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Tolerance of the barnacle *Balanus amphitrite amphitrite* to salinity and temperature stress: effects of previous experience

Jian-Wen Qiu, Pei-Yuan Qian*

Department of Biology, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

ABSTRACT: We conducted 4 experiments to study the effects of salinity and temperature on the barnacle *Balanus amphitrite amphitrite* Darwin, with particular focus on the effects of stress experienced in one life-stage on the performance of the next life-stage. At 15°C, typical winter water temperature in Hong Kong, larvae exhibited low survivorship, adults molted infrequently, and only a low percentage of individuals had developing ovaries and embryos. However, at 30°C, typical summertime temperature in Hong Kong, larvae developed rapidly, survivorship was high, adults molted frequently, and a high percentage of individuals had developing ovaries and embryos. These results suggest that low winter temperature may be a limiting factor responsible for cessation of recruitment, whereas high summer temperature is unlikely to be the cause for the decline in recruitment. Salinity produced significant detrimental effects on both survival and development at ≤10‰. In the 15 to 35‰ S range, however, none of the stages tested exhibited signs of stress. Salinity is a limiting factor for the survival and development of *B. a. amphitrite* in Hong Kong only during mid-summer when salinity in the surface water can drop to below 10‰. Exposing embryos to different salinities produced differential effects on larvae. For larvae cultured at 10‰ S, both survivorship and time of development were independent of the salinity that the embryos had experienced; for larvae cultured at 15 and 35‰ S, exposing embryos to 10‰ S led to lower larval survivorship and longer larval development times. Exposing cypris larvae to 10‰ S did not alter juvenile growth but did result in lower survivorship. Osmotic stress experienced in one life-stage can be passed over to the next life-stage. In bioassays involving the use of *B. a. amphitrite*, results of the tested life-stage may be affected by stress experienced in a previous life-stage.

KEY WORDS: Barnacle · *Balanus* · Larvae · Life-history · Salinity · Stress

INTRODUCTION

Marine invertebrates living in coastal areas may experience substantial fluctuations in environmental conditions. The effects of biotic and abiotic factors such as predation, competition, temperature, salinity, food, and pollution on the survival, growth, and reproduction of marine invertebrates are well documented (Thorson 1946, 1966, Kinne 1964, 1971, Foster 1987, Pechenik 1987, Barnes 1989, Boidron-Métairon 1995, Morgan 1995). However, effects of environmental stress experienced in one developmental stage of the life-cycle on the performance of later stages have been examined only in few marine invertebrate species (Bacon 1971, Bayne 1972, Helm et al. 1973, Roller & Stickle 1993, 1994, Hintz & Lawrence 1994, Pechenik et al. 1996a,b, 1998). Some of these studies have shown that stress experienced by a given stage has detrimental effects on later stages (Bayne 1972, Helm et al. 1973, Pechenik et al. 1996a,b, 1998). Others, however, have suggested that the development of a given stage is independent of the stress experienced by a previous stage (Roller & Stickle 1993, 1994, Hintz & Lawrence 1994). Bacon (1971) showed that exposing embryos of the barnacle *Balanus eburneus* to low or high salinity stress increased the resistance of the larvae to similar adverse salinity conditions. If the embryos developed at 40‰ S, and the subsequent larval stage was maintained at 40‰ S, the survivorship was increased when

*E-mail: boqianpy@ust.hk

compared with the larvae maintained at 6 and 12‰ S. Conversely, if the embryos developed at 6‰ S, and the following larval stage was kept at 6‰ S, these larvae had higher survivorship when compared with larvae maintained at 12 to 40‰ S. Similar acclimation phenomena, however, have not been reported in other barnacle species.

Balanus amphitrite amphitrite is a barnacle species commonly found in tropical and subtropical estuaries. Its embryos exhibit normal development at wide ranges of temperature (14.5 to 31.5°C, Patel & Crisp 1960) and salinity (15 to 70‰, Crisp & Costlow 1963), with slower developmental rates at lower temperatures and at salinities (≤15‰ and > 40‰ (Crisp & Costlow 1963). In studies of *B. a. amphitrite* larval development within 10 to 30‰ S and 15 to 30°C ranges, survival was low at the lowest tested salinity and temperature (Anil et al. 1995). These studies suggest that the early developmental stages of *B. a. amphitrite* can tolerate a wide range of salinities and temperatures. However, it is not known how cyprids and adults respond to similar salinity and temperature conditions or whether they can acclimate to such potential stress. In Hong Kong waters, recruitment of barnacle larvae into mixed adult populations of *B. a. amphitrite*, *B. variegatus* and *B. trigonus* takes place from April to September (Wu 1974, Vrijmoed 1975), with a decline in mid-summer. Greene & Morton (1976) attributed this decline to the high temperature and low salinity prevailing in the region at this time of the year. However, no follow-up study has been conducted in order to clarify which of the 2 factors plays a more important role in determining the settlement pattern of these barnacles and which life-stage is most vulnerable to these adverse salinity and temperature conditions.

In this study, we examined the response of several life-stages of *Balanus amphitrite amphitrite* to a combination of salinity and temperature treatments. The salinity and temperature ranges in the experiments were selected to represent those experienced by barnacles in Hong Kong waters (Morton & Morton 1983). Our objectives were: (1) to define the salinity and temperature conditions which limit recruitment in the field, and (2) to determine whether exposure in one life-stage to stressful conditions will lead to altered performance in the next life-stage.

MATERIALS AND METHODS

Expt 1: effects of salinity and temperature on adults. Three-month-old adult *Balanus amphitrite amphitrite* were obtained by rearing the nauplii to cyprids (Qiu & Qian 1997), inducing the cyprids to settle and metamorphose on polystyrene Petri dishes, and feeding the juveniles with the diatom *Skeletonema costatum* for 10 d post-metamorphosis before switching to brine shrimp (*Artemia* sp.) nauplii. All pre-experimental cultures were conducted in an air-conditioned room (23°C) in 35‰ S. At the onset of the experiment, the adults had reached an average size of 5.72 ± 0.33 mm (mean ± SD, n = 20), all were noted to have ovaries and 25% of individuals also had developing embryos. The experiment consisted of 5 salinity (Expt 1A: 5, 10, 15, 20, 35‰ S; all at 25°C) and 3 temperature (Expt 1B: 15, 25, 30°C; all at 35‰ S) treatments. Each treatment consisted of 3 replicates, and each replicate contained 10 individuals placed in a beaker containing 0.2 l seawater; a total of 210 individuals were used. These salinities and temperatures were obtained by diluting 0.22 µm filtered natural seawater (35‰ S) with double-distilled water, and by placing culture beakers in incubators (Powers Scientific SD33SE) at the designated temperatures. Barnacles were fed newly hatched brine shrimp nauplii at 500 to 800 barnacle^{-1} d^{-1}. The salinity of the seawater was maintained by replacing the water every other day for 1 mo. Upon replacement of water, numbers of molts and the dead in each beaker were enumerated and removed. The beakers were covered with aluminum foil; the pre-mixed water for each salinity treatment was maintained at the corresponding temperature for at least 2 h prior to water replacement; brine shrimp nauplii were concentrated using a net (mesh diameter: 90 µm) before being transferred into the beakers. These steps were aimed at minimizing fluctuations in salinity and temperature. At the end of the experiment, all surviving individuals were dissected in order to determine the stage of reproduction. O_0, O_1, and O_2 represent ovary absent, present as a thin layer of ovarian tissue, and filling at least a third of the mantle cavity, respectively. E_0, E_1 and E_2 stand for embryo absent, embryo without eyes, and embryo with eyes, respectively.

Expt 2: salinity and temperature stress on adults and consequent effects on embryonic development. Embryos from selected treatments in Expt 1 were used in Expt 2 if found at the early stages of E_1 (i.e. embryos with divided yolk cells, corresponding to Stages 5 to 7 in Crisp 1954; within 12 h of release from ovary, pers. obs.) (see Fig. 3). Removal of embryos from the parent makes *in vitro* observation possible without resulting in harmful effects on development (Patel & Crisp 1960). Paired egg masses from each individual were divided into small clusters of 20 to 30 embryos, and each cluster was transferred into a glass vial containing 5 ml seawater. The vials were capped and placed on shakers (80 rpm) to promote uniform embryonic development, and each shaker was kept in an incubator at a designated temperature. The experiment could be divided into two 2-factor experiments: salinity

effects were tested in Expt 2A and temperature effects were tested in Expt 2B (Fig. 3). In Expt 2A, temperature was kept constant at 25°C; there were 9 salinity treatments resulting from combinations of the 3 adult conditioning salinities with the 3 embryonic incubation salinities. In Expt 2B, salinity was kept constant at 35‰; there were 4 temperature treatments resulting from combinations of 2 adult conditioning temperatures with 2 embryonic incubation temperatures. Each treatment was set up in triplicate. Salinities were obtained by diluting artificial seawater (35‰ S) with double-distilled water. The antibiotic Crystamycin (22.5 mg l^{-1} Penicillin G and 37.5 mg l^{-1} Streptomycin sulfate) was used to inhibit bacterial growth. The cultures were checked every 12 h so that the times of first hatching were recorded. Embryos were transferred to fresh medium daily until disintegration or emergence of nauplii was observed.

Expt 3: salinity and temperature stress on embryos and consequent effects on larval development. Twenty-five adults cultured at 23°C and 35‰ S were dissected. Whole egg masses, when found at Stages 5 to 7 (Crisp 1954), were incubated at 10, 15, 35‰ S (all at 25°C) and 15 and 30°C (both at 35‰ S) until they hatched into nauplii. These nauplii were then incubated at different combinations of salinity and temperature to investigate the effects of exposure to combinations of salinity and temperature during the embryonic development on larval development (see Fig. 4). In total, 13 treatments were set up, each treatment containing 5 replicates and each replicate containing 10 larvae. The experimental design and incubation method were similar to those in Expt 2, except that the diatom *Skeletonema costatum* was used as larval food at a concentration of 10^5 cells ml^{-1}. The medium was changed daily, and the treatment was terminated when all larvae were observed to have metamorphosed into juveniles or died.

Expt 4: salinity and temperature stress on cypris larvae and consequent effects on juvenile development. Cyprids obtained by rearing nauplii at 25°C and 35‰ S were maintained in Petri dishes (diameter: 5 cm) containing 15 ml seawater at various salinities and temperatures (see Fig. 5). Cyprids not metamorphosed into juveniles within 24 h were decanted. The experiment can be divided into 2 single-factor experiments: Expt 4A, conditioning cyprids to 3 salinity treatments (10, 15, 35‰ S; all at 25°C); Expt 4B, conditioning cyprids to 2 temperature treatments (15 and 30°C; both at 35‰ S). In total, there were 5 treatments each with 4 replicates of 10 to 15 one-day-old juveniles. Juveniles were reared on diets of *Skeletonema costatum* for 3 wk followed by *Artemia* sp. for the 4th wk, under constant laboratory conditions (25°C and 35‰ S) to examine the impact of exposing cypris larvae to salinity and temperature stress on juvenile survival and growth.

Data analysis. Data were tested for normality (Shapiro-Wilk test, in Zar 1996) before proceeding with further statistical analysis. None of these data sets were normally distributed even after arcsine and logarithmic transformations, and so nonparametric analyses were performed. The normal approximation to the Mann-Whitney test (Zar 1996) was used to compare the means between 2 treatments. For comparison of more than 2 means, ANOVA (Zar 1996) was employed using ranked data (Conover & Iman 1981, SAS 1988), followed by Tukey tests (Zar 1996). Chi-square analysis of contingency tables was used to compare differences in ratios among treatments (Zar 1996). In Expt 1, a 1-way ANOVA was used to compare difference in molting among salinity or temperature treatments, as well as in percentages of individuals bearing ovaries or embryos; Chi-square analysis was used to test the differences in reproductive parameters among treatments. In Expts 2 and 3, duration of development and percentage of individuals completing embryonic or larval development under different salinity or temperature treatments were compared using a 2-way ANOVA (Expt 2: adult culture condition × embryonic culture condition; Expt 3: embryonic culture condition × larval culture condition). In Expt 4, differences in juvenile growth after exposing cyprids to salinity or temperature stress were compared using a 1-way ANOVA.

RESULTS

Expt 1: effects of salinity and temperature on adults

Survival

Of the 210 individuals tested, 189 survived to the end of the experiment. At 5‰ S and 25°C, the opercular valves of all individuals were closed from the time of immersion to the 3rd day when they were found dead. No cirral beating was observed. Dead individuals had characteristically loose opercular valves and partially extended cirral fans. At 10‰ S and 25°C, the opercular valves were closed at the time of immersion but by the 6th hour most individuals had resumed normal cirral activity; only 23.3% of individuals in this treatment died throughout the course of the experiment and the greatest mortality (16.6%) occurred within the 1st week. At all higher salinities (15, 20, 35%) and other temperatures (15, 30°C), normal cirral beating was observed within the 1st hour after transfer into the medium and only 4 out of 150 individuals died during the experiment; these deaths occurred sporadically throughout the whole experimental period.

Molting

Both salinity and temperature significantly affected molting frequency. At 25°C, the number of total molts per individual during the 4 wk observation period significantly decreased with decreasing salinity (1-way ANOVA, $F_{3,9} = 4.57$, p = 0.038) (Fig. 1A), being highest at 15 to 35‰ S (4.23 to 4.51) and smallest at 10‰ S (3.36). At 35‰ S, the number of cumulative molts per individual significantly decreased with the decrease in temperature (1-way ANOVA, $F_{2,6} = 29.45$, p = 0.001), being largest at 30°C (5.48), intermediate at 25°C (4.51), and smallest at 15°C (1.63) (Fig. 1B). Temperature had a larger effect than salinity on molting over the ranges tested.

Reproductive traits

Percentage of individuals with ovaries (O_1 + O_2) ranged from 59 to 100% (Fig. 2). For the low salinity ($\chi^2_3 = 41.114$, p = 0.0001) and low temperature ($\chi^2_2 = 71.128$, p = 0.0001) treatments this percentage was lower: only 59% in the 35‰ S and 15°C treatment, and 75 to 82% in the 10 to 15‰ S and 25°C treatments, but over 90% in the 20‰ S and 25°C, and 35‰ S and 25 to 30°C treatments (Fig. 2). The ratio of the number of individuals possessing O_1 to that of those possessing O_2 varied between 0.38 and 1.33, apparently not correlated to salinity ($\chi^2_3 = 4.184$, p = 0.2423) or temperature ($\chi^2_2 = 0.104$, p = 0.9494). Percentages of individuals having mature ovaries (O_2) were significantly different among salinity (1-way ANOVA, $F_{2,9} = 4.91$, p = 0.032) and temperature (1-way ANOVA, $F_{2,6} = 8.68$, p = 0.0171) treatments (Fig. 2). At 25°C, individuals possessing O_2 represented only 22.7% at 10‰ S but 34.5 to 46.8% at 15 to 35‰ S. The percentage of individuals with O_1 ranged from 32.1 to 59.1% and was not significantly different among salinity (1-way ANOVA, $F_{2,9} = 1.36$, p = 0.3220) or temperature (1-way ANOVA, $F_{2,6} = 0.50$, p = 0.630) treatments.

The percentage of brooding individuals ($E_1 + E_2$) (27.3 to 48.3%) was significantly lower at the low salinity (1-way ANOVA, $F_{2,9} = 12.99$, p = 0.0019) and low temperature (1-way ANOVA, $F_{2,6} = 5.74$, p = 0.0420) treatments (Fig. 2). At 25°C, brooding was lowest at 10‰ S (27.3%) but higher at 15 to 35‰ S (40.0 to 46.4%). At 35‰ S, brooding was lowest at 15°C (27.6‰) but higher at 25 and 30°C (41.4 to 48.3%). The ratios for the number

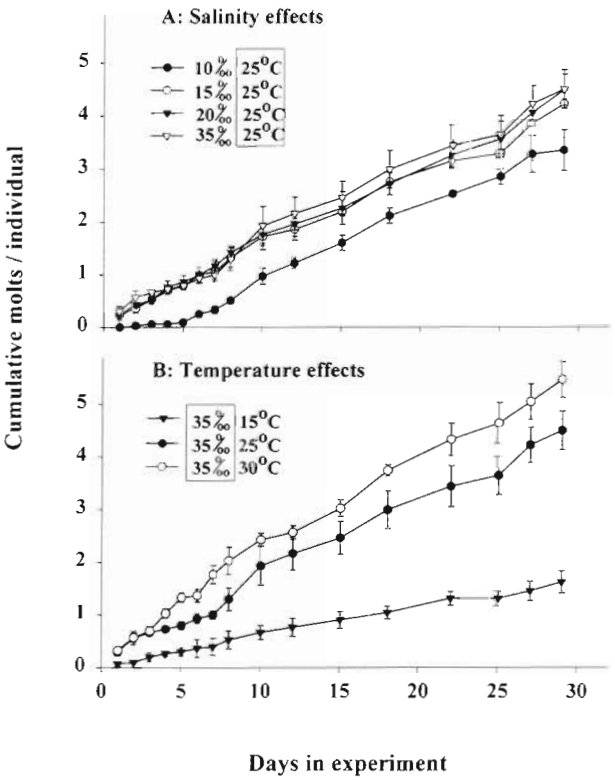

Fig. 1. *Balanus amphitrite amphitrite*: Expt 1. effects of (A) salinity and (B) temperature on the molting of adults during the 4 wk experiment. Data are plotted as mean cumulative molts per individual ± SD. Each treatment consisted of 3 replicates and each replicate consisted of 10 individuals

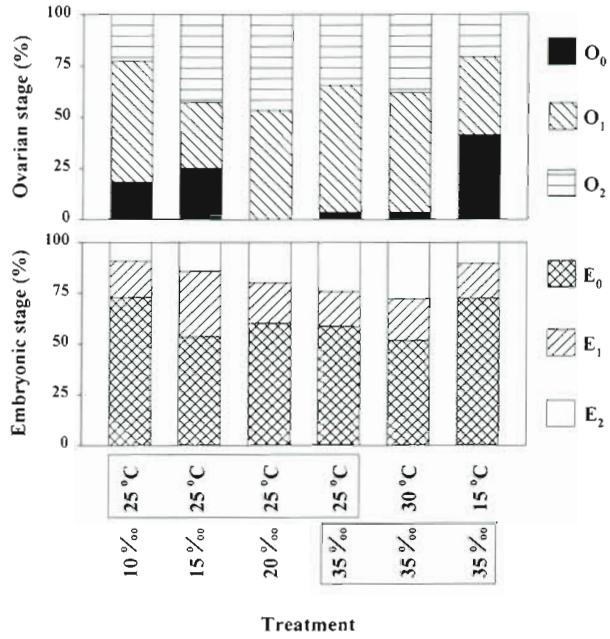

Fig. 2. *Balanus amphitrite amphitrite*: Expt 1: effects of salinity and temperature on the reproduction of adults. Data in each treatment were pooled for the calculation of percentages. O_0, O_1, and O_2 represent ovary absent, present as a thin layer of ovarian tissue, and filling at least a third of the mantle cavity, respectively. E_0, E_1 and E_2 stand for embryo absent, embryo without eyes, and embryo with eyes, respectively

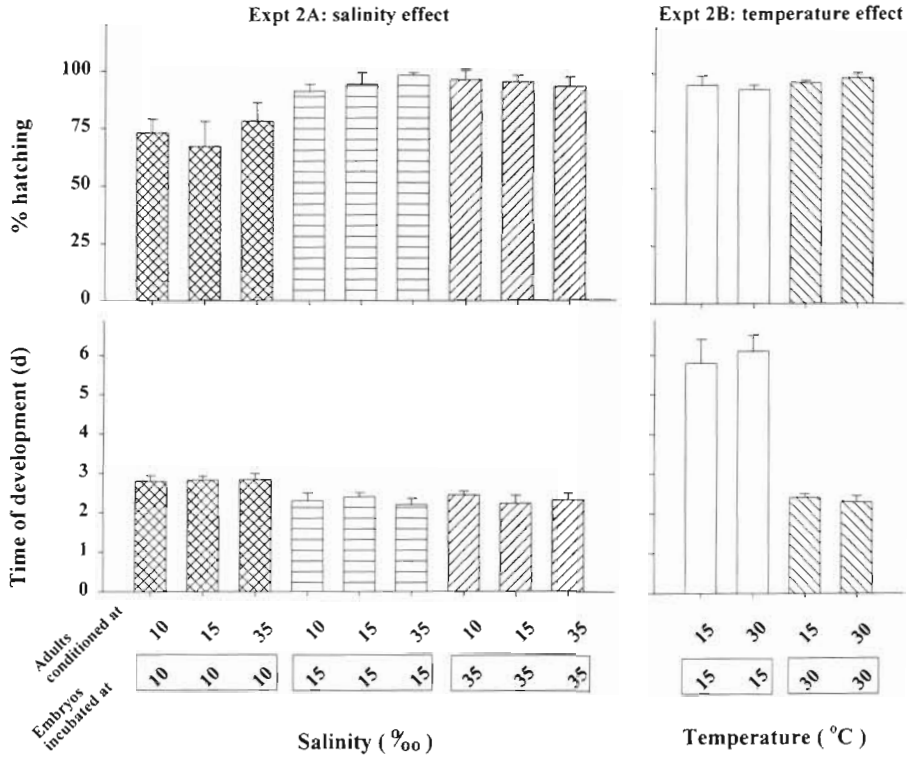

Fig. 3. *Balanus amphitrite amphitrite*: Expt 2: effects of exposing adults to salinity and temperature stress on embryonic hatching (%) and time to complete embryonic development. Expt 2A: salinity effects. Expt 2B: temperature effects. Data are plotted as mean + SD. Each treatment consisted of 3 replicates and each replicate consisted of 20 to 30 embryos

of individuals possessing E_1 to those possessing E_2 varied between 0.44 and 1.40, and were apparently not correlated with salinity ($\chi^2_3 = 2.374$, $p = 0.4985$) or temperature ($\chi^2_2 = 1.005$, $p = 0.605$).

Expt 2: salinity and temperature stress on adults and consequent effects on embryonic development

Hatching ranged from 67 to 78% in the embryos incubated at 10‰ S to between 91 and 98% at higher salinities, disregarding the salinity or temperature conditions that the adults had experienced (Fig. 3, Table 1). Time to hatching in the embryos was not related to the salinity or temperature conditions experienced by the parents, but was significantly affected by the salinity and temperature conditions experienced during embryonic development: it was longer in the 10‰ S treatment (2.80 to 2.84 d) than in higher salinity treatments (2.20 to 2.45 d); and much longer (5.80 to 6.10 d) for the embryos incubated at 15°C than those at 25 to 30°C (2.23 to 2.45 d) (Fig. 3, Table 1).

Expt 3: salinity and temperature stress on embryos and consequent effects on larval development

Survival to the time of metamorphosis ranged from 20 to 94% (Fig. 4) and was significantly affected by both salinity and temperature when rearing larvae (Table 2). Survival did not vary with the temperature in embryonic culture, but varied with the salinity in embryonic culture (Table 2). Larvae reared at 10‰ S had lower survivorship (20 to 32%) than those cultured at 15 or 35‰ S (58 to 90%); while larvae reared at 15°C had lower survivorship (54 to 56%) than those cultured at 25 or 30°C (66 to 94%). Time of larval development varied between 4.61 and 17.85 d. It did not vary with the temperature experienced by the embryos (Fig. 4), but varied with the salinity the embryos experienced and the salinity in larval culture (Fig. 4, Table 2). A breakdown of the 2-way data in Expt 3A according to salinity in the larval culture showed that embryonic experience at different salinities had different effects on both larval survival and larval development time. For the larvae cultured at 10‰ S, both survival ($F_{2,12} = 1.79$, $p = 0.4092$) and duration of development ($F_{2,12} = 0.87$, $p = 0.4444$) were independent of the salinity experienced by embryos; for the larvae cultured at 15 and 35‰ S, survival was lower and duration of development was longer for the embryos that had been exposed to 10‰ S as compared to the embryos that had been exposed to 15 or 35‰ S (15‰ S: survival: $F_{2,12} = 6.86$, $p = 0.0324$, duration of development: $F_{2,12} = 16.33$, $p = 0.0004$; 35% S: survival: $F_{2,12} = 7.44$, $p = 0.0244$, duration of development: $F_{2,12} = 15.18$, $p = 0.0005$).

Table 1. *Balanus amphitrite amphitrite*. 2-way ANOVA results: effects of exposing adults to salinity and temperature stress on the tolerance of embryos. There were 3 replicates per treatment; each replicate started with 20 to 30 embryos. When there was no interaction between factors, Tukey tests were performed. Treatments that do not differ at 0.05 level are connected by underlining (NS: not significant)

Source	Treatment	df	*F*	p
Percent hatching				
Salinity effect				
Adult	10 15 35	2	1.11	0.3499(NS)
Embryo	10 15 35	2	32.49	0.0001
Adult × Embryo		4	2.1	0.1234(NS)
Error		18		
Temperature effect				
Adult	15 30	1	1.17	0.3114(NS)
Embryo	15 30	1	0.11	0.7481(NS)
Adult × Embryo		1	1.17	0.3114(NS)
Error		8		
Days to hatching				
Salinity effect				
Adult	10 15 35	2	0.01	0.7928(NS)
Embryo	10 15 35	2	18.33	0.0001
Adult × Embryo		4	0.47	0.7539(NS)
Error		18		
Temperature effect				
Adult	15 30	1	0.02	0.8819(NS)
Embryo	15 30	1	30.49	0.0006
Adult × Embryo		1	0.59	0.4651(NS)
Error		8		

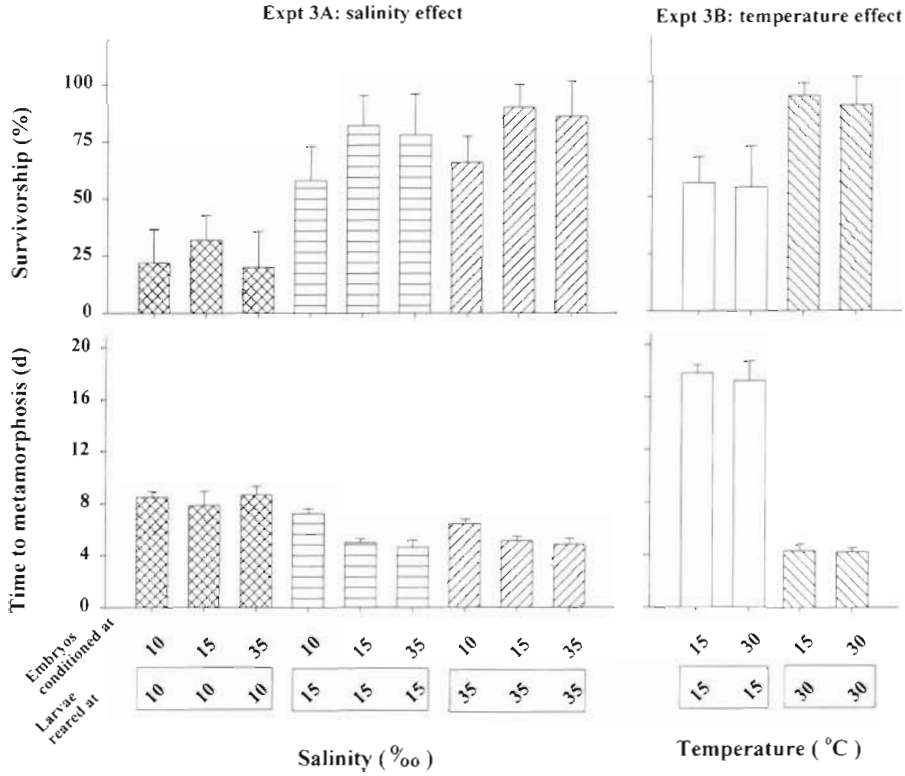

Fig. 4. *Balanus amphitrite amphitrite*: Expt 3: effects of exposing embryos to salinity and temperature stress on larval survival and time to complete larval development. Expt 3A: salinity effects. Expt 3B: temperature effects. Data are plotted as mean + SD. Each treatment consisted of 5 replicates and each replicate consisted of 10 larvae

Table 2. *Balanus amphitrite amphitrite*. 2-way ANOVA results: effects of exposing embryos to salinity and temperature stress on the tolerance of larvae. There were 5 replicates per treatment; each replicate started with 10 Stage II nauplii. When there was no interaction between factors, Tukey tests were performed. Treatments that do not differ at 0.05 level are connected by underlining. (NS: not significant)

Source	Treatment			df	*F*	p
Survivorship (%)						
Salinity effect						
Embryo	10	15	35	2	8.64	0.0009
Larva	10	15	35	2	61.00	0.0001
Embryo × Larva				4	1.43	0.2431(NS)
Error				36		
Temperature effect						
Embryo		15	30	1	0.23	0.6399(NS)
Larva		15	30	1	43.66	0.0001
Embryo × Larva				1	0.02	0.8933(NS)
Error				16		
Days to metamorphosis						
Salinity effect						
Embryo	10	15	35	2	21.38	0.0001
Larva	10	15	35	2	83.25	0.0001
Embryo × Larva				4	9.28	0.0001
Error				35		
Temperature effect						
Embryo		15	30	1	0.61	0.4852(NS)
Larva		15	30	1	50.63	0.0001
Embryo × Larva				1	0.05	0.8337
Error				16		

Expt 4: salinity and temperature stress on cypris larvae and consequent effects on juvenile development

During the 4 wk experimental period, juvenile survival ranged from 53.3 to 94.2% (Fig. 5). Juvenile survival was significantly affected by salinity (1-way ANOVA, $F_{2,9} = 13.37$, $p = 0.0020$) but not by temperature (normal approximation to the Mann-Whitney test, $Z = 1.0164$, $p = 0.3094$) the cyprids had experienced. Survival was higher in the juveniles which had been conditioned at 15 and 35‰ S (88.3 to 94.2%) as cyprids as compared to those which had spent the cyprid stage at 10‰ S (53.3%) (Fig. 5). Most juvenile mortality occurred in the first 2 wk. Body length in the 4th week varied between 5.7 and 5.9 mm, however, this did not vary with the salinity (1-way ANOVA, $F_{2,9} = 0.55$, $p = 0.5967$) or temperature (normal approximation to the Mann-Whitney test, $Z = 1.691$, $p = 0.4116$) that the cyprids had experienced (Fig. 5).

DISCUSSION

The salinity and temperature ranges used in our laboratory experiments represented those encountered in local waters. The data showed that high temperature (30°C) resulted in increased survival and growth of embryos, larvae and adults, indicating the depressed reproduction of the field populations in mid-summer was not caused by high temperature, as previously suggested by Greene & Morton (1976). Low temperature (15°C), however, led to complicated effects: it did not produce a decline in survivorship of embryos or adults but caused a decrease in larval survivorship, molting frequency, and percentage of adults bearing developing ovaries and embryos. From the survey of a local field population conducted from August 1997 to July 1998, we found that in summer up to 45% of individuals possessed embryos while in winter less than 5% of individuals possessed embryos (Qiu & Qian unpubl. data). Although our laboratory experimental results suggest that a substantial percentage of embryos maintained under winter temperature condition would be viable, they would require a much longer time to complete embryonic development (Fig. 3). Furthermore, larvae released into the water column at low temperature might suffer high mortality and longer larval development (Fig. 4), increasing the chance of being carried by currents into unfavorable habitats or being preyed on by carnivores. Similar effects of low temperature on the embryonic and larval development of this species have been reported (Patel & Crisp 1960, Crisp & Costlow 1963, Anil et al. 1995). Crisp & Cost-

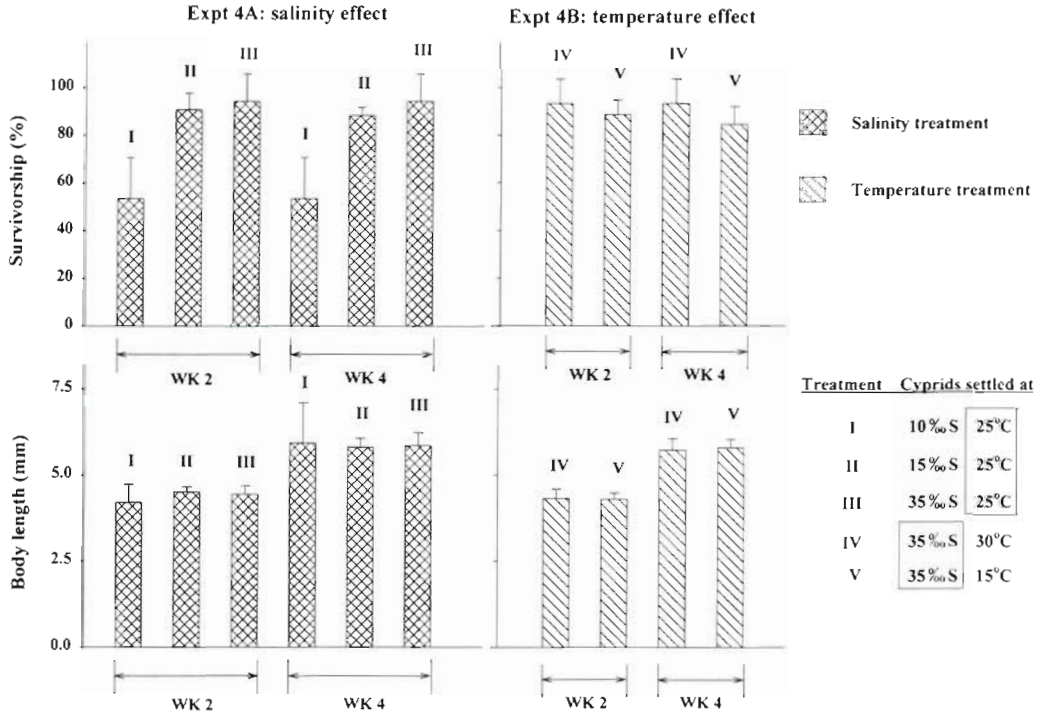

Fig. 5. *Balanus amphitrite amphitrite*: Expt 4: effects of exposing cyprids to salinity and temperature stress on juvenile survival and growth. Expt 4A: salinity effects. Expt 4B: temperature effects. Data are plotted as mean + SD. Each treatment consisted of 4 replicates and each replicate consisted of 10 to 15 juveniles

low (1963) found that most embryos could complete embryonic development at 15°C but development was at a substantially slower rate. Anil et al. (1995) found that this species could complete larval development at 15°C with high mortality (>50%) and much-increased development time. Low temperature, therefore, could be the biggest contributing factor for the cessation of settlement of this species in winter.

Previous studies have demonstrated that the early developmental stages of this species can tolerate a wide range of salinities (15 to 60‰ in embryonic development, Crisp & Costlow 1963; 10 to 30‰ in larval development, upper limit not tested, Anil et al. 1995). Since earlier stages of many invertebrates are usually more sensitive to stress (Gosselin & Qian 1997, Qiu & Qian 1998), we included a 5‰ S treatment in the experiment (Expt 1) designed to test the tolerance of the adults. All adults died at 5‰ S, and detrimental effects could still be found at 10‰ S: lower survivorship in the 1st wk of the experiment, fewer molts, and lower percentage of individuals possessing ovaries and embryos. At 15 to 35‰ S, however, the adults had high survivorship, molted frequently, and had a high percentage of reproducing individuals. Our results on early stages were similar to those of previous studies (Crisp & Costlow 1963, Anil et al. 1995): embryos and larvae had lower survivorship and developed slower at 10‰ S, but ≥ 15‰ S did not cause detrimental effects. All 4 stages tested were similar in having a strong capacity to tolerate hypo-osmotic pressure, although the mechanisms for osmotic regulation may vary among these stages with different levels of organizational complexity. The lower salinity limit for this species seems to be 10‰ S, below which it is unable to regulate the osmotic pressure. The relative insensitivity of this species to low salinity may explain the only slight decline of barnacle settlement in summer (Wu 1974, Vrijmoed 1975), when, in Hong Kong, the rainfall can reach 1000 mm mo^{-1}.

If an individual is transferred to a new environment and exposed to a shift in 1 or more environmental parameters, the individual may respond by gradually shifting its functions, such as feeding rate or respiration rate. Such compensatory mechanisms were observed in Expt 1: most adults resumed cirral beating by the 6th hour after immersion in 10‰ S seawater, and mortality of most adults at this low salinity occurred only in the 1st wk of exposure. Although stress still existed, as shown by a lower molting frequency and a lower percentage of reproductive individuals, no further mortality occurred, indicating that the remaining individuals had acclimated to the low salinity. These acclimation effects occurred within 1 developmental stage. In another barnacle, *Balanus eburneus*, however, the sur-

vival of larvae within 48 h post-hatching depended on the salinity experienced by the embryos during the pre-liberation period: larvae that hatched at 6‰ S survived better at 6‰ S than those hatched at 12 to 40‰ S; larvae that hatched at 40‰ S survived better at 40‰ S than those kept at 6 and 12‰ S (Bacon 1971). Bacon's findings (Bacon 1971) suggest that acclimation in one developmental stage may increase the resistance to adverse condition in the next stage. Our data, however, show that in *Balanus amphitrite amphitrite* survivorship was reduced and time to complete larval development was prolonged when embryos had been maintained at a low salinity of 10‰ S; exposure of cypris larvae to 10‰ S also led to lower survivorship in the juveniles. Exposure to hypo-osmotic stress in *B. a. amphitrite* during a given developmental stage may thus lead to declined performance of the next stage. The exposure of *B. a. amphitrite* larvae to low salinity could thus produce a detrimental effect when juveniles recruit into the adult population. Similarly, the prolongation of larval development due to exposure to low salinity during embryonic development could increase the likelihood of being preyed on or misrouted to an unfavorable habitat. Since the larvae have often been used in settlement or toxicity assays (Rittschof et al. 1984, 1986, Holmström et al. 1992, Clare et al. 1994, Kon et al. 1995, Sasikumar et al. 1995), when obtaining the larvae for bioassays, care should be taken to avoid using adults collected from, or cultured in, low salinity environments.

In summary, our data suggest that low temperature and low salinity might be responsible for the decline in reproduction of *Balanus amphitrite amphitrite* in the winter and summer in Hong Kong, respectively. Exposure of one life-stage to low salinity stress could decrease the performance of the next life-stage. Apart from nutritional stress (Jarrett & Pechenik 1997, Pechenik et al. 1998), decreased barnacle performances might also be caused by embryonic or larval exposure to osmotic pressure.

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