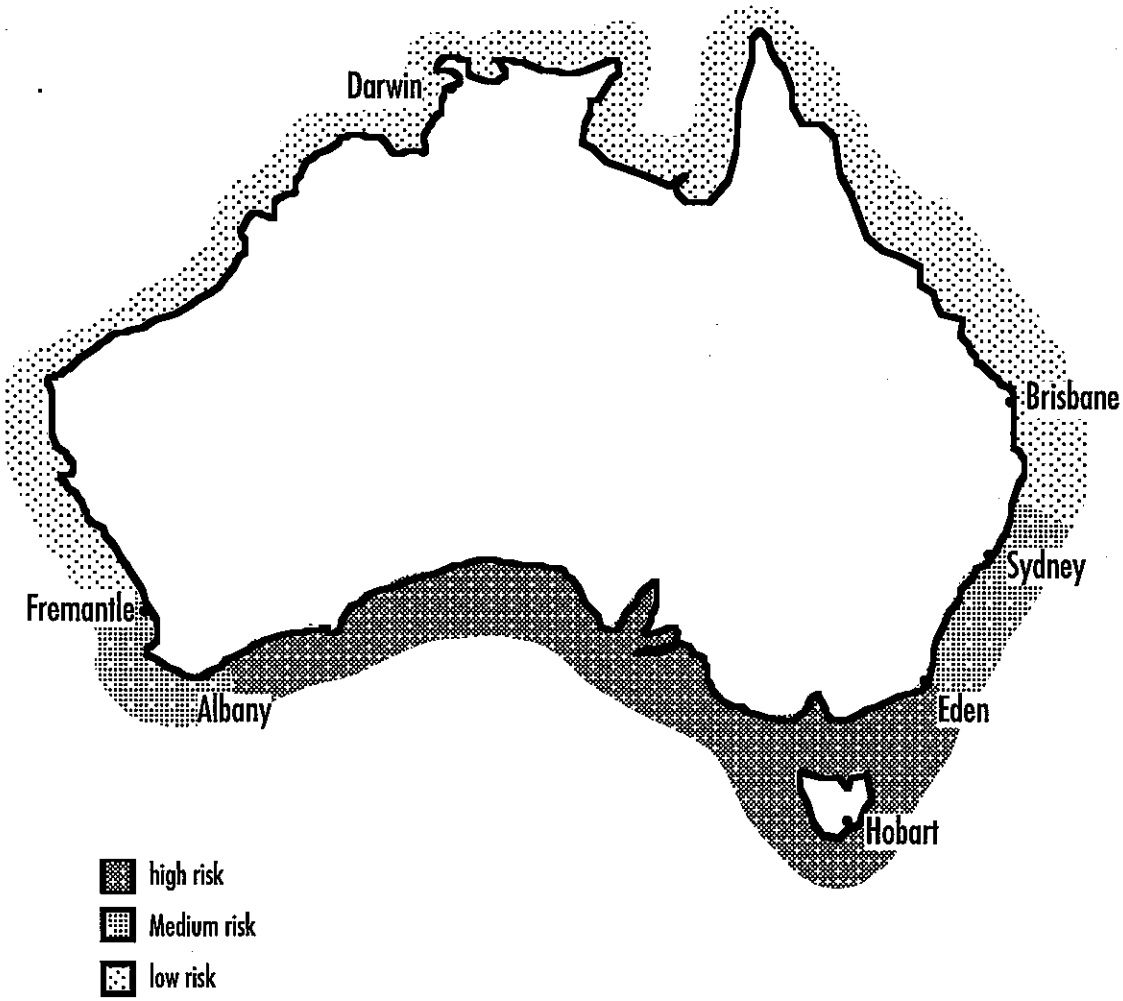

Figure 9. Potential distribution of *A. amurensis* in Australian waters.

3.5. POTENTIAL FOR FURTHER INTRODUCTIONS FROM NATIVE SOURCE REGIONS

The introduction of *A. amurensis* to south eastern Tasmania poses the question why this region was initially, successfully colonised whereas other ports within southern Australia were not. This is particularly intriguing given that ports in south eastern Tasmania (and Hobart in particular) receive far less ballast water discharge than mainland counterparts (Kerr 1994), and hence presumably, fewer opportunities for successful inoculation.

The timing of spawning in native source localities and the temperature tolerance of larvae may have played a vital role. Based on genetic similarity, Ward & Andrew (1995) considered central eastern Honshu to be the most likely source region for SE Tasmanian populations of *A. amurensis*. The peak spawning period for *A. amurensis* in this region of Japan (and hence,

presumably the period of peak larval availability) is February–March (Hatanaka & Kosaka 1959, Kim 1968,). This corresponds to the timing of maximum water temperatures in southern Australia. Assuming that the 16.5°C isotherm represents the likely limit for larval survival, then southern Tasmania represents the only region in southern Australia where water temperatures are suitable for survival during that period.

This suggests that the risk of successful inoculations of *A. amurensis* larvae from this region of Japan to southern mainland Australian ports is low. However, further introductions from more northern Japanese source localities may be possible given the later spawning by populations in such regions thus aligning larval availability with cooler conditions in southern Australia.

Given that *A. amurensis* in southern Tasmania have now aligned their spawning period with the austral winter, the risk of ballast mediated introductions from the Derwent River to southern Australian ports bounded by the 16.5°C isotherm during winter/spring is high.

4. RECOMMENDATIONS FOR FURTHER RESEARCH

The temperature and salinity tolerances of larval stages, whilst important, are only two of a suite of components that will determine the successful spread and establishment of *A. amurensis* in southern Australia. The following research areas are recommended in order to facilitate a more complete assessment:

- How well do *A. amurensis* larvae survive in ballast water?
- What are the cues for spawning and will *A. amurensis* spawn at temperatures above 12°C?
- To what extent will *A. amurensis* acclimatise to Australian conditions and what are the implications for distribution?
- What are the cues and requirements for settlement?
- What are the salinity and temperature tolerances of juveniles and adults?
- What determines the susceptibility of host environments to invasion?

Some of these topics are obviously not unique to *A. amurensis* and are required to assess the risks of successful introduction and spread of non-indigenous marine species in general. Given this, a more general approach to these research areas (i.e., examining a range of target species rather than just *A. amurensis*) is recommended.

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