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Combined effects of salinity, temperature and food on early development of the polychaete *Hydroides elegans*

Jian-Wen Qiu*, Pei-Yuan Qian

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

ABSTRACT: Effects of salinity, temperature and food concentration on early development of the polychaete *Hydroides elegans* (Haswell) were examined in 4 laboratory experiments. Three 2-factor experiments tested the effects of salinity (15 to 35‰) and temperature (15 to 30°C) on the survival and duration of development of *H. elegans* from newly-released oocyte to 2-cell, 2-cell to blastula, and blastula to trochophore stages respectively. A fourth 3-factor experiment tested the effects of salinity (15 to 35‰), temperature (15 to 30°C), and concentration of the single-cell alga *Isochrysis galbana* (0 to 10^6 cells ml^{-1}) on survival, settlement, and duration of development from trochophore to newly-settled juvenile. Within the experimental range, temperature had no effect on survivorship, but low temperature led to longer duration of development. Low salinity reduced survivorship and settlement, and lengthened the duration of development. Low food concentration reduced survivorship and settlement, and lengthened the duration of development from trochophore to newly-settled juvenile. At concentrations $\leq 10^3$ cells ml^{-1}, >35% larvae survived through the 10 d experiment but lost their ability to become competent. Percentages of trochophores reaching settlement were similar at 10^4, 10^5, and 10^6 cells ml^{-1}. Duration of development was shortest at concentrations of 10^5 cells ml^{-1}, while trochophores at 10^4 and 10^6 cells ml^{-1} had similar but longer durations of development. Our data suggest that in Hong Kong waters, the decrease in salinity during the summer seems to override the benefits of high temperature and to be responsible for the decline in *H. elegans* settlement. The increase in phytoplankton concentration from early spring to early summer may contribute to the formation of settlement peaks. Temperature, however, does not seem to be a limiting factor for early development and settlement of *H. elegans*.

KEY WORDS: *Hydroides* · Development · Salinity · Temperature · Food

INTRODUCTION

Hydroides elegans (Haswell) (Polychaeta: Serpulidae) is a tube-building polychaete species conspicuous in tropical and subtropical coastal fouling communities. In most places, settlement of *H. elegans* peaks in summer or autumn (Skerman 1958[1], Wisley 1958[1], Reish 1961[1], Kawahara 1969, Li et al. 1982, Zhang et al. 1984). In Hong Kong, however, settlement of *H. elegans* peaks in early spring to early summer (Greene & Morton 1976, Hon 1978, Qian unpubl. data) This difference in settlement time suggests that reproduction and recruitment of *H. elegans* in Hong Kong waters may be influenced by environmental factors specific to this area.

In the past several decades, laboratory studies on *Hydroides* spp. have mainly focused on systematics (Zibrowius 1973, Imajima 1976, Fauchald 1977, Knight-Jones et al. 1991, ten Hove et al. 1991), oogenesis and fertilization (Nordbaek 1956), maturation (Leone 1970), light reaction (Wisley 1958, Miura & Tajihara 1984), tube formation (Hedley 1956), salinity tolerance in adults (Hill 1967, Mak & Huang 1982, Mohan & Aruna 1994), larval settlement (Scheltema et al. 1981,

*E-mail: bojwqiu@usthk.ust.hk

[1]*H. elegans* was identified as *H. norvegica* (see ten Hove 1974, Imajima 1976 for reviews)

Hurlbut 1991, Hadfield et al. 1994), and description of early life history (Haswell 1888, Wisely 1958, Miura & Kajihara 1981). Less attention has been paid to the mechanism by which environmental factors such as temperature, salinity, and food availability affect embryonic and larval development, and consequently influence larval settlement and population dynamics in the field.

Marine invertebrate larvae are usually more sensitive to stress than adults and juveniles of the same species, and such sensitivity may help us explain seasonal or annual variations in recruitment success in the field (cf. reviews by Kinne 1970, 1971, Pechenik 1987, Strathmann 1987). The objectives of this study were to examine the developmental sensitivity of *Hydroides elegans* to environmental factors and to determine if the results on developmental sensitivity explain the settlement and population dynamics patterns observed in Hong Kong waters. Specifically, we examined how temperature and salinity influence embryonic development (from newly-released oocyte to the hatching of trochophore) and how temperature, salinity and food concentration affect larval development (from trochophore to newly-settled juvenile) of *H. elegans*.

MATERIAL AND METHODS

Rearing procedures. Procedures for obtaining oocytes and sperm were adopted from Hadfield et al. (1994). *Hydroides elegans* attached to nylon ropes in Port Shelter, Hong Kong (22° 19' N, 114° 16' W) were brought back to the laboratory on March 20, 1996 (salinity and temperature in the field: 21°C and 35‰, respectively). Tubes containing live worms were carefully removed from the ropes, placed individually into petri dishes containing 10 ml, 0.22 µm filtered seawater (salinity: 35‰), and gently broken up in the laboratory where temperature was 22°C. Both males and females released gametes into the seawater within 15 min. For the following experiments, distinct stages developed from the same batch of gametes were used: newly-released oocyte, 2-cell, blastula, and trochophore (Table 1). Oocytes from 4 females and sperm from 3 males were combined for the experiments. To examine effects of salinity and temperature on fertilization and first cleavage, the newly-released oocytes were transferred into small dishes with 10 ml seawater, and 50 µl of seawater containing sperm was pipetted into the dishes. The cultures were maintained at selected salinities and temperatures (Expt I). Meanwhile, the newly-released oocytes were also transferred into a beaker containing 600 ml seawater, and 3 ml seawater containing sperm were pipetted into the beaker where fertilization took place. When the fertilized eggs reached the 2-cell embryo, blastula, and trochophore stages, they were used in Expts II, III, & IV, respectively. Two-cell embryos and blastulae were transferred into small dishes with 10 ml filtered seawater, and maintained at selected salinities and temperatures (Expts II & III, respectively). Early trochophores were transferred into dishes with 10 ml filtered seawater, and maintained at selected food concentrations, salinities and temperatures (Expt IV). A previous study had shown that at 20°C, it takes approximately 1 h for development from fertilization to 2-cell, 4 h from 2-cell to blastula, 8 h from blastula to trochophore and 6 d from trochophore to competence (Wisely 1958). To allow for flexibility in time of development under different salinity and temperature treatments, our experiments for these 4 stages were run for considerably longer periods (12 h, 24 h, 36 h, and 10 d, respectively). The experimental ranges in salinity (15 to 35‰) and temperature (15 to 30°C) were comparable to those encountered in the field (Morton & Morton 1983). The single-cell chrysophyte *Isochrysis galbana* (Tahitian strain) at exponential phase was used to feed the trochophores. *Isochrysis galbana* concentrations of 0 to 10^6 cells ml^{-1} were selected to cover the full range of food concentrations encountered in the field. Setup of the 4 experiments is detailed below and summarized in Table 1

Expt I: Newly-released oocyte to 2-cell. Expt I was designed to examine how salinity and temperature affect survival and duration of development of newly-released oocytes. The experimental setup followed a 2-factor design: combinations of 4 temperature levels (15, 20, 25, 30°C) and 5 salinity levels (15, 20, 25, 30, 35‰). These salinities and temperatures were obtained by diluting filtered natural seawater (35‰) with double-distilled water, and were placed into incubators (Powers Scientific SD33SE) at the 4 designated temperatures. Each of the 20 treatments consisted of 3 replicates, each replicate with 40 oocytes; a total of 2400 newly-released oocytes were used. Developmental stages were checked every half hour after onset of the experiment, until the fertilized oocytes had undergone fertilization and cleavage or disintegrated in 12 h.

Expt II: Two-cell to blastula. Expt II was designed to examine the effects of salinity and temperature on survival and duration of development of 2-cell embryos. The experimental design was the same as in Expt I. Cultures were checked at 3, 4, 5, 6, 8, 10, 12, 14, 16 and 24 h after the onset of experiment, until the 2-cell embryos had developed into blastulae or disintegrated in 24 h.

Expt III: Blastula to trochophore. Expt III was designed to examine the effects of salinity and temperature on survival and duration of development of blastulae. The experimental design was the same as in

Table 1. Experimental protocols and schedules

Experiment	Stage at onset	Experimental period (d)	Design	Experimental regime Sal. (‰)	Temp. (°C)	Food (cells ml^{-1})
I	Newly-released oocyte	0.5	2-factor	35	30	
				30	25	
				25	20	
				20	15	
				15		
II	2-cell	1	2-factor	35	30	
				30	25	
				25	20	
				20	15	
				15		
III	Blastula	1.5	2-factor	35	30	
				30	25	
				25	20	
				20	15	
				15		
IV	Trochophore	10	3-factor	35	30	10^6
				30	25	10^5
				25	20	10^4
				20	15	10^3
				15		10^2
						0

Expt I. The cultures were checked at 8, 10, 12, 24 and 36 h after the onset of experiment, until the blastulae had developed into trochophores or disintegrated in 36 h.

Expt IV: Trochophore to newly-settled juvenile. Expt IV was designed to examine the effects of food concentration, salinity and temperature on survivorship, duration of development of trochophores, and percent trochophores that settled. Experimental setup followed a 3-factor design: combinations of 6 *Isochrysis galbana* concentrations (0, 10^2, 10^3, 10^4, 10^5, and 10^6 cells ml^{-1}), 4 temperature levels (15, 20, 25, and 30°C) and 5 salinity levels (15, 20, 25, 30, and 35‰). Each of the 120 treatments consisted of 2 replicates, each replicate with 20 oocytes; a total of 4800 trochophores were used. Larvae were transferred to fresh media every 2 d. At each transfer, numbers of surviving larvae were counted. Previous research indicated that *Hydroides elegans* larvae become competent 6 to 8 d post-fertilization (Wisely 1958, Hadfield et al. 1994) and preferentially settled on biologically filmed surfaces (Hadfield et al. 1994). Therefore, starting on Day 2 of the experiment, dishes coated with 2 d old film obtained by soaking dishes in fresh seawater were used to induce settlement. These dishes were then checked daily and the numbers of newly-settled juveniles were counted. Percentage of larvae settled in each dish was calculated as the total numbers of settled juveniles divided by 20 (the total number of trochophore larvae at the beginning of experiment) times 100.

At the end of the 10 d experiment, over 35% of the larvae in the $\leq 10^3$ cells ml^{-1} treatments survived (except at salinity of 15‰ where trochophores all died) but did not grow well. *Isochrysis galbana* at a concentration of 10^5 cells ml^{-1} were fed to these larvae for an additional 7 d, to test whether the effect of starvation was reversible.

Statistical analysis. Since the data (survivorship, percent reaching settlement, and duration of development) did not meet the normality assumption of parametric analysis, they were analyzed using nonparametric statistics. This was done by transforming values to ranks and then applying parametric statistics on the data, as described in Zar (1984) and SAS (1988). A 2-way ANOVA (analysis of variance) (Zar 1984) was used to detect salinity and temperature effects on duration of development and survivorship in Expts I, II, and III. A 3-way ANOVA (Zar 1984) was used to analyze food, salinity and temperature effects on survivorship, percent settlement, and duration of development of trochophores in Expt IV. The Tukey multiple comparisons test (Zar 1984) was used to compare treatment means in cases where factors did not significantly interact. When interaction between (among) factors was significant ($p > 0.05$), the influence of each factor on treatment means was tested at fixed levels of the other factor(s).

RESULTS

Expt I: Newly-released oocyte to 2-cell

Although the experiment was run for only 12 h, all oocytes that failed to undergo fertilization and cleavage were dead and had disintegrated within this period.

Temperature (15 to 30°C) had no effect on survivorship from newly-released oocyte to 2-cell (Table 2, Fig. 1A). However, duration of development was significantly longer in the 15°C treatment than in the 3 higher temperature treatments (Fig. 1B).

Salinity had a significant influence on both survivorship and duration of development from newly-released oocyte to 2-cell (Table 2). At salinities ≤20‰, none of the newly-released oocytes attained first cleavage. At 25‰, 85 to 90% oocytes successfully developed into 2-cell. At 30 and 35‰, over 97% developed into 2-cell (Fig. 1A). The duration of development was longer at 25‰ (2.00 ± 0.59 h) than at 30 and 35‰ (0.88 ± 0.31 h and 0.73 ± 0.29 h, respectively) (Fig. 1B).

No interaction between temperature and salinity on survivorship or duration of development was detected in the experiment (Table 2, Fig 1B).

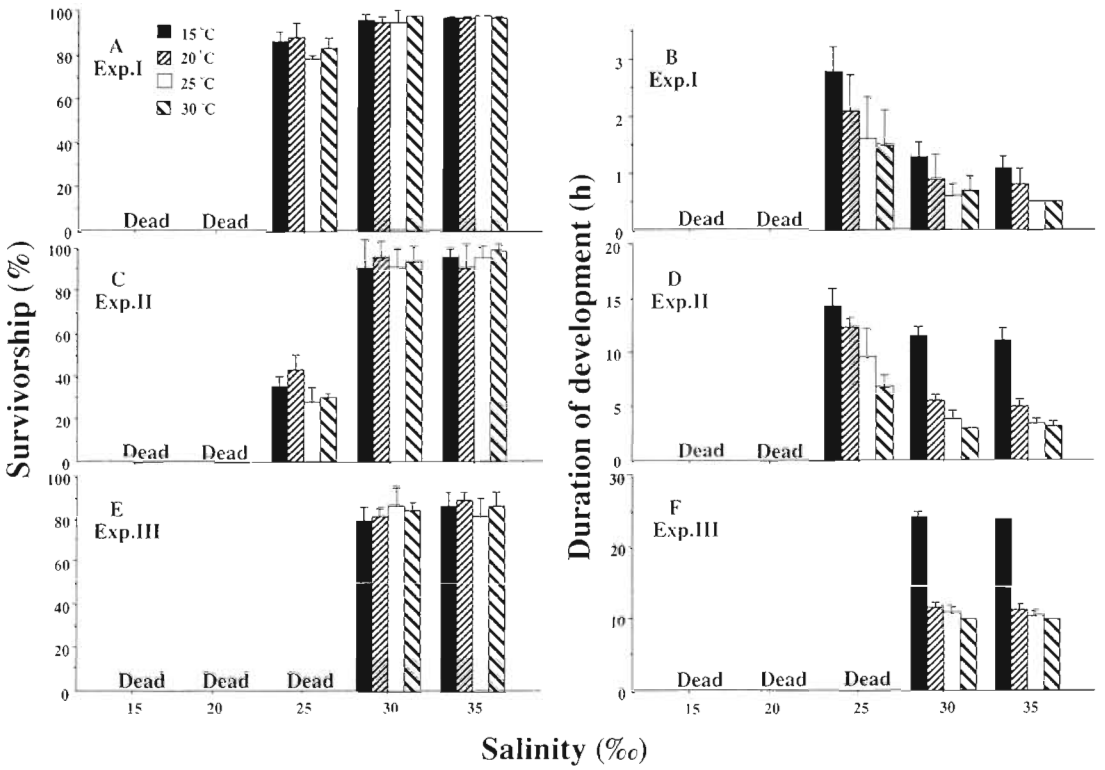

Fig. 1. *Hydroides elegans*. Effects of salinity and temperature on fertilization and embryonic development. Expt I: Newly-released oocyte to 2-cell. (A) Survivorship; (B) duration of development. Oocytes all died at salinities ≤20‰. Expt II: 2-cell to blastula. (C) Survivorship; (D) duration of development. Two-cell embryos all died at salinities ≤20‰. Expt III: Blastula to trochophore. (E) Survivorship; (F) duration of development. Blastulae all died at salinities ≤25‰. Data are plotted as mean + SD of 3 replicate cultures (each started with 40 individuals)

Expt II: Two-cell to blastula

Temperature (15 to 30°C) did not affect survivorship during development from 2-cell stage to blastula (Table 2, Fig. 1C). However, duration of development was the longest in the 15°C treatment, and progressively shorter with increasing temperature (Fig. 1D).

Salinity strongly affected survivorship and duration of development from 2-cell to blastula (Table 2). None of the 2-cell embryos had gone through subsequent cleavages at salinities ≤20‰. At 25‰, about 34% of the 2-cell embryos successfully developed into blastulae. At 30 and 35‰, over 90% of the 2-cell embryos successfully developed into blastulae (Fig. 1C). Duration of development was longer at 25‰ than at 30 and 35‰ (Fig. 1D).

No interaction between temperature and salinity on survivorship was detected in Expt II (Table 2). However, interaction of temperature and salinity on duration of development were observed, with the quickest development achieved under high temperature and high salinity conditions (Table 2, Fig. 1D).

Expt III: Blastula to trochophore

Survivorship during development from blastula to trochophore was not affected by temperature (15 to 30°C) (Table 2, Fig. 1E). However, duration of development was longest in the 15°C treatment, and progressively shorter with increasing temperature (Table 2, Fig. 1F).

Salinity significantly affected survivorship (Table 2). None of the blastulae developed to trochophores at salinities ≤25‰. At 30 and 35‰, over 83% of the blastulae developed into trochophores (Fig. 1E). Duration of development was similar at 30 and 35‰ (Fig. 1F).

Temperature and salinity had no interactive affect on survivorship or duration of development (Table 2, Fig. 1E, F).

Expt IV: Trochophore to newly-settled juvenile

Survivorship of trochophores was not significantly affected by temperature (15 to 30°C) (Table 3, Fig. 2). However, both the percent reaching settlement and

Table 2. *Hydroides elegans*. Summary of 2-way ANOVA results on effects of salinity and temperature on survivorship and duration of development in fertilization and embryonic development. When there is no interaction between factors, values that do not differ at 0.05 level in Tukey tests are joined by an underline. NS: not significant; NA: data not available due to mortality of entire treatment

	Treatments					df	*F*	p
Expt I: Newly-released egg to 2-cell								
Survivorship								
Salinity (‰)	35	30	25	20	15	4	180.85	0.0001
Temperature (°C)	30	25	20	15		3	0.26	0.8505 (NS)
Salinity × Temperature						12	1.11	0.3826 (NS)
Duration of development								
Salinity (‰)	35	30	25	NA	NA	2	49.91	0.0001
Temperature (°C)	30	25	20	15		3	15.53	0.0001
Salinity × Temperature						6	0.25	0.9579 (NS)
Expt II: 2-cell to blastula								
Survivorship								
Salinity (‰)	35	30	25	20	15	4	157.83	0.0001
Temperature (°C)	30	25	20	15		3	0.51	0.6797 (NS)
Salinity × Temperature						12	0.64	0.7971 (NS)
Duration of development								
Salinity (‰)	35	30	25	NA	NA	2	133.93	0.0001
Temperature (°C)	30	25	20	15		3	133.34	0.0001
Salinity × Temperature						6	4.47	0.0001
Expt III: Blastula to trochophore								
Survivorship								
Salinity (‰)	35	30	25	20	15	4	153.29	0.0001
Temperature (°C)	30	25	20	15		3	0.21	0.8906 (NS)
Salinity × Temperature						12	0.79	0.6547 (NS)
Duration of development								
Salinity (‰)	35	30	NA	NA	NA	1	0.02	0.8890 (NS)
Temperature (°C)	30	25	20	15		3	34.69	0.0001
Salinity × Temperature						3	0.50	0.6883 (NS)

Table 3. *Hydroides elegans*. Summary of 3-way ANOVA results for Expt IV on effects of salinity and temperature, and food, on survivorship, percent reaching settlement, and duration of development in trochophores. When there is no interaction between factors, values that do not differ at 0.05 level in Tukey tests are joined by an underline. NS: not significant; NA: data not available due to mortality of entire treatment

	Treatments						df	*F*	p
Survivorship									
Food ration (cells ml^{-1})	10^6	10^5	10^4	10^3	10^2	0	5	56.25	0.0001
Salinity (‰)	35	30	25	20	15		4	370.47	0.0001
Temperature (°C)	30	25	20	15			3	2.48	0.0643 (NS)
Food × Salinity							20	6.40	0.0001
Food × Temperature							15	0.32	0.9919 (NS)
Salinity × Temperature							12	1.90	0.0407
Food × Salinity × Temperature							60	0.43	0.9998 (NS)
Percent reaching settlement									
Food ration (cells ml^{-1})	10^6	10^5	10^4	NA	NA	NA	2	0.33	0.7720 (NS)
Salinity (‰)	35	30	25	20	NA		3	44.05	0.0001
Temperature (°C)	30	25	20	15			3	3.40	0.0251
Food × Salinity							6	0.54	0.7779 (NS)
Food × Temperature							6	0.10	0.9960 (NS)
Salinity × Temperature							9	1.03	0.4277 (NS)
Food × Salinity × Temperature							18	0.21	0.9997 (NS)
Duration of development									
Food ration (cells ml^{-1})	10^6	10^5	10^4	NA	NA	NA	2	13.37	0.0001
Salinity (‰)	35	30	25	20	NA		3	77.24	0.0001
Temperature (°C)	30	25	20	15			3	103.68	0.0001
Food × Salinity							6	14.29	0.0001
Food × Temperature							6	21.21	0.0001
Salinity × Temperature							9	8.14	0.0407
Food × Salinity × Temperature							18	2.87	0.0001

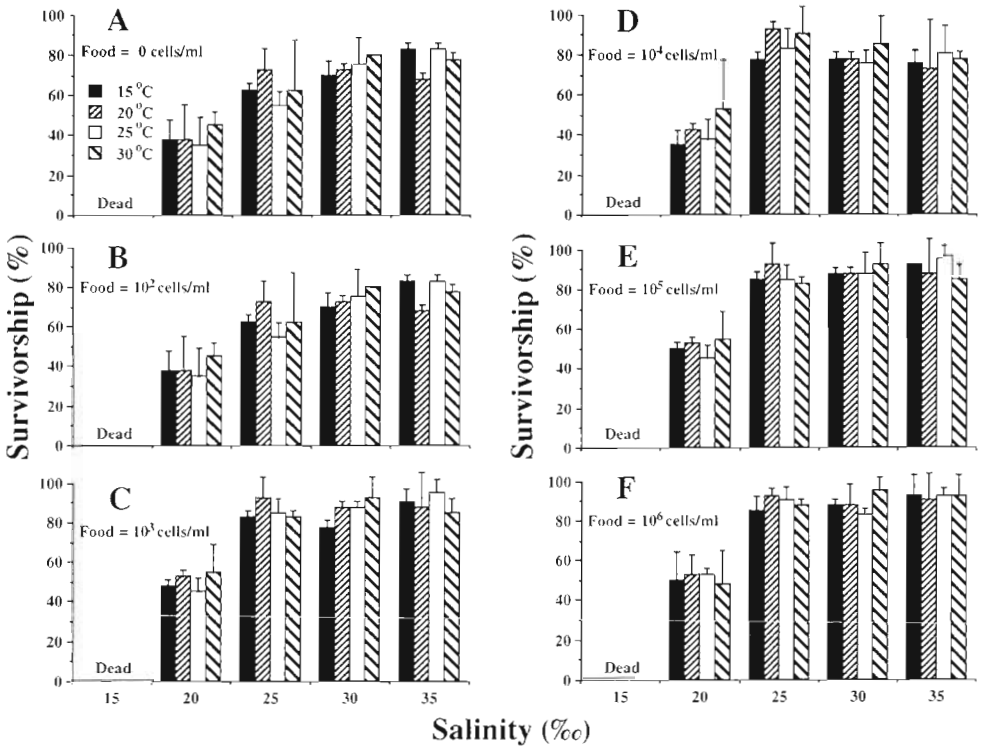

Fig. 2. *Hydroides elegans*. Expt IV: Effects of food concentration, salinity and temperature on survivorship from trochophore to newly-settled juvenile. Trochophores all died at salinity of 15‰. Data are plotted as means +SD of 2 replicate cultures (each started with 20 individuals)

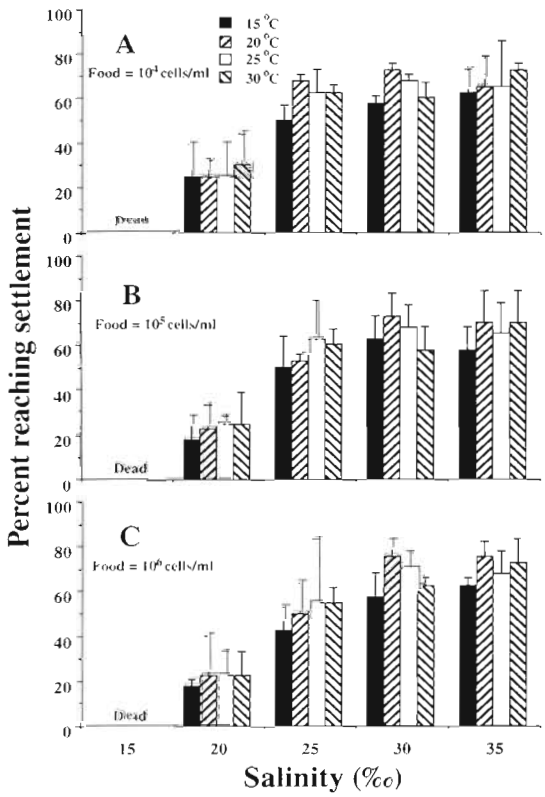

Fig. 3. *Hydroides elegans*. Expt IV: Effects of food concentration, salinity and temperature on percent reaching settlement from trochophore to newly-settled juvenile. Data are plotted as means + SD of 2 replicate cultures (each started with 20 individuals)

the duration of development were affected by temperature (Table 3). Percent trochophores reaching settlement was lower at the 15°C treatment than at the 3 higher temperature treatments (Fig 3). Duration of development was longest at 15°C, but progressively shorter with increasing temperature (Fig. 4).

Salinity strongly affected survivorship, percent reaching settlement, as well as duration of development (Table 3). None of the trochophores survived at 15‰. At 20‰, less than 40% survived to the end of experiment; at 25 to 35‰, 80 to 100% trochophores survived. Not all trochophores that survived through the experiment settled and began sessile life: only about 20 to 30% of the trochophores settled at 20‰; 42 to 67% settled at 25‰; and 57 to 75% settled at 30 and 35‰. Duration of development was about 7.5 d at 20‰; 6.7 d at 25‰; and 6.1 d at 30 and 35‰.

Food concentration affected survivorship, percent settlement and duration of development (Table 3, Figs. 2 to 4). Survivorship of trochophores increased with increasing *Isochrysis galbana* concentrations from 0 to 10^6 cells ml^{-1}. Except at 15‰ salinity where all trochophores died, at food concentrations $\leq 10^3$ cells ml^{-1}, 35 to 95% larvae survived through the 10 d experiment (Fig. 2). However, none of the survivors in treatments with $\leq 10^3$ cells ml^{-1} were able to settle. Percent reaching settlement was similar at algal concentrations $\geq 10^4$ cells ml^{-1}. Trochophores at food concen-

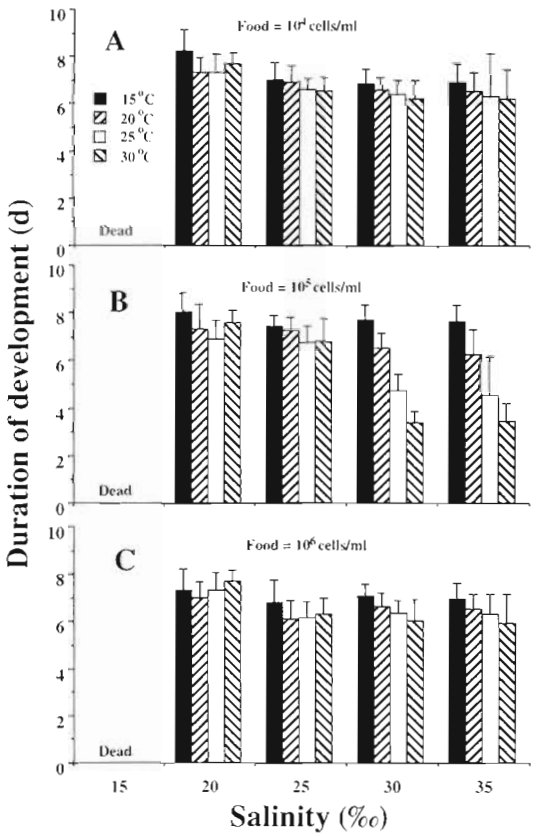

Fig. 4. *Hydroides elegans*. Expt IV: Effects of food concentration, salinity and temperature on duration of development from trochophore to newly-settled juvenile. Data are plotted as means + SD of 2 replicate cultures (each started with 20 individuals)

tration of 10^5 cells ml^{-1} developed fastest. Those at 10^4 and 10^6 cells ml^{-1} had a similar but longer duration of development.

Interaction between food and salinity, and between salinity and temperature was detected for survivorship, with the highest survivorship found at higher salinities and higher food concentrations (Table 3, Figs. 2 to 4). Interactions between food and salinity, between food and temperature, between salinity and temperature, and among food, salinity and temperature were detected for duration of development, with the fastest rate of development (3.43 ± 0.79 and 3.39 ± 0.5 d) found at 30°C temperature, 30 and 35‰ salinity, and 10^5 cells ml^{-1} *Isochrysis galbana* (Table 3, Fig. 4).

At the end of the 10 d experiment, none of the survivors at food concentrations $\leq 10^3$ cells ml^{-1} had settled. When feeding was resumed for an additional 7 d with *Isochrysis galbana* (10^5 cells ml^{-1}), settlement still did not occur These larvae gradually died off. Few larvae survived to Day 7, and those that did had become sluggish.

DISCUSSION

In many places, peaks of *Hydroides elegans* settlement occur in summer or autumn (Skerman 1958, Wisely 1958, Reish 1961, Kawahara 1969, Li et al. 1982, Zhang et al. 1984), and coincide with the high water temperature. In Hong Kong, however, settlement of *H. elegans* peaks in early spring to early summer when the seawater temperature is still low and salinity is high (Greene & Morton 1976, Hon 1978, Qian unpubl. data). Structures submerged in Hong Kong waters for several months during winter and spring can be coated by *H. elegans* with a thickness and wet-weight reaching 3.8 cm and 12.5 kg m^{-2}, respectively (Wang & Huang 1993). However, only few individuals survive through the summer (Greene & Morton 1976). The sharp decline in *H. elegans* adult population and larval settlement in summer could be due to the drastic drop in salinity caused by heavy rainfall and large amount of fresh water input from the Pearl River during these months. This argument is supported by both laboratory experiments and field surveys. For example, Mak & Huang (1982) exposed naked *H. elegans* adults to salinities from 0 to 26‰ for up to 50 h and concluded that the lowest salinity in which *H. elegans* populations could grow was between 15 and 20‰. We carried out a similar experiment, but used *H. elegans* adults with intact tubes (Qiu & Qian unpubl. data). Worms were placed in 24 combinations of salinity (10 to 35‰) and temperature (15 to 30°C) for 72 h. Temperature had no effect on survival. Over 85% of adult survival was obtained at salinities ≥20‰, but at salinities ≤15‰, all worms died within 24 h. A survey (Wang & Huang 1993) of the fouling community in Hong Kong and adjacent waters showed that *H. elegans* did not occur in the western waters where fresh water input from the Pearl River is high, and the salinity can drop to below 10‰ during the wet summer season. *H. elegans* occurred only in the central and eastern waters where salinity is closer to oceanic level (Fig. 5). The experiments and field survey suggest that low salinity in summer may adversely affect the survival of *H. elegans* in Hong Kong waters.

Our experimental salinities (15 to 35‰) and temperatures (15 to 30°C) and food concentration (0 to 10^6 cells ml^{-1}) were selected to cover the ranges that *Hydroides elegans* larvae would likely experience in Hong Kong waters (Morton & Morton 1983, Thompson 1986, Cai 1990). Low salinity reduced survivorship and percent reaching settlement, and lengthened duration of development. The tolerances to experimental salinities, however, were different among developmental stages. Development from newly-released oocyte to 2-cell, from 2-cell to blastula, from blastula to trochophore, and from trochophore to newly-settled juvenile failed

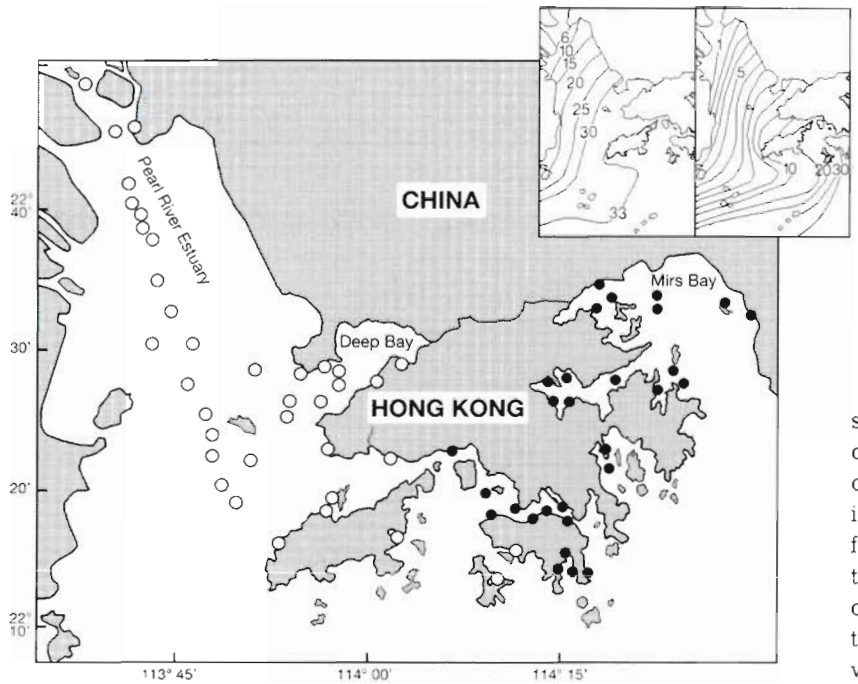

Fig. 5. Map drawn using data in Wang & Huang (1993) showing the distribution of *Hydroides elegans*, and, above, salinity profiles in January (left) and July (right) in Hong Kong and adjacent waters. (●) Presence of *H. elegans*, (○) absence of *H. elegans*

at salinities of ≤20, ≤20, ≤25, and 15‰, respectively (Figs. 1 & 2). Among these 4 larval stages, blastula to trochophore appears most likely to be limited by low salinity, as the lower limit for development to trochophore was 25 to 30‰, higher than the lower salinity limits for other embryonic stages and larval stage. Our results suggest that salinity can be the limiting factor for the early development of *H. elegans* in this area. The decline in salinity in Hong Kong waters during the summer monsoon could be responsible for the decline in settlement of *H. elegans*. Since the salinity resistance of the blastulae (25‰) was much lower than that of the juveniles (15‰) and adults (15‰), the blastula stage during embryonic development is most likely to be affected when salinity drops in the field. However, it still remains unclear how salinity and temperature will affect the reproductive output of *H. elegans* adult, which may alternatively affect the settlement in the field.

In contrast, although temperature effects were evident on the duration of development in both embryonic and larval stages, in which higher temperature led to faster rate of development (Figs. 1 to 3, Table 2), temperature had no effect on survivorship. Even at the lowest temperature (15°C), there was still a considerable number of embryos and larvae developed through the respective stage (Figs. 1 to 4). Our results suggest that temperature is not a limiting factor for early development and settlement of *Hydroides elegans* in this area, and may not be responsible for the decline of settlement in field populations during the summer.

Food concentration affected survivorship, percent reaching settlement and duration of development. Survivorship of trochophores increased with increasing *Isochrysis galbana* concentrations from 0 to 10^6 cells ml^{-1}. At concentrations $\leq 10^3$ cells ml^{-1}, no settlement occurred. This indicates that there is a threshold of food concentration below which larval development and settlement is compromised. In Hong Kong waters, the settlement peak commences in early spring, coinciding with the spring phytoplankton bloom (Thompson 1986, Cai 1990) which produced concentrations close to or above 10^3 cells ml^{-1}. Adverse effects of food limitation have been shown in many invertebrate species (cf. reviews by Pechenik 1987, Strathmann 1987). Extended duration of starvation may cause irreversible damage to larvae. For example, the larvae of 2 other polychaetes, *Capitella capitata* and *Polydora ligni*, were studied in laboratory experiments (Qian & Chia 1991). Although larvae survived at a wide range of phytoplankton concentrations (0.5 times to 50 times ambient seawater concentration), settlement occurred only at phytoplankton ≥10 times ambient seawater concentration for *C. capitata* and ≥5 times ambient concentration for *P. ligni*. Food-limited larvae of both species had higher mortality, grew poorly, had a lower settling rate, and a prolonged larval life span. In this study, larvae that were allowed to resume feeding after being kept in the culture with little or no *I. galbana* for 10 d did not settle. It appears that 10 d of food limitation exceeds the point at which *H. elegans* larvae retain the ability to develop to competence. For *C. capitata* and *P. ligni*, however, over 50% larvae were still able to complete metamorphosis if feeding resumed after 1 or 2 wk of starvation (Qian & Chia 1993).

Interaction between temperature and salinity on survivorship of *Hydroides elegans* was not detected in the experiments. However, interaction of temperature and salinity on duration of embryonic development from 2 cell to blastula (Table 2, Fig. 1d) was observed, with

the quickest development achieved under high temperature and high salinity conditions. Interactions between food and salinity, between food and temperature, between salinity and temperature, and among food, salinity and temperature were also detected for duration of larval development, with the fastest development occurring at a temperature of 30°C, salinity ≥30‰, and 10^5 cells ml^{-1} *Isochrysis galbana* (Table 3, Fig. 4).

Interaction among environmental factors may significantly influence larval settlement patterns of *Hydroides elegans*. In places where seasonal salinity fluctuations are minor, the population dynamics of *H. elegans* may be more dependent on the interaction of temperature and food. This explains the occurrence of settlement peaks in summer or autumn in many places (Skerman 1958, Wisley 1958, Reish 1961, Kawahara 1969, Li et al. 1982, Zhang et al. 1984). In Hong Kong waters, however, the drop in salinity during the summer appears to override the benefits of high temperature and results in the mortality of embryos, and eventually the decline in *H. elegans* settlement. From winter to early summer, the temperature in Hong Kong waters (>15°C) does not limit early development of *H. elegans* and salinity is optimal (≅34‰). A peak in settlement commences in early spring, corresponding to spring phytoplankton bloom (Thompson 1986, Cai 1990). This suggests that the increase in phytoplankton concentration from early spring to early summer may contribute to the formation of settlement peaks. Temperature, however, does not seem to be a limiting factor for early development and settlement of *H. elegans* in this area.

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