Marine Biology 115, 287–294 (1993)

Marine Biology
International Journal on Life in Oceans and Coastal Waters

Influence of delayed metamorphosis on survival and growth of juvenile barnacles *Balanus amphitrite*

J. A. Pechenik [1], D. Rittschof [2], A. R. Schmidt [2]

[1] Biology Department, Tufts University, Medford, Massachusetts 02155, USA
[2] Duke University Marine Laboratory, Beaufort, North Carolina 28516, USA

Received: 3 June 1992 / Accepted: 10 September 1992

Abstract. Competent cyprid larvae of the barnacle *Balanus amphitrite* Darwin were prevented from metamorphosing in the laboratory for 3 or 5 d using three different techniques (holding at low temperature, crowding, and detaining on a silanized surface). We then assessed the effects of prolonging larval life on post-metamorphic growth and survival, in comparison with control individuals that metamorphosed soon after they were competent to do so. Seven experiments were conducted over 2 yr (July 1987 to September 1989). In all experiments (each with six replicates per treatment), postponing larval metamorphosis for 3 or 5 d dramatically depressed post-metamorphic growth rate ($P < 0.05$), although metamorphic success and post-metamorphic survival were not affected ($P > 0.10$). The results suggest that *B. amphitrite* cyprids deferring their metamorphosis in the field may be less successful in competing for space, at least during the first few weeks of postlarval life.

Introduction

Following their metamorphosis to the cyprid stage, barnacle larvae subsist on stored nutrients until they locate a substrate and complete metamorphosis (Lucas et al. 1979, Crisp 1988). Cypris larvae exhibit settlement behaviors in response to flow, pressure, light, and surface texture and color (reviewed by Crisp 1974, Rittschof et al. 1984, Wethey 1986, Mullineaux and Butman 1991). In addition, chemical cues associated with adult barnacles (Knight-Jones 1953, Larman and Gabbott 1975, Moyse and Hui 1981, Yule and Crisp 1983, Dineen and Hines 1992), "footprints" left by exploring cyprids (Yule and Walker 1985), and bacterial films (Maki et al. 1988, 1989) can stimulate or inhibit larval settlement, as can the inherent physical and chemical properties (such as contour and critical surface energy) of the attachment surface (Wethey 1986, Rittschof and Costlow 1989, Roberts et al. 1991).

In the laboratory, newly competent invertebrate larvae will metamorphose upon contacting specific external cues but will typically postpone metamorphosis and keep swimming in their absence (reviewed by Pechenik 1990). For barnacles, the energy lost while larval life is prolonged eventually compromises the ability to metamorphose successfully, once larval energy reserves fall below the level needed to pay the energetic costs of metamorphosing (Lucas et al. 1979, Crisp 1988). *Balanus balanoides*, for example, can remain in the cyprid stage for up to 28 d at 10 °C before this energy limit is reached; thereafter, larvae do not metamorphose successfully (Lucas et al. 1979).

In the laboratory, prolonging the larval life of at least some invertebrates with lecithotrophic larvae results in lower post-settlement survival (the polychaete *Capitella* sp. I: Pechenik and Cerulli 1991) or reduced post-metamorphic growth rate (the bryozoan *Bugula stolonifera*: Woollacott et al. 1989). We examine here the consequences of extending the cyprid stage of *Balanus amphitrite* for 3 to 5 d in the laboratory, reporting effects on metamorphic success, juvenile survival, and juvenile growth.

Materials and methods

Balanus amphitrite Darwin, an acorn barnacle occurring on both coasts of the U.S. and throughout the world, exhibits six feeding naupliar stages and a terminal, non-feeding cyprid stage (Rittschof et al. 1984, Raimondi 1992). Nauplii were obtained from adult *B. amphitrite* held at the Duke University Marine Laboratory at 28 °C and cultured as described elsewhere (Branscomb and Rittschof 1984, Rittschof et al. 1984). Most larvae metamorphosed to the cyprid stage within 4 d. Cyprids were then haphazardly subsampled from a large batch culture. Some were immediately allowed to metamorphose on untreated, 5 cm diameter polystyrene dishes at 28 °C; these individuals served as controls in our experiments.

Metamorphosis of the remaining larvae in each experiment was prevented for the next 3 or 5 d, using one or more of three different techniques (referred to as 6C, DMS, or CRD). For treatment 6C, cyprids were maintained at 6 °C; this prevented metamorphosis, and presumably depressed metabolic and developmental rates substan-

tially as well. In treatment DMS, cyprids were held at 23 °C in glass vials whose surfaces were silanized with a multilayer of dimethyl silane. Coating glass with dimethyl silane converts the surface from hydrophilic to lipophylic, so that it binds weakly to the larval carapace. The contact area between the larvae and the glass surface is small, and the strength of attachment only slightly exceeds the ability of a larvae to dislodge itself by swimming. Entrapped larvae can spin in place or move slowly across the surface on their sides. However, they cannot contact the surface with their antennules and do not metamorphose. The DMS treatment should not have directly depressed either developmental rate or metabolic rate. Finally, in treatment CRD ("crowded"), metamorphosis was prevented by maintaining cyprids in small volumes of water at concentrations exceeding 400 larvae ml^{-1} at 23 °C; this treatment probably has a relatively minor effect on metabolic rate. After 3 or 5 d, we promoted metamorphosis by either: (1) warming larvae to 28 °C (treatment 6C); (2) gently rinsing trapped larvae (treatment DMS) off the surface and incubating them at 10 larvae ml^{-1} in 5 cm diameter polystrene dishes at 28 °C; or (3) diluting larvae to approximately 10 larvae ml^{-1} and raising the temperature to 28 °C (treatment CRD).

The percentage of cyprids attaching to the surfaces was recorded in four experiments; most cyprids that attach securely metamorphose to the feeding juvenile stage within 6 h (Rittschof personal observation). Six replicates of metamorphosed juveniles were established per treatment, held at room temperature (22 to 25 °C), and fed either the naked flagellate *Dunaliella tertiolecta* or the chain-forming diatom *Skeletonema costatum*.

In six of the seven experiments conducted, all juveniles were shipped overnight Federal Express to Tufts University 2 to 3 d after the 5-d delay treatment group of cyprids was induced to metamorphose; juveniles were then reared for an additional 1 to 4 wk on a diet of *Dunaliella tertiolecta* at room temperature (22 to 25 °C). The number of juveniles $dish^{-1}$ was recorded upon arrival; dishes typically arrived with 20 to 50 individuals. If any dishes contained more than 25 individuals or if some individuals were in close proximity, excess individuals were removed and the remaining individuals were thinned so that no barnacle was closer than about 0.5 cm to its nearest neighbor. This equalized the number of individuals on each dish and minimized the potential effects of crowding on growth rates and shell shape (Bertness 1989, Bertness et al. 1991). We also removed any individuals growing on the sides of the dishes. There were usually at least 11 barnacles remaining in each dish.

Dishes of juveniles were attached to strips of plexiglass (4 to 5 dishes $strip^{-1}$), which were then suspended vertically in a single 20-liter glass aquarium; dishes within treatment groups were haphazardly distributed among plexiglass strips and within the aquarium tank to compensate for potentially localized differences in aeration and water circulation. There was no temporal overlap between experiments, so that dishes from different experiments were never mixed together in the aquarium. Seawater (ca. 30 ppt salinity) was collected from Nahant, Massachusetts and filtered to 1 μm before use. Water was changed every other day, and phytoplankton was added daily to a concentration of approximately 5×10^4 cells ml^{-1}. At each water change, all individuals were gently brushed to remove algal debris and biological films, and the dishes were then haphazardly redistributed on the plexiglass strips and within the tank. All individuals within an experiment were thus reared under comparable conditions within the same body of water. Barnacles were typically 1 to 2 mm in basis length (diameter from rostrum to carina) when placed in the rearing tank, and typically doubled in size by the end of each experiment, following 1 to 4 wk of juvenile rearing. At the end of an experiment, all barnacles in each dish were measured (Exp. I, IV, V, and VI only), carefully removed from the substratum, rinsed in distilled water, and grouped in pre-weighed foil pans for weighing. Individuals prevented from metamorphosing for 3 or 5 d were reared for 3 or 5 d longer than controls. Thus, within an experiment, barnacles in all treatments were reared for the same amount of time after they metamorphosed.

Pans of juveniles were dried at 75 °C for 48 h and then weighed to the nearest 0.1 mg on a Cahn Model 21 electrobalance, with indicating $CaSO_4$ (Drierite) placed in the weighing chamber to deter specimen rehydration. Subsequent drying for an additional 24 h caused no further weight loss. Pans were reweighed after ashing for 6 to 8 h at 525 °C, and individual tissue weight was estimated from the total weight lost (Paine 1964), divided by the number of ind. pan^{-1}.

The final experiment (Exp VI) differed from the previous six in that we considered whether rearing conditions were less suitable at Tufts University than at the Duke University marine laboratory; such a difference in rearing conditions would selectively disadvantage individuals whose metamorphosis had been prevented since they would have been deprived of up to 5 d of post-metamorphic culture under optimal conditions. Exp VI was therefore similar to previous experiments, except that juveniles were reared for 2 wk at the Duke Lab and then sent to Tufts for immediate measurement and weighing.

In the final experiment we also preserved some cyprid larvae from each treatment group just before other individuals in the same cohort were allowed to metamorphose. About 150 cyprids from each treatment were preserved in 10% formalin saturated with sodium borate to maintain a pH of about 8. These individuals were then rinsed, weighed in groups of 20 (with six replicates $treatment^{-1}$), ashed for 5 h at 525 °C, and reweighed as before to determine the average amount of weight lost when cyprids were prevented from metamorphosing for 3- or 5-d periods.

The relationship between basis length and ash-free dry weight of post-metamorphic individuals was determined in a separate study by measuring, weighing, and ashing 19 individuals selected from a group whose cyprids were allowed to metamorphose within 12 h of their molt to that stage. The individuals were killed from 1 to 3 wk after metamorphosis, and ranged between about 2.8 and 5.4 mm in basis length.

Table 1 summarizes the treatments used and measurements made in each of the seven experiments conducted. Treatment effects were assessed by one-way ANOVA followed by Bonferroni pairwise comparisons where appropriate.

Results

Larval weight loss, attachment, and metamorphosis

We detected no significant effect of delaying metamorphosis on mean cyprid weight ($F = 0.48$; $df = 2.15$; $P = 0.63$); the average dry tissue weight per cyprid was 4.12 μg $ind.^{-1}$ ($SE = 0.11$; $N = 18$ replicates).

The percentage of cyprids that attached when offered suitable substrate was typically greater ($P < 0.05$) for older larvae than for control larvae whose metamorphosis had not been postponed (Fig. 1). This reflects the declining selectivity of aging cyprids (Rittschof et al. 1984, Crisp 1988). Percent attachment was not significantly affected by the treatments used to prevent metamorphosis (Fig. 1, $P > 0.10$), implying that cyprids were not damaged by the treatments. Moreover, postponing metamorphosis for 3 d vs 5 d typically had little effect on mean percent attachment. In Expt IV, however, a much higher percentage of the cyprids held for 5 d at high concentrations (treatment CRD) attached to the substrate compared with larvae from the other two treatments settling on the same day; many of the oldest cyprids from the other two treatments in this experiment (6C and DMS, 5 d delay) may have lost the ability to metamorphose in comparison with individuals whose metamorphosis was prevented for only 3 d (Fig. 1).

Table 1. *Balanus amphitrite*. Summary of experiments. Date of experiment given in first column. + indicates that designated treatments were performed or that designated measurements were made

Experiment		Metamorphosis prevented		Treatments used			Percent attached	Percent recovered	Final basis diameters	Final tissue weights
		3 d	5 d	6C	DMS	CRD				
A	(Jul 1987)	+	+	+	+	+ (3 d only)	+	−	Experiment terminated due to poor juvenile survival	
I	(Aug 1987)	+	+	−	+	−	−	−	+	+
II	(Dec 1987)	+	+	−	+	−	+	+	−	+
III	(Aug 1988)	+	+	+	+	+ (3 d only)	−	+	−	+
IV	(Nov 1988)	+	+	+	+	+	+	+	+	+
V	(Apr 1989)	+	+	+ (5 d only)	−	+	+	+	+	+
VI	(Aug 1989)	−	+	+	−	−	−	−	+	+

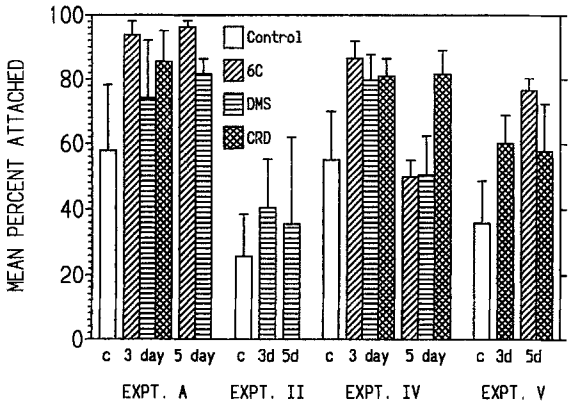

Fig. 1. *Balanus amphitrite*. Influence of prolonged larval life and treatment used to prevent metamorphosis on attachment of cyprids to polystyrene dishes. Each bar represents the mean (±SD) of six replicates, typically involving a total of 300 to 600 cyprids. Treatments (6C, DMS, CRD) are described in "Materials and methods"

Survival of metamorphosed individuals

Loss of individuals reflects either natural mortality or accidental dislodgement during routine cleaning, so that the percentages reported may underestimate juvenile survival. Most of the barnacles arriving at Tufts (and not thinned out) were recovered at the end of the study for weighing, indicating that juveniles survived well in all treatments; in the four experiments in which initial numbers were recorded, an average of 85 to 100% of those individuals were recovered for measuring and/or weighing after 1 to 4 wk of laboratory growth.

In Expts II and III, duration of larval life had no statistically significant effect on percentage of individuals recovered ($P > 0.10$). In Expts IV and V, however, recovery of individuals prevented from metamorphosing for 5 d was significantly lower ($P < 0.05$) than that of control individuals (those allowed to metamorphose on Day 0); in Expt IV we recovered 100 (±0)% of control individuals and only 86 (±11)% of 5-d delay individuals, while in Expt V we recovered 98 (±4)% of control individuals and only 85 (±6)% of 5-d delay individuals (mean % ± SD, $N = 6$ replicates treatment^{-1}). Statistically significant ($P < 0.05$) treatment effects on percent recovery were also observed, but effects of any particular treatment were not consistent within or among experiments (Table 2). Even so, at least 85% of the individuals survived to the end of all four experiments in which survival was estimated.

Influence of delayed metamorphosis on shell growth

Control barnacles grew substantially in all experiments. In Expt IV, for example, control barnacles averaged 2150 μm in basis length (SD = 314 μm, $N = 12$) when first measured, and over 3900 μm in basis length (SD = 584 μm, $N = 122$) ca. 3 wk later. The large variation in growth of control barnacles among experiments likely reflects variation in room temperature and experiment duration. Comparisons must be made within experiments, not among experiments. In no case did we detect any significant within-treatment variation in final basis lengths for control or treated groups (5 to 6 dishes of barnacles treatment^{-1}, averaging 21 barnacles dish^{-1}) ($P > 0.10$). Where multiple treatments were used to prevent metamorphosis (e.g. Expts IV and V), treatment effects on basis growth were significant only in Expt IV: in that experiment, final basis diameters were smaller if metamorphosis was prevented by maintaining cyprids at low temperature (treatment 6C), but no such temperature effect was observed in Expt V (Table 3).

Most importantly, statistically significant treatment effects were minor compared with the effect of delayed metamorphosis on subsequent shell growth (Fig. 2). For Fig. 2, we have combined data from all treatments to facilitate presentation; examining the effect of delaying metamorphosis for any particular treatment does not alter our conclusions.

Table 2. *Balanus amphitrite.* Effect of treatment used to prevent metamorphosis on juvenile survival (estimated from recovery of juveniles at the end of an experiment). Horizontal lines link treatment groups that are statistically equivalent (using Bonferroni's multiple comparison procedure) ($P>0.10$)

Experiment	Delay period	Treatments	ANOVA results
III	3 d[a]	6C CRD DMS	$F=7.5$ (2, 15) $P=0.0056$
	5 d	6C DMS	$F=2.1$ (1, 10) $P=0.17$
IV	3 d	DMS CRD 6C	$F=0.82$ (2, 15) $P=0.46$
	5 d[a]	CRD DMS 6C	$F=5.5$ (2, 15) $P=0.02$
V	5 d	CRD 6C	$F=1.4$ (1, 9) $P=0.26$

[a] Source of treatment differences ($P<0.05$) indicated in column 3

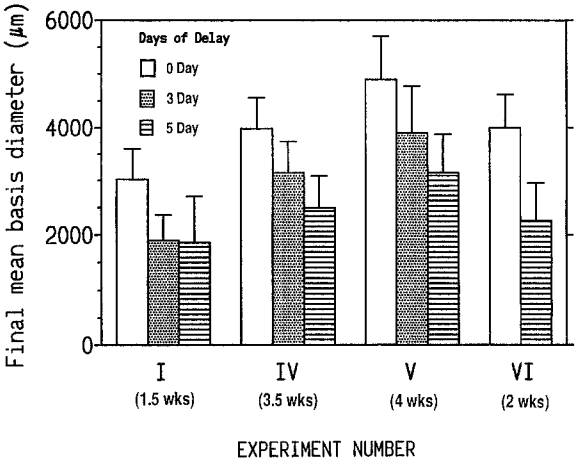

Fig. 2. *Balanus amphitrite.* Influence of delayed metamorphosis on basis growth. Each bar represents the mean (±SD) of six to eight replicates and measurements on 67 to 152 ind. treatment^{-1}. Numbers in parentheses below X-axis indicate duration of juvenile rearing period

Table 3. *Balanus amphitrite.* Effect of treatment used to prevent metamorphosis on final mean basis diameter. For statistical comparisons, horizontal lines connect statistically equivalent ($P>0.10$) treatments (Bonferroni multiple comparisons test)

Experiment	Treatment	Mean shell length (µm) ±1 SD	N	Statistical comparisons	ANOVA results
IV					
3d delay	6C	3051±604	152	CRD DMS 6C	$F=9.61$
	CRD	3388±640	125		df=2,349
	DMS	3196±692	75		$P<0.0001$
5d delay	6C	2290±518	122	CRD DMS 6C	$F=15.1$
	CRD	2688±570	111		df=2, 297
	DMS	2558±623	67		$P=0.0001$
V					
5d delay	6C	3091±68	110	CRD 6C	$F=0.33$
	CRD	3147±730	99		df=1, 207
					$P=0.57$

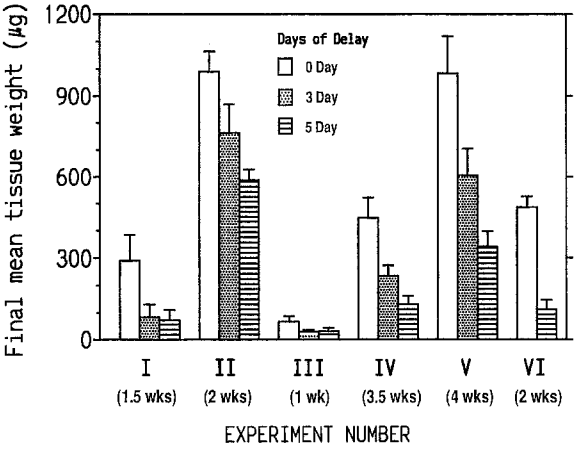

Fig. 3. *Balanus amphitrite.* Influence of delayed metamorphosis on tissue growth. Each bar represents the mean (±SD) of five to six replicates, typically with six to 18 individuals weighed in each. Numbers in parentheses below X-axis indicate duration of juvenile rearing period

In every experiment, barnacles grew more slowly if metamorphosis was prevented for at least 3 d (Fig. 2), regardless of treatment. In Expts IV and V, preventing metamorphosis for 5 d slowed the average juvenile growth rate even more ($P<0.05$). Note that the observed effects of postponing metamorphosis on growth were also marked in Expt VI, in which juveniles were maintained entirely at the Duke University Marine Laboratory. Thus, the suppressed growth rates clearly result from postponing metamorphosis; they are not shipping artifacts.

Influence of postponing metamorphosis on juvenile growth rate

Our data are most complete with respect to the effects of delayed metamorphosis on post-metamorphic rates of tissue growth, in that we have data for six experiments. With the exception of Expt IV, results were statistically equivalent ($P>0.10$) regardless of how metamorphosis was prevented; in Expt IV, juveniles held at low temperature (treatment 6C) weighed less, on average, than did individuals from the other two treatments, whether metamorphosis was prevented for 3 d or 5 d (Table 4). When treatment effects were observed, those effects were minor compared with the effects of postponing metamorphosis, so that we have combined data from all treatments for graphical presentation; the conclusions are identical when the effects of postponing metamorphosis are considered separately for the different treatments.

In all six experiments (and for every treatment within an experiment), postponing metamorphosis for 3 d correlated with dramatically decreased rates of tissue growth compared with growth of control individuals (Fig. 3). In Expts IV and V, tissue growth rates were further depressed ($P<0.05$) by postponing metamorphosis for an additional 2 d (Table 5).

Table 4. *Balanus amphitrite*. Effect of treatment used to prevent metamorphosis on final juvenile tissue weights. For statistical comparisons, horizontal lines connect statistically equivalent means (Bonferroni multiple comparisons test)

Experiment	Treatment	Mean weight (μg) ±SD	*N*	Statistical comparison	ANOVA (df)
III					
3 d delay	DMS	24.4± 7.1	7	No difference between means	$F=2.24$ (2, 16)
	6C	26.6± 8.1	6		$P=0.13$
	CRD	32.9± 6.8	6		
5 d delay	DMS	29.9±16.9	6	No difference between means	$F=0.02$ (1, 10)
	6C	28.7± 8.3	6		$P>0.80$
IV					
3 d delay	DMS	233.0±31.0	5		
	6C	205.8±44.2	5		
	CRD	268.0± 8.9	6	6C DMS CRD	$F=5.77$ (2, 13)
					$P=0.02$
5 d delay	DMS	142.0±33.6	6	No difference between means	$F=4.57$ (2, 15)
	6C	102.2±15.4	6		$P=0.03$
	CRD	148.4±33.2	6		
V					
5 d delay	6C	314.8±52.7	6	No difference between means	$F=0.60$ (1, 10)
	CRD	339.5±57.4	6		$P=0.46$

Table 5. *Balanus amphitrite*. Effect of delayed metamorphosis on final mean tissue weight (data from all treatments combined). In the final column, horizontal lines connect treatment groups with statistically equivalent ($P>0.10$) means (Bonferroni multiple comparison tests)

Experiment	ANOVA	Statistical comparison		
I	$F=$ 18.3 (2, 13)	0 d	3 d	5 d
II	$F=$ 25.0 (2, 9)	0 d	3 d	5 d
III	$F=$ 33.1 (2, 39)	0 d	3 d	5 d
IV	$F=$ 29.3 (2, 35)	0 d	3 d	5 d
V	$F=$106.4 (2, 21)	0 d	3 d	5 d
VI	$F=$ 99.8 (2, 13)	0 d	5 d	

Table 6. *Balanus amphitrite*. Influence of prolonged larval life and method of postponing metamorphosis on the relationship between basis diameter and ash-free dry weight. In all cases where significant differences were found, actual weights exceeded predicted weights

Experiment	Delay period	Treatment	*F*-value (df)	Significance (*P*)
I	0 d	Control	$F=$ 0.005 (1, 7)	>0.80
	3 d	DMS	$F=$ 0.003 (1, 7)	>0.80
	5 d	DMS	$F=$ 0.008 (1, 7)	0.78
IV	0 d	Control	$F=$ 2.5 (1, 10)	0.14
	3 d	6C[a]	$F=$ 5.1 (1, 9)	0.05
		CRD[a]	$F=$ 24.2 (1, 9)	<0.001
		DMS[a]	$F=$ 7.8 (1, 10)	0.02
	5 d	6C	$F=$ 0.16 (1, 10)	0.70
		CRD	$F=$ 1.41 (1, 10)	0.26
		DMS	$F=$ 0.72 (1, 10)	0.41
V	0 d	Control	$F=$ 3.7 (1, 10)	0.09
	3 d	CRD	$F=$ 3.7 (1, 10)	0.08
	5 d	CRD	$F=$ 4.5 (1, 10)	0.06
VI	0 d	Control	$F=$ 0.28 (1, 11)	0.60
	5 d	6C	$F=$ 0.23 (1, 9)	0.64

[a] Means are significantly different

Effects of postponing metamorphosis on juvenile morphology

The relationship between juvenile tissue weight and basis diameter for control barnacles (no delay) was: $\log Y=2.621 \log X-6.765$ ($r^2=0.96$, $N=19$), where Y is tissue weight in μg and X is basis diameter in μm. To determine whether delaying metamorphosis had any effect on the pattern of individual juvenile growth, this equation was used to predict mean final tissue weights from the basis diameter data obtained in Expts I, IV, V, and VI. These predicted values were then compared (one-way analysis of variance) with mean tissue weights determined directly.

Although measured mean tissue weights differed significantly from weights predicted from basis length measurements in several instances, there is no indication that any particular treatment consistently altered the relationship between length and tissue weight (Table 6).

Discussion

The capacity of competent larvae to delay metamorphosis has traditionally been viewed in terms of benefits likely accruing to the individual and to the species. These benefits include increased likelihood of locating potentially suitable habitats for metamorphosis and increased dispersal potential, with concomitant increased gene flow and decreased extinction rates (reviewed by Thorson 1950, Scheltema 1971, 1986, Hansen 1978, Hedgecock 1986, Jablonski 1986). To the extent that post-metamor-

phic development is not compromised by delaying metamorphosis, these benefits may be fully realized. The gastropod *Crepidula fornicata*, for example, sustains no measurable cost from delaying metamorphosis in terms of survival, metabolism, or growth rate, at least for the first few weeks of juvenile life (Pechenik and Eyster 1989). Similarly, prolonging larval life of the nudibranch *Phestilla sibogae* for up to 3 wk did not affect time to reproductive maturity, length of adult life, or adult reproductive output (Miller and Hadfield 1990). Larvae of *C. fornicata* and *P. sibogae* feed throughout their planktonic lives, so that postponing metamorphosis imposes no apparent nutritional burden upon the larvae. Similarly, prolonging the larval period has no apparent effect on post-metamorphic growth in the coral reef fish *Semicossyphus pulcher* (Cowen 1991).

In contrast, the bryozoan *Bugula stolonifera* developed more slowly following metamorphosis if its larvae were kept swimming for as little as 8 to 10 h after their emergence from the parent ovicell (Woollacott et al. 1989). Prolonging larval life of the polychaete *Capitella* sp. I by 3 to 5 d at 25 °C also affected post-metamorphic fitness: post-settlement survival was reduced, although growth rate, fecundity, and time to reproductive maturity were not affected (Pechenik and Cerulli 1991). The larvae of these species cannot feed on particulates, so that the effect of prolonged swimming on growth or survival may somehow be mediated through nutritional stress. Surprisingly, delaying metamorphosis of the polychaete *Polydora ligni* in the laboratory decreased the ability of larvae to metamorphose successfully, decreased post-metamorphic growth rates, and decreased the extent of gonadal development (Qian et al. 1990), despite the fact that these are planktotrophic larvae; the detrimental effects observed could reflect nutritional inadequacy of the food provided (or other suboptimal culture conditions) during the larval period, a possibility that applies to experiments with any planktotrophic species showing reduced post-metamorphic fitness with an extended planktonic period.

The present data for *Balanus amphitrite* are consistent with detrimental effects of prolonged larval life being mediated through nutritional stress, since cyprid larvae do not feed (Lucas et al. 1979). The loss of ability to attach to substrates implied for many of the cyprids in Expt IV (Fig. 1, treatments 6C and DMS) is consistent with the idea of nutritional stress, but the effect was not observed in other experiments. Moreover, it is puzzling that the effect of deferring metamorphosis was comparably pronounced regardless of how we prevented metamorphosis. We might expect a less pronounced effect in treatment 6C, since nutrient stores should be depleted much more slowly at reduced temperature. Depression of juvenile growth rates may be mediated through different mechanisms in the different treatments. The abnormal metamorphosis documented by Crisp (1988) when cyprids of *B. amphitrite* were maintained for at least several days at 4 °C was not observed for cyprids maintained at 6 °C in our experiments.

Although Expt VI assessed whether our results might simply reflect a difference in juvenile rearing conditions between our two laboratories, we did not control for variation in degree of crowding during the juvenile culture period in any experiment. In each experiment, the first individuals induced to metamorphose (Day 0 controls) did not compete for food with individuals from other treatment groups for their first 3 d of post-metamorphic development, but the last individuals induced to metamorphose (on Day 5) were always placed among already-growing juveniles from all other treatments, and so may have experienced some competition for food. Therefore, we cannot rule out the possibility that our experimental design favors the growth of individuals that have experienced the shortest planktonic periods. This seems unlikely, however, as we added phytoplankton to the aquarium twice daily, and phytoplankton concentrations were always at least 1.5×10^4 cells ml^{-1} in spot checks during the experiment. Moreover, as juveniles were removed for weight determinations toward the end of each experiment, the number of juveniles remaining in the aquarium obviously diminished, giving a compensatory potential growth advantage to those barnacles with the longest period of planktonic life.

Barnacles have been widely used in studying the factors that determine the dynamics of intertidal populations (e.g. Connell 1961, 1985, Strathmann et al. 1981, Wethey 1983, 1984, Gaines and Roughgarden 1987, Bertness 1989, Hughes 1990, Raimondi 1990, 1991, Sutherland 1990, Bertness et al. 1991). These studies have focused on the roles of larval supply, predation, competition, food supply, and susceptibility to physical stress in determining the survival and growth of barnacle individuals and populations. Our results suggest another factor of potential importance: Delaying metamorphosis may be a double-edged sword for *Balanus amphitrite*, increasing an individual's chance of locating a site appropriate for metamorphosis, but simultaneously reducing that individual's ability to compete for space during at least the first few weeks of juvenile life.

Lucas et al. (1979) prevented cyprids from metamorphosing by violently agitating larval cultures. The three techniques we used to deter metamorphosis mimic, to some extent, situations that may occur naturally. For example, preventing metamorphosis by low temperature likely occurs over much of the northern and southern extremes of the range of *Balanus amphitrite*. In Beaufort, North Carolina, *B. amphitrite* cyprids are collected throughout the year (Sutherland 1984), with seawater temperatures falling below 6 °C for extended periods. The crowding technique (treatment CRD) of preventing metamorphosis probably exploits the known inhibition of cyprid attachment by high concentrations of barnacle settlement pheromone (Tegtmeyer and Rittschof 1989); the pheromone is apparently associated with temporary adhesive secreted onto a surface by exploring cyprids (Yule and Walker 1985). Finally, *B. amphitrite* commonly become entrapped in surface films, a situation analogous to our DMS treatment; in both cases, entrapped larvae do not metamorphose.

In the field, cyprids defer metamorphosis naturally, rejecting some substrates that lack certain chemical or physical characteristics (Crisp 1974, 1988, Mullineaux

and Butman 1991, Raimondi 1991, Roberts et al. 1991) and rejecting others that discourage metamorphosis directly (Moyse and Hui 1981, Maki et al. 1988). In some cases, competent cyprids may simply be advected away from the coastline and encounter no solid substrate suitable for metamorphosis (Roughgarden et al. 1988). The extent to which metamorphosis of *B. amphitrite* is postponed in the field and the extent to which the costs of delaying metamorphosis documented here are realized remain to be evaluated.

Acknowledgements. We thank K. M. Zimmerman and L. S. Eyster for dedicated and careful laboratory assistance and V. Ricciardone for helping to type the manuscript and tables. The comments of L. S. Eyster, R. Olson's 1992 larval ecology class, and two anonymous reviewers on a draft of the manuscript are gratefully acknowledged.

Literature cited

Bertness, M. D. (1989). Intraspecific competition and facilitation in a northern acorn barnacle population. Ecology 70: 257–268

Bertness, M. D., Gaines, S. D., Bermudez, D., Sandford, E. (1991). Extreme spatial variation in the growth and reproductive output of the acorn barnacle *Semibalanus balanoides*. Mar. Ecol. Prog. Ser. 75: 91–100

Branscomb, E. S., Rittschof, D. (1984). An investigation of low frequency sound waves as a means of inhibiting barnacle settlement. J. exp. mar. Biol. Ecol. 79: 149–154

Connell, J. H. (1961). The influence of interspecific competition and other factors on the distribution of the barnacle *Chthamalus stellatus*. Ecology 42: 710–723

Connell, J. H. (1985). The consequences of variation in initial settlement vs. post-settlement mortality in rocky intertidal communities. J. exp. mar. Biol. Ecol. 93: 11–45

Cowen, R. K. (1991). Variation in the planktonic larval duration of the temperate wrasse *Semicossyphus pulcher*. Mar. Ecol. Prog. Ser. 69: 9–15

Crisp. D. J. (1974). Factors influencing the settlement of marine invertebrate larvae. In: Grant, P. T., Mackie, A. M. (eds.) Chemoreception in marine organisms. Academic Press, New York, p. 177–265

Crisp, D. J. (1988). Reduced discrimination of laboratory-reared cyprids of the barnacle *Balanus amphitrite amphitrite* Darwin, Crustacea Cirripedia, with a description of a common abnormality. In: Thompson, M. F., Sarojini, R., Nagabushanam, R. (eds.) Marine biodeterioration. Oxford and IBH Publ. Co., New Delhi, p. 409–432

Dineen, J. F. Jr., Hines, A. H. (1992). Interactive effects of salinity and adult extract upon settlement of the estuarine barnacle *Balanus improvisus* (Darwin, 1854). J. exp. mar. Biol. Ecol. 156: 239–252

Gaines, S. D., Roughgarden, J. (1987). Fish in offshore kelp forests affect recruitment to intertidal barnacle populations. Science, N. Y. 235: 479–481

Hansen, T. A. (1978). Larval dispersal and species longevity in lower tertiary gastropods. Science, N. Y. 199: 885–887

Hedgecock, D. (1986). Is gene flow from pelagic larval dispersal important in the adaptation and evolution of marine invertebrates? Bull. mar. Sci. 39: 550–564

Hughes, T. P. (1990). Recruitment limitation, mortality, and population regulation in open systems: a case study. Ecology 71: 12–20

Jablonski, D. (1986) Larval ecology and macroevolution in marine invertebrates. Bull. mar. Sci. 39: 565–587

Knight-Jones, E. W. (1953). Laboratory experiments on gregariousness during settling in *Balanus balanoides* and other barnacles. J. exp. Biol. 30: 584–598

Larman, V. N., Gabbott, P. A. (1975). Settlement of cyprid larvae of *Balanus balanoides* and *Elminius modestus* induced by extracts of adult barnacles and other marine animals. J. mar. biol. Ass. U.K. 55: 183–189

Lucas, M. I., Walker, G., Holland, D. L., Crisp. D. J. (1979). An energy budget for the free-swimming and metamorphosing larvae of *Balanus balanoides* (Crustacea: Cirripedia). Mar. Biol. 55: 221–229

Maki, J. S., Rittschof, D., Costlow, J. D., Mitchell, R. (1988). Inhibition of attachment of larval barnacles, *Balanus amphitrite*, by bacterial surface films. Mar. Biol. 97: 199–206

Maki, J. S., Rittschof, D., Schmidt, A. R., Snyder, A. G., Mitchell, R. (1989). Factors controlling attachment of bryozoan larvae: a comparison of bacterial films and unfilmed surfaces. Biol. Bull. mar. biol. Lab., Woods Hole 177: 295–302

Miller, S. E., Hadfield, M. G. (1990). Developmental arrest during larval life and life-span extension in a marine mollusc. Science, N. Y. 248: 356–358

Moyse, J., Hui, E. (1981). Avoidance by *Balanus balanoides* cyprids of settlement on consepecific adults. J. mar. biol. Ass. U.K. 61: 449–460

Mullineaux, L. S., Butman, C. A. (1991). Initial contact, exploration and attachment of barnacle (*Balanus amphitrite*) cyprids settling in flow. Mar. Biol. 110: 93–103

Paine, R. T. (1964). Ash and caloric determinations of sponge and opisthobranch tissues. Ecology 45: 384–387

Pechenik, J. A. (1990). Delayed metamorphosis by larvae of benthic marine invertebrates: does it occur? Is there a price to pay? Ophelia 32: 63–94

Pechenik, J. A., Cerulli, T. R. (1991). Influence of delayed metamorphosis on survival, growth, and reproduction of the marine polychaete *Capitella* sp. I. J. exp. mar. Biol. Ecol. 151: 17–27

Pechenik, J. A., Eyster, L. S. (1989). Influence of delayed metamorphosis on the growth and metabolism of young *Crepidula fornicata* (Gastropoda) juveniles. Biol. Bull. mar. biol. Lab., Woods Hole 176: 14–24

Qian, P.-Y., McEdward, L. R., Chia, F.-S. (1990). Effects of delayed settlement on survival, growth, and reproduction in the spionid polychaete, *Polydora ligni*. Invert. Reprod. Dev. 18: 147–152

Raimondi, P. T. (1990). Patterns, mechanisms, consequences of variability in settlement and recruitment of an intertidal barnacle. Ecol. Monogr. 60: 283–309

Raimondi, P. T. (1991). Settlement behavior of *Chthmalus anisopoma* larvae largely determines the adult distribution. Oecologia 85: 349–360

Raimondi, P. T. (1992) Adult plasticity and rapid larval evolution in a recently isolated barnacle population. Biol. Bull. mar. biol. Lab., Woods Hole 182: 210–220

Rittschof, D., Branscomb, E. S., Costlow, J. D. (1984). Settlement and behaviour in relation to flow and surface in larval barnacles, *Balanus amphitrite* Darwin. J. exp. mar. Biol. Ecol. 82: 131–146

Rittschof, D., Costlow, J. D. (1989). Surface determination of macroinvertebrate larval settlement. In: Proc. 21st Eur. mar. Biol. Symp. 411–416 [Styczynska-Jurewicz, E. (ed.) Instituto de Ciencias de Mar, Barcelona]

Roberts, D., Rittschof, D., Holm, E., Schmidt, A. R. (1991). Factors influencing initial larval settlement: temporal, spatial, and surface molecular components. J. exp. mar. Biol. Ecol. 150: 203–211

Roughgarden, J., Gaines, S., Possingham, H. (1988). Recruitment dynamics in complex life cycles. Science, N.Y. 241: 1460–1466

Scheltema, R. S. (1971). Larval dispersal as a means of genetic exchange between geographically separated populations of shallow-water benthic marine gastropods. Biol. Bull. mar. biol. Lab., Woods Hole 140: 284–322

Scheltema, R. S. (1986). On dispersal and planktonic larvae of benthic invertebrates: an eclectic overview and summary of problems. Bull. mar. Sci. 39: 290–322

Strathmann, R. R., Branscomb, E. S., Vedder, K. (1981). Fatal errors in set as a cost of dispersal and the influence of intertidal flora on set of barnacles. Oecologia 48: 13–18

Sutherland, J. P. (1984). The structure and stability of marine macrofouling communities. In: Costlow, J. D., Tipper, R. C. (eds.) Marine biodeterioration: an interdisciplinary study. U.S. Naval Inst. Press, Annapolis, Maryland

Sutherland, J. P. (1990). Recruitment regulates demographic variation in a tropical intertidal barnacle. Ecology 71: 955–972

Tegtmeyer, K., Rittschof, D. (1989). Synthetic peptide analogs to barnacle settlement pheromone. Peptides 9: 1403–1406

Thorson, G. (1950). Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25: 1–45

Wethey, D. (1983). Intrapopulation variation in growth of sessile organisms: natural populations of the intertidal barnacle *Balanus balanoides*. Oikos 40: 14–23

Wethey, D. (1984). Spatial pattern in barnacle settlement: day to day changes during the settlement season. J. mar. biol. Ass. U.K. 64: 687–698

Wethey, D. (1986). Ranking of settlement cues by barnacle larvae: influence of surface contour. Bull. mar. Sci. 39: 393–400

Woollacott, R. M., Pechenik, J. A., Imbalzano, K. M. (1989). Effects of the duration of larval swimming period on early colony development in *Bugula stolonifera* (Bryozoa: Cheilostomata). Mar. Biol. 102: 57–63

Yule, A. B., Crisp. D. J. (1983). Adhesion of cypris larvae of the barnacle, *Balanus balanoides*, to clean and arthropodin treated surfaces. J. mar. biol. Ass. U.K. 63: 261–271

Yule, A. B., Walker, G. (1985). Settlement of *Balanus balanoides*: the effect of cyprid antennular secretion. J. mar. biol. Ass. U.K. 65: 707–712

Communicated by J. Grassle, New Brunswick