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DRAMATIC PHENOTYPIC PLASTICITY IN BARNACLE LEGS (*BALANUS GLANDULA* DARWIN): MAGNITUDE, AGE DEPENDENCE, AND SPEED OF RESPONSE

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Abstract.—The precise dependence of barnacle leg form on flow suggests the wave-swept environment imposes strong selection on suspension feeding limbs. I conducted three experiments to determine the mechanism, age dependence, and response time of cirrus variation in the acorn barnacle *Balanus glandula*. (1) To test whether cirrus variation arises via genetic or environmental mechanisms, I transplanted juvenile barnacles from one wave-exposed and one protected population into high and low flow conditions. Both populations exhibited similar abilities to modify cirri in response to experimental velocities: transplanted barnacles grew legs up to 84% longer in low flow. A small (up to 24%), but significant difference between source populations suggested slight genetic divergence in leg form. (2) Because flow is heterogeneous over space and time, I tested whether cirrus plasticity was limited to juveniles by transplanting both juveniles and adults from exposed and protected shores into quiet water. Remarkably, both juveniles and adults from the wave-exposed population produced legs over 100% longer than the original population, whereas protected barnacles remained unchanged. (3) A third transplant of adults into quiet water demonstrated that wave-exposed *B. glandula* modified cirrus form very quickly—within 18 days, or one to two molts. Results from these experiments suggest that variation in cirrus form is largely environmentally induced, but genetic differences may account for some variation observed among field populations; spatial and temporal flow heterogeneity appear to have selected for extreme flexibility of feeding form throughout a barnacle's life; and flow heterogeneity in the wave-swept environment appears to have selected for rapid ecophenotypic responses in the form of feeding structures.

Key words.—Barnacle, environmental heterogeneity, genotype-by-environment interaction, phenotypic plasticity, suspension feeding, wave-swept.

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The causes of phenotypic variation in heterogeneous environments—whether due to genetic variation, phenotypic plasticity, or their interaction—are an ongoing challenge in evolutionary ecology (Schlichting and Pigliucci 1998). In spatially or temporally fluctuating environments, many species exhibit distinct forms under different conditions (Stearns 1989; Travis 1994; Schlichting and Pigliucci 1998). The wave-swept environment is extremely heterogeneous in both time and space (Denny 1994) and phenotypic plasticity may be common among invertebrates from wave-swept shores. For example, in many invertebrates, body size, growth and reproductive rate, shell shape, and tenacity are modified in response to wave exposure (Palumbi 1984; Bertness et al. 1991; Trussell 1997, 2000). Although feeding form varies with water velocity in suspension feeding barnacles (Arsenault et al. 2001; Marchinko and Palmer 2003) and bryozoans (Okamura and Partridge 1999), the cause of these differences—ecophenotypic, or genetic differentiation—is unclear. However, phenotypic plasticity is strongly suspected (N. D. Pentcheff, unpubl. ms.).

Suspension-feeding invertebrates rely on ambient flow and particle flux to provide food. Flow plays a large role in the dynamics of particle capture and is likely crucial to the evolution of filtering elements in suspension feeders (LaBarbera 1984; Cheer and Koehl 1987; Shimeta and Jumars 1991). Water flow is spatially variable (horizontally) along shorelines, and the larvae of many suspension feeders may settle under a wide range of flow conditions, from virtually still in protected bays to extremely fast (over 14 m/sec) on very exposed shores (Denny 1994). As a consequence, variation in feeding structures with water velocity (Okamura and Partridge 1999; Arsenault et al. 2001; Marchinko and Palmer 2003) may arise in three ways: (1) larvae choose to settle under specific flow conditions that match a genetically rigid phenotype (differential settlement), (2) larvae settle regardless of flow conditions and suffer higher mortality rates if cirrus genotype does not match flow conditions (selective mortality), or (3) larvae settle regardless of flow conditions and feeding phenotype changes to suit local flow conditions (phenotypic plasticity). However, water velocity also varies within a single location due to seasonality, storm events, the settlement, and aggregation of other animals (Thomason et al. 1998), and along the vertical slope of the intertidal (Denny 1988). Therefore, variation in feeding form may also arise via phenotypic plasticity after differential settlement or selective mortality has taken place.

Barnacles are suspension feeders that live on rocky shores throughout the world. They feed by extending long, feathery appendages into ambient currents that may vary from low flow, in protected bays, to extremely high flow on wave-exposed shores. Substantial variation of feeding (cirrus) form with water velocity is now known for four species of intertidal barnacles (Marchinko and Palmer 2003): populations from protected inlets possess long, thin cirri with long setae, whereas wave-exposed populations posses short, stout cirri with short setae. This dependence of cirrus form appears adaptive because it increases the surface area of the feeding fan in low flow where particle flux is limited, while possibly reducing the damage caused by greater drag force under high flow (Marchinko and Palmer 2003). In one species, *Balanus glandula*, water velocity accounts for an exceptional 95–99% of leg length variation among sites of different wave exposure, suggesting the trade-offs among environments are quite severe (Arsenault et al. 2001). Although preliminary results

suggest the variation is ecophenotypic (N. D. Pentcheff, unpubl. ms.), the magnitude, age or size dependence, and the time-course of the response remains unknown.

Because barnacles are well known to be morphologically plastic in response to environmental conditions (Barnes and Barnes 1962; Crisp and Bourget 1985; Lively 1986a,b), possess a long larval dispersal period (Strathmann 1987), are well known to settle based on environmental cues (Crisp 1955; Neal and Yule 1994; Neal et al. 1996), and show significant differences in genetic structure along a single shoreline (Schmidt and Rand 2001), variation in cirrus form could arise via genetic differences among populations (differential settlement or selective mortality), phenotypic plasticity, or some combination of both mechanisms. Therefore, in this study, I address four questions using the most variable species, *Balanus glandula*: (1) Is the mechanism responsible for variation in limb length genetic, phenotypic, or a combination of both? (2) Is this response similar for the other two cirrus characters, seta length and ramus diameter, which also vary with water velocity? (3) Does the capacity for plasticity depend on size/age? (4) How rapid is the phenotypic response?

Materials and Methods

Study Species

Balanus glandula ranges from the Aleutian Islands in Alaska to Baja California and is commonly found in the midintertidal from quiet bays to extremely wave-exposed shores (Barnes and Barnes 1956). Like all balanomorph barnacles, *B. glandula* feeds using the cirral net, which consists of six pairs of biramous cirri. This species extends the posterior three pairs of captorial cirri (legs 4, 5, and 6) into currents to feed on micro-and macroscopic plankton and other particles transported by ambient currents (Barnes 1959; Anderson and Southward 1987). The anterior three cirri transfer food from the captorial cirri to the mouth (Anderson and Southward 1987). Variation in the length of captorial cirri with wave exposure is extreme in magnitude and precision (Arsenault et al. 2001), with protected shore populations possessing legs up to 80% longer than wave-exposed ones (Marchinko and Palmer 2003), and up to 99% of the variation in leg length is explained statistically by wave action (Arsenault et al. 2001).

Collection Sites and Experimental Location

For each experiment, I collected barnacles from two populations in Barkley Sound, Vancouver Island, British Columbia, Canada: one protected shore (Bamfield Inlet) and one wave-exposed shore (Sepping's Island) were chosen for the extreme differences in leg length documented by Marchinko and Palmer (2003). The protected shore collection site (48°50′06″N, 125°08′20″W) was located on the west-facing shore near the mouth of Bamfield Inlet. Water velocity (1.2 cm/s) was produced mainly by tidal currents and leg length of the population was 4.1 mm at a body mass of 0.0096 g (Marchinko and Palmer 2003). The exposed shore site (48°50′50″N, 125°12′50″W) was located on the southwest-facing shore of Sepping's Island, and characterized by high flow (4.41×10^2 cm/sec; for details see Arsenault et al. 2001) produced by breaking waves. Leg length of the exposed shore population was 2.7 mm at a body mass of 0.0096 g (Marchinko and Palmer 2003).

Three separate common-garden experiments ran from 14 April to 16 September 2001 under the Bamfield Marine Station research dock, approximately 100 m south of the protected shore collection site. Three-dimensional, instantaneous water velocity was measured over six days (9 May to 5 June 2001) using an acoustic doppler velocimeter (ADV; SonTek/YSI Inc., Yellow Springs, OH; 10-MHz), operating at 25 Hz for five minutes each day. Five of the six sampling days coincided (± two days) with the two spring tides during this period and sampling times were between one and two hours after slack tide. These measurements should provide a reasonable estimate of the maximal velocity of tidal currents in the experimental location. Velocity was calculated each day according to the equation:

$$(u^2 + v^2 + w^2)^{1/2},$$

where u, v, and w represent velocity in the x, y, and z direction, and then averaged over the six sampling days for a mean (± SE) ambient water velocity of 0.96 ± 0.1 cm per sec.

Barnacle Collection and Manipulation

Balanus glandula were collected as juveniles (mean ± SE basal diameter 2.8 ± 0.20 mm, $N = 36$) and adults (mean ± SE basal diameter 9.6 ± 0.23 mm, $N = 63$) on mussel shells, *Mytilus californianus* and *Mytilus trossulus*, from the lower one-third of the *B. glandula* zone. Only solitary individuals (shell plates not fused with neighboring individuals) were used in each experiment. Juveniles were collected within 7–10 days of settlement (settlement surveys were carried out every 7–10 days) on 9 April (experiment one) and 30 May 2001 (experiment two). Adults were also collected on 30 May (experiment two) and on 6 August 2001 (experiment three). After collection, mussel shells were cut with a variable-speed MultiPro rotary tool (Dremel, Racine, WI) so only one barnacle occupied each mussel fragment. Fragments varied in area, but their thickness did not exceed 2 mm. Mussel fragments (with barnacles) were left overnight in running seawater, then air-dried and glued (Krazy Glue, Elmer's Products, Columbus, OH) to Plexiglas plates according to each experiment (see details below). After gluing, the basal diameter of each barnacle was measured with vernier calipers and the Plexiglas plates were submerged at the experimental location in Bamfield Inlet according to each experiment (see details below). The time from collection to submersion did not exceed 48 hours.

Measurements of Cirrus Form and Body Size

Measurements of cirrus dimensions were taken on the sixth cirrus from the left side of the body and should be representative of all captorial cirri, as all three vary similarly with water velocity (Arsenault et al. 2001; Marchinko and Palmer 2003). The sixth cirrus was dissected from the prosoma and measured at 25–50× magnification using a dissecting scope mounted with a camera lucida, and a digitizing tablet with a precision of 20 dots per mm. I measured three traits on each

cirrus (for drawing see Marchinko and Palmer 2003): (1) ramus length, the curvilinear distance traced from base to tip on the dorsal side of the ramus; (2) ramus diameter, the distance between the dorsal and ventral side of the first (basal) segment of each ramus; and (3) seta length, the length of the longest seta on the middle three segments of the endopodite. In experiments two and three, only data for ramus length are shown. To obtain a single value of each trait for each individual, both ramus length and diameter were averaged from both the endo- and exopodite of each cirrus, whereas seta length was averaged over the middle three segments from a single ramus. Prior to experimentation, adult barnacles collected from both exposed and protected shore populations on 9 April (experiment one) and 6 August (experiments two and three) were also dissected to measure cirral form of the original, pretreatment populations.

To avoid the confounding effects of environmental variation on shell shape common among barnacles (Crisp and Bourget 1985; Lively 1986b), body size was estimated as prosomal wet mass. To measure prosomal wet mass, the soft tissue (prosoma) was separated from all shell plates and from any egg mass that was present, blotted dry for 20 sec on a dry Kimwipe (Kimberly-Clark, Dallas, TX), and weighed to the nearest 0.1 mg. Because barnacles were killed to measure prosomal wet mass, basal diameter of the shell (distance from the carinal to rostral plate) was used to distinguish between juvenile and adult barnacles prior to experiments.

Experiment 1: Genetic versus Environmental Control of Cirrus Form

To test whether cirrus variation arises via phenotypic plasticity or genetic divergence, I transplanted juvenile *B. glandula* from the exposed and protected shore sites into high (35.4 cm/sec; see below) and low flow (0.96 cm/sec; see above) growth environments at the experimental Bamfield Inlet location. To simulate low flow conditions, mussel fragments containing a single barnacle were glued (see above) to the underside of a 15 × 30-cm Plexiglas plate. Six barnacles from each population were arranged in a single line parallel to the short axis of the plate so each barnacle was 2 cm from its closest neighbor. The plate was constantly submerged 15 cm below water surface in ambient flow (0.96 cm/sec), 1.5 m from the high flow setup, for 35 days (11 April to 16 May 2001). Flow visualization with dye, and ADV measurements, during the experimental period confirmed that the generation of high flow had little influence on ambient velocity in the low flow growth environment 1.5 m away.

High flow in Bamfield Inlet was simulated using a rotating disk and paddle. Mussel fragments containing single barnacles were glued to the underside of a 60-cm^2 Plexiglas plate. Ten barnacles from each population were arranged with cirral nets perpendicular to flow on one of four quadrants of a stationary disk (two quadrants for each population; Fig. 1A). A circle of 25-cm radius was drawn so that in each quadrant, five barnacles were placed 1 cm inside and five more 1 cm outside the circle, with a 2-cm space between each barnacle in any direction. To control for the effects of neighbors on flow pattern, the leading two and last two bar-

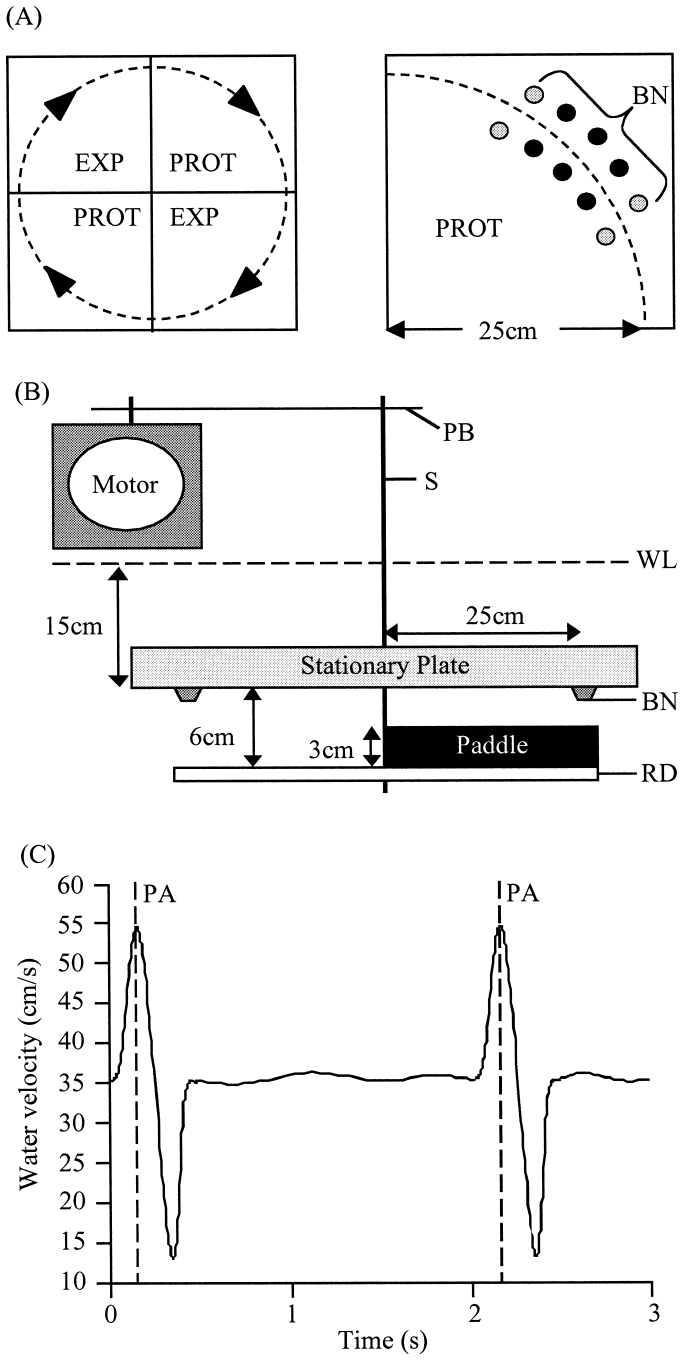

FIG. 1. Experimental setup and velocity schematic of high flow conditions created using a rotating disk and paddle submerged in Bamfield Inlet. (A) Arrangement of barnacles (BN) into four quadrants on the stationary Plexiglas plate (EXP, exposed shore; PROT, protected shore). The dotted line is 25 cm from the plate's center; arrows indicate the direction of flow. Black dots indicate barnacles that were measured; gray, barnacles that were not (see Materials and Methods). (B) Barnacles were fixed to a stationary plate and submerged 15 cm below water level (WL) while a rotating disk (RD) and paddle generated a mean flow of 35.4 cm per sec with a periodic increase (54.1 cm/sec) and subsequent decrease (14.1 cm/sec) in velocity each time the paddle passed (every 1.9 sec; PA in Fig. 1C). PB, pulley and belt; S, stainless steel shaft.

nacles in each group were not measured (Fig. 1A). Thus, measurements were only taken on barnacles that had a neighbor 2 cm in front (facing flow) and 2 cm behind (the middle six barnacles of each quadrant). At the end of the experiment only six individuals per population were measured because of mortality from algal abrasion (*Macrocystis integrifolia* became tangled in the rotating disk). Rotation was stopped no more than twice a week to clear algae, and for no longer than a one-hour period each time.

Barnacles on the stationary Plexiglas plate were submerged 15 cm under water, 6 cm above a rotating Plexiglas disk (radius 25 cm) fixed with a paddle (3 cm high) along one radius (Fig. 1B). The disk was rotated clockwise at 32 rpm by a stainless steel shaft attached to a 1.5V DC motor (Fig. 1B). The rotating disk and paddle generated a mean ± SE water velocity of 35.4 ± 0.62 cm per sec ($N = 19$), with a periodic increase (54.1 ± 0.70 cm/sec, $N = 19$) and subsequent decrease (14.1 ± 0.77 cm/sec, $N = 19$) in water velocity each time the paddle passed (every 1.9 sec; Fig. 1C). Flow was measured 9 mm below the stationary plate and 25 cm from the center of the disk, using a Nixon Streamflo analog meter with a series 403 low-speed propeller probe 15 mm in diameter (Novonic Instruments, Gloucester, England). Flow measurements were read once every minute for 19 min (separate trials were run for mean, maximum, and minimum velocities) in a tank of fresh water 2 m in diameter. Since a propeller rotates at an angular velocity proportional to a fluid's speed and hydrodynamic forces are proportional to density (Denny 1988), velocity measured in fresh water should closely approximate velocity in salt water (salt water is 2.3% denser than fresh water).

Leg dimensions were analyzed using a two-factor analysis of covariance (ANCOVA): covariate = log(prosomal wet mass), main effects = source population and growth environment (low or high flow), dependent variable = log(cirrus dimension). Significant differences in cirrus dimension between growth environments would suggest cirrus variation is under environmental control, whereas significant differences between populations would suggest cirrus variation is genetically controlled. A significant population by growth environment interaction would suggest an asymmetry in the ability of the two populations to respond to flow differences. A one-factor ANCOVA, followed by a Bonferroni test for multiple comparisons, was employed separately for each population to test whether cirrus dimensions produced under high and low treatments were significantly different from pretreatment populations (collected on 11 April). Thus, the influence of experimental procedure on cirrus form was tested by comparing protected shore barnacles grown in low flow to those untreated from the original protected shore population (100 m away) in Bamfield Inlet. If significant differences exist between these two groups, any change in cirrus form may be confounded by some step in the experimental procedure.

Experiment 2: Age Dependence of the Response

To determine whether the capacity to change ramus length depended on age, I transplanted both juveniles and adults from the exposed shore site into ambient (low flow) conditions in Bamfield Inlet. Juvenile and adult barnacles from the protected shore population in Bamfield Inlet were also transplanted back to Bamfield Inlet to test for artifacts due to experimental conditions. Mussel fragments containing a single barnacle were glued (see above) on the underside of a 15 × 30-cm Plexiglas plate. Six barnacles from each population were arranged in a single line parallel to the short axis. Each barnacle was 2 cm from its closest neighbor and lines were arranged from left to right as follows: protected shore juveniles, exposed shore adults, protected shore adults, exposed shore juveniles. The plate was constantly submerged 15 cm below the water surface for 51 days (1 June to 22 July 2001), after which barnacles were collected and weighed, and ramus length was measured as described above. Only five adults from each population survived the experiment.

Data were analyzed using a two-factor ANCOVA: covariate = log(prosomal wet mass), main effects = source population and transplant age (juvenile or adult), dependent variable = log(ramus length). Significant differences in ramus length between exposed shore juveniles and adults would suggest that age affects a barnacle's ability to modify cirri, whereas differences in the protected shore population would suggest experimental procedure influenced cirrus form. A one-factor ANCOVA was employed separately for each population to test whether ramus length differed between transplanted and original, pretreatment populations (tested against barnacles collected on 6 August 2001 for experiment 3). Significant differences between pretreatment and transplanted exposed shore barnacles would reconfirm that ramus length is influenced by water flow, whereas significant differences between pretreatment and transplanted barnacles from the protected shore population would suggest that changes in cirrus form in either population were confounded by experimental procedure.

Experiment 3: Response Time

To examine how quickly barnacles from the exposed shore population modified ramus length, I transplanted exposed-shore adults into low flow conditions in Bamfield Inlet. Protected shore adults were also transplanted back to Bamfield Inlet to test for experimental artifacts. Barnacles were collected on 6 August 2001, glued and arranged on two 15 × 30-cm Plexiglas plates in the same manner as experiment 2, with the exception that only adults were transplanted. Plates were constantly submerged 15 cm below the water surface and six barnacles from each population were sampled destructively at four different time intervals (7, 18, 25, and 35 days after transplant, from 8 August to 12 September 2001). Ramus length of six untreated barnacles from both populations (collected on 6 August) were included as pretreatment values (at day 0). After barnacles were sampled, they were weighed and ramus length was measured as described above. Differences in sample size were due to mortality during the experiment.

Because I was no longer concerned with source-population differences, data were analyzed using a one-factor ANCOVA for each site separately: covariate = log(prosomal wet mass), main effect = collection interval, dependent variable = log(ramus length). Because individual barnacles were not followed over time (i.e., subsamples of different individuals

were taken at each time interval), treating each collection interval as an independent sample in the analysis was appropriate. Significant ANCOVA results were followed by a Bonferroni test for multiple comparisons to assess at which time interval barnacles produced significantly different-sized legs from the original population. Thus, four comparisons of mean leg length were made between 0–7, 0–18, 0–25, and 0–35 days after transplant (Bonferroni adjusted $\alpha = 0.0125$).

Results

Experiment 1: Two-Way Transplant (High versus Low Flow Environments)

Juvenile *B. glandula* from both the protected and the exposed shore populations grew longer rami (66% and 84% respectively) and seta (54% and 52%) under low flow compared to high flow conditions (Fig. 2). Differences in ramus and seta length between growth environments were highly significant ($P < 0.001$; Table 1). Although both populations produced shorter rami and setae when grown under high flow, individuals from the protected shore grew consistently and significantly longer rami (24%) and setae (11%) than those from the exposed shore population, regardless of growth environment ($P < 0.019$; Table 1).

Ramus and seta length of pretreatment barnacles from both populations were significantly different from those grown under low and high flow environments when analyzing populations separately ($P < 0.001$). Multiple comparisons (Bonferroni adjusted $\alpha = 0.016$) revealed that protected-shore barnacles grown under high flow possessed rami and setae significantly shorter ($P < 0.001$), while those grown under low flow conditions were statistically indistinguishable ($P > 0.095$) from the pretreatment protected shore population (Fig. 2). Multiple comparisons also revealed that barnacles from the exposed shore possessed significantly longer rami and setae ($P < 0.001$) when grown under low flow conditions. Remarkably, those grown experimentally under high flow possessed limbs significantly shorter ($P < 0.001$), than the pretreatment, exposed-shore individuals (Fig. 2).

Growth environment and source population had no effect on ramus diameter ($P = 0.53$ and 0.098, respectively; Table 1; Fig. 2), unlike the pattern observed for ramus and seta length. Surprisingly, ramus diameter of the transplanted exposed-shore barnacles was consistently, although not significantly, smaller than those transplanted from the protected shore; contrary to the trend of increased ramus diameter in exposed shore populations observed here (bars in Fig. 2) and in previous work (Marchinko and Palmer 2003). Ramus diameter of transplanted barnacles from both exposed and protected shores was significantly different from their respective pretreatment populations ($P = 0.01$ and 0.033, respectively). Multiple comparisons (Bonferroni adjusted $\alpha = 0.016$) revealed that exposed-shore barnacles under both high and low flow had significantly thinner rami than the original, pretreatment population, whereas protected-shore barnacles transplanted to low flow had significantly thicker rami than protected-shore barnacles before treatment.

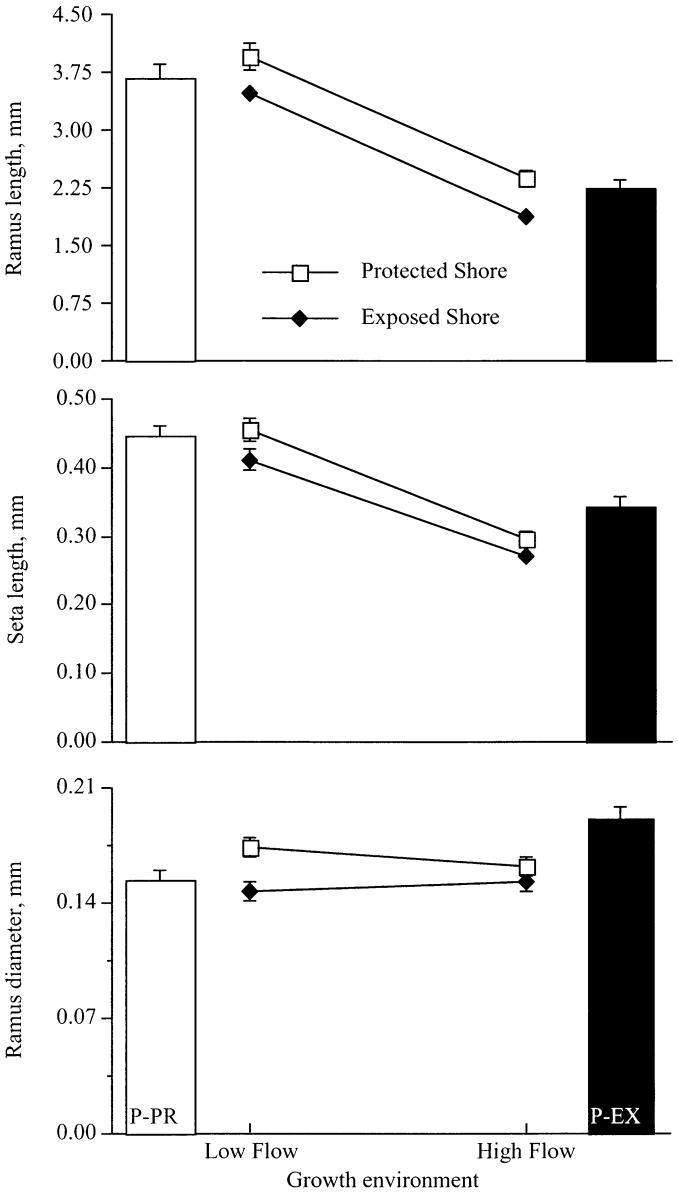

FIG. 2. Cirrus dimensions of *Balanus glandula* from two populations (one wave-exposed and one protected) before (bars) and 35 days after (points and lines) continuous exposure to low flow (0.96 cm/sec) and high flow (35.7 cm/sec) growth environments. Each point or bar represents the mean (± SE) of six individuals adjusted to a common prosomal wet mass of 0.0062 g by analysis of covariance (see Table 1). Standard errors were less than the symbol size where absent. Points connected by lines represent barnacles grown experimentally under different flow conditions. Bars represent cirrus dimensions measured in pretreatment individuals (P-PR, pretreatment, protected; P-EX, pretreatment, exposed).

Experiment 2: One-Way Transplant to Low Flow (Juveniles versus Adults)

No significant differences in ramus length were found between barnacles transplanted as juveniles or adults from either the protected- or exposed-shore source populations ($P = 0.46$; Table 2; Fig. 3). Population differences were significant ($P = 0.002$; Table 2), and similar to experiment 1: exposed-shore barnacles had consistently shorter rami than

TABLE 1. Results from two-factor analysis of covariance testing for differences in cirrus form of *Balanus glandula* from two populations (one wave-exposed and one protected) 35 days after transplant to high (35.4 cm/sec) and low (0.96 cm/sec) flow conditions in Bamfield Inlet (see Fig. 2). All variables were $\log_{10}$ transformed before analysis. Interactions with the covariate were all nonsignificant (i.e., slopes were equal) and are not included in this table.

Source of variation	Ramus length			Seta length		Ramus diameter	
	df	MS	*P*	MS	*P*	MS	*P*
Source population	1	0.019	<0.001	0.010	0.019	0.003	0.098
Growth environment (high vs. low flow)	1	0.193	<0.001	0.099	<0.001	4.2×10^{-4}	0.530
Log(prosomal wet mass, g)	1	0.024	<0.001	0.014	0.007	0.015	0.001
Source population × growth environment	1	9.5×10^{-7}	0.975	0.0002	0.725	0.003	0.099
Error	19	0.001		0.001		0.001	

protected shore barnacles. Within-population comparisons of treated to pretreatment barnacles showed ramus lengths of transplanted exposed-shore barnacles were significantly longer than pretreatment ($P < 0.001$), while rami of treated and pretreatment protected-shore barnacles were statistically indistinguishable ($P = 0.59$).

Experiment 3: One-Way Transplant to Low Flow (Response Time)

Ramus length of adult *B. glandula* from the exposed-shore population began to increase around 18 days after transplant to quiet water in Bamfield Inlet (Figs. 4 and 5). Treatment (number of days after transplant) had a significant effect on exposed-shore barnacles ($P = 0.0016$; Table 3) and multiple comparisons (Bonferroni adjusted $\alpha = 0.0125$) revealed that barnacles sampled 18, 25, and 35 days after transplant possessed rami significantly longer than pretreatment barnacles ($P < 0.007$; Fig. 4). Moreover, the homogeneity of standard error in mean ramus length among time intervals (Fig. 4) suggests that changes in ramus length of all exposed-shore barnacles occurred within a similar time period. Treatment had no significant effect on ramus length of protected-shore barnacles ($P = 0.285$; Table 3; Fig. 4).

Because the transplant of Bamfield Inlet (protected shore) barnacles back into ambient (low) flow in Bamfield Inlet had no effect on cirrus form (other than ramus diameter), experimental procedure appears not to confound the results for ramus and seta length in all three experiments.

TABLE 2. Results from two-factor analysis of covariance testing for differences in ramus length between adult and juvenile *Balanus glandula* from two populations (one wave-exposed and one protected) 51 days after transplant to quiet water in Bamfield Inlet (see Fig. 3). All variables were $\log_{10}$ transformed before analysis. Interactions with the covariate were nonsignificant (i.e., slopes were equal) and are not included in this table.

Source of variation	df	Mean square	*P*
Source population	1	0.010	0.002
Transplant age (juveniles vs. adults)	1	4.4×10^{-4}	0.464
Log(prosomal wet mass, g)	1	0.035	<0.001
Source population × transplant age	1	0.002	0.168
Error	17	0.001	

DISCUSSION

Genotypic or Ecophenotypic Response?

The extreme modification of ramus and seta length in response to changes in water velocity (Figs. 1–5) is clearly ecophenotypic. Both protected- and exposed-shore populations of *B. glandula* grew longer limbs (up to 84%) when raised in low versus high flow growth environments. Induced differences in cirrus form agree with prior reports of among-population differences for *B. glandula* (90%, Arsenault et al. 2001; 80%, (Marchinko and Palmer 2003), strongly suggesting that the majority of cirrus variation observed in nature arises from environmentally induced phenotypic plasticity. Moreover, quantitative evidence of flow-induced changes in cirrus form of *B. glandula*, coupled with form dependence on wave exposure in four barnacle species (Marchinko and Palmer 2003), suggests that spatial and temporal variation in flow along coastlines played a large role in selection for phenotypically plastic feeding fans in barnacles.

Although the adjustment of cirrus form to different flow

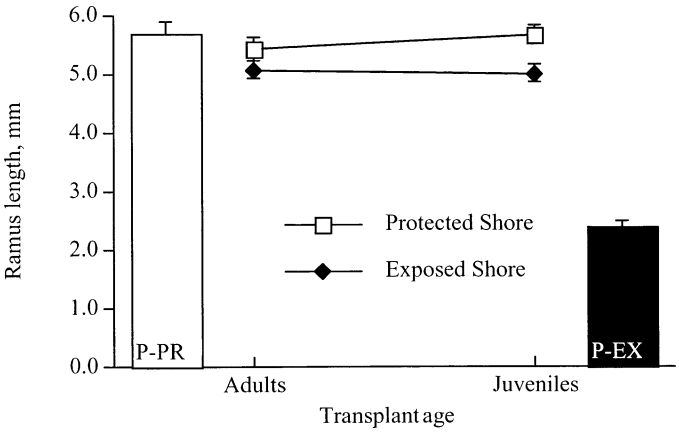

FIG. 3. Feeding leg length of adult and juvenile *Balanus glandula* from two populations (one wave-exposed and one protected) before (bars) and 51 days after (points and lines) transplant to low flow conditions (0.96 cm/sec) in Bamfield Inlet. Each point or bar represents mean ramus length (± SE) adjusted to a common prosomal wet mass of 0.0262 g by analysis of covariance (see Table 2). Standard errors were less than the symbol size where absent. $n = 6$ for original populations and juveniles, $n = 5$ for adults. Points connected by lines represent barnacles grown experimentally under different flow conditions. Bars represent cirrus dimensions measured in pretreatment individuals (P-PR, pretreatment, protected; P-EX, pretreatment, exposed).

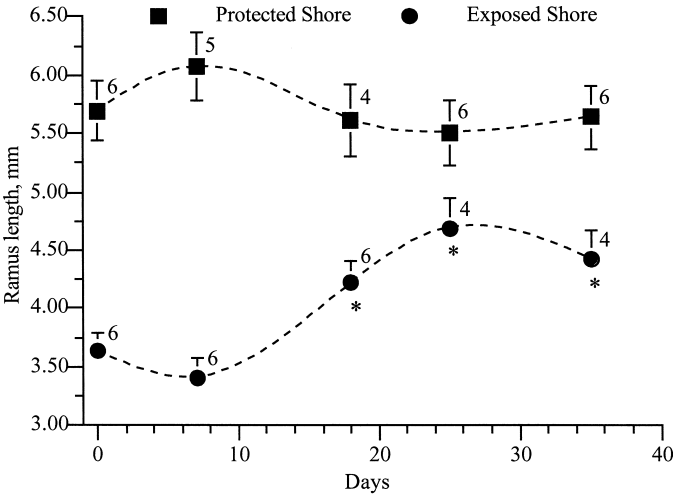

FIG. 4. Feeding leg length of exposed- and protected-shore *Balanus glandula* 0, 7, 18, 25, and 35 days after transplant to low flow conditions (0.96 cm/sec) in Bamfield Inlet. Each point represents mean ramus length (± SE) of barnacles adjusted to a common prosomal wet mass of 0.0259 g by analysis of covariance (see Table 3). Points with an asterisk underneath (exposed shore only) were significantly different from the pretreatment (0 days) population ($P < 0.007$; Bonferroni adjusted $\alpha = 0.0125$; see Materials and Methods). Differences in ramus length of protected shore barnacles were nonsignificant ($P = 0.285$; Table 3). Numbers above each point represent sample size for each group.

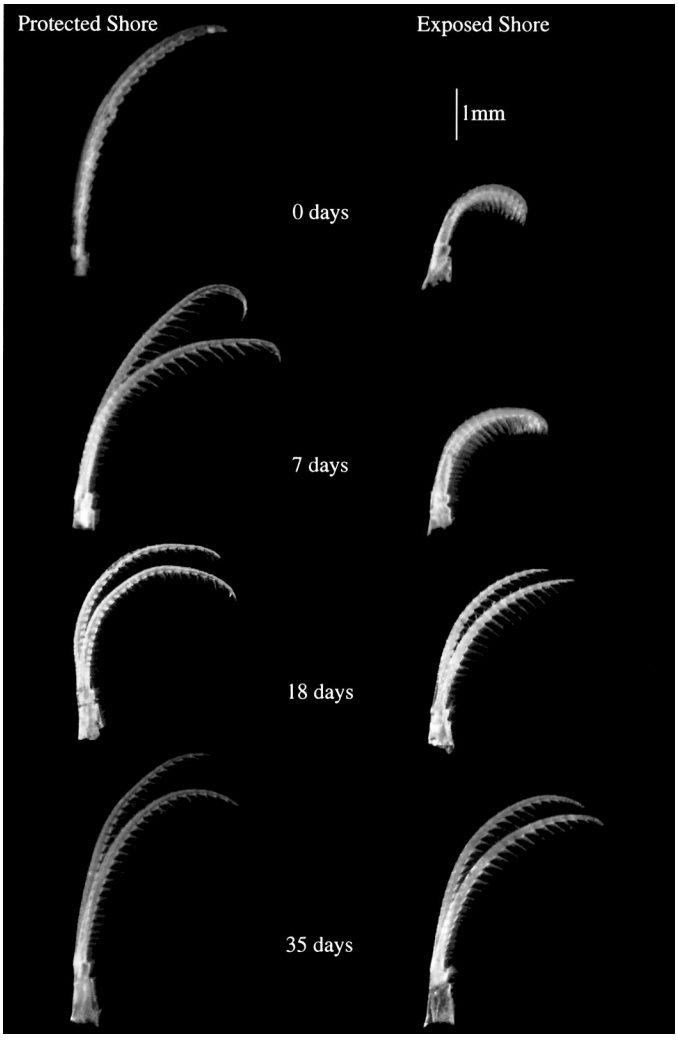

FIG. 5. Leg 6 of *Balanus glandula* from wave-exposed and protected shore populations before (0 days) and 7, 18, and 35 days after transplant to low flow conditions (0.96 cm/sec) in Bamfield Inlet. Leg length 25 days after transplant is not represented because of sizable differences in prosomal wet mass between sites; however, adjusted ramus length was similar to those 35 days after transplant (see Fig. 4). Prosomal wet mass (in grams listed from 0–35 days) was as follows: protected shore, 0.024, 0.021, 0.024, and 0.025; exposed shore, 0.023, 0.026, 0.026, and 0.032. Each photograph was taken at 90X magnification.

regimes is clear, both hydrodynamic forces and food availability are correlated with changes in velocity. A smaller feeding net may decrease drag in high flow, but may also maintain particle capture rate in higher flows because of greater particle flux. Increases in both water velocity and food availability induces production of fewer rays in the labral fan of suspension-feeding larval black flies (Zhang and Malmqvist 1997; Lucas and Hunter 1999). N. D. Pentcheff (unpubl. ms.) suggested that food supply rather than flow velocity may be the primary stimulus inducing changes in leg length, but these results need to be confirmed. Without separating the effects of water velocity and food availability, it is impossible to conclude whether one or the other is the primary stimulus for cirrus modification. However, cirrus dimensions are clearly influenced by flow around them.

Despite the large influence of flow on cirrus morphology, slight genetic differences between populations may be responsible for the small, but significant ($P < 0.019$; Tables 1 and 2) difference in ramus and seta length between populations (protected shore > exposed shore; Figs. 2 and 3). Of the two possible mechanisms for generating such genetic differences (see above), selective mortality is likely responsible, for two reasons. First, in *Semibalanus balanoides*, selective mortality in response to environmental conditions occurs within two weeks after settlement (Schmidt and Rand 2001) and therefore may have taken place before juvenile *B. glandula* were collected during this study. Second, onshore settlement discrimination is not predicted by the position of larvae in offshore waters (Delafontaine and Flemming 1989), and larval choice must take place at small scales after barnacles reach the shore (Neal et al. 1996). Thus, the long larval dispersal of *B. glandula* (Strathmann 1987) likely provides substantial time for larvae to settle throughout wave-exposure gradients. If small differences in the genes controlling cirrus form exist between populations, genotype may account for a small fraction of the exposure-dependent variation in cirrus form observed in nature (Arsenault et al. 2001; Marchinko and Palmer 2003). Additionally, juveniles may have received environmental cues within the seven to 10 days prior to collection that influenced the growth trajectory of cirrus form. Such cues may limit the absolute dimensions of the cirral fan, while maintaining a similar range of plasticity between exposed- and protected-shore barnacles. Unfortunately, from these data it is impossible to conclude whether the slight differences in phenotype between sites arises from wave-exposure-induced mortality of early juveniles or via other

TABLE 3. Results from one-factor analysis of covariance testing for differences in ramus length of wave-exposed and protected-shore *Balanus glandula* at 0, 7, 18, 25, and 35 days after transplant to quiet water in Bamfield Inlet (see Figs. 4 and 5). All variables were $\log_{10}$ transformed before analysis.

	Exposed shore			Protected shore		
Source of variation	df	Mean square	*P*	df	Mean square	*P*
Sampling interval (0, 7, 18, 25, 35 days)	4	0.019	0.0016	4	0.002	0.285
Log(prosomal wet mass, g)	1	0.101	0.0001	1	0.048	<0.001
Error	20	0.003		21	0.001	
Equality of slopes*	4	0.002	0.6128	4	0.001	0.657

* When testing for equality of slopes, the error degrees of freedom were 16 and 17 (for exposed and protected shores respectively) and the error mean squares were 6.7 and 7.4% larger than tabled here.

environmental cues or variables that correlate with wave exposure.

Alternatively, insufficient time may have passed for complete convergence of cirrus form between populations. Because cirrus form should only change during growth at each molt, and juvenile molting rate is nearly twice that of adults (Crisp and Patel 1961), juveniles will have more opportunity to modify cirrus form in a given time period. However, in adults ramus length begins to change within 18 days, and since ramus length of juveniles and adults were statistically indistinguishable ($P = 0.46$; Table 2) after 51 days (Fig. 3), experiment length appears not to have prevented convergence in cirrus form.

Range of Phenotypic Plasticity

Surprisingly, exposed-shore barnacles transplanted to high flow had even shorter limbs ($P < 0.001$; Fig. 2) than individuals experiencing extreme flow under breaking waves in their original habitat (Sepping's Island). This has two significant implications. First, by possessing the shortest limbs under experimental high flow (Fig. 2), the breadth of cirrus plasticity appears even more extreme than documented in the field study by Arsenault et al. (2001). Thus, *B. glandula* likely have the ability to feed and survive on shores experiencing greater wave exposure than those sampled by Arsenault et al. (2001). Second, because maximum recorded velocity at Sepping's Island (4.6 m/sec; Arsenault et al. 2001) was much greater than maximum experimental high flow (0.54 m/sec), production of shorter limbs in experimental high flow suggests barnacles choose to feed in considerably lower velocities after waves break, or in their backwash. Alternatively, differences between pretreatment and high flow transplants from the exposed shore might be an artifact of differences in velocity generated by a rotating disk and paddle versus flow under breaking waves. For example, the shorter period of experimental high flow, 1.9 sec versus 7–10 sec of average ocean swell (Denny 1988) may provide less opportunity to feed at the slower velocities that would normally occur after each wave breaks. Regardless of the mechanism, cirrus form appears even more plastic than previously thought (Arsenault et al. 2001).

Contrary to the patterns observed in ramus and seta length, transplant to low and high flow had no significant effect on ramus diameter ($P = 0.098$; Table 1). However, ramus diameter of transplanted juveniles from both populations was relatively thin and similar to pretreatment, protected-shore individuals (Fig. 2). Unfortunately, explanation of this phenomenon is difficult, since it remains unknown whether ramus diameter increases through cuticle thickening or via an increase in diameter of the fluid-filled compartment of the ramus. There are three reasonable explanations, two involving a trade-off between respiratory needs on protected shores versus leg damage in wave-exposed shores. First, if cirri function as a surface for gas exchange (Anderson and Southward 1987), the increased water temperature and lower oxygen concentration (Walton-Smith 1974) associated with protected bays may demand production of a thinner cuticle to meet respiratory needs. Second, juvenile barnacles from wave-exposed shores may rely on environmental cues for producing thicker rami to reduce physical damage. If such cues were not present in the protected habitat, exposed-shore juveniles raised in quiet water would possess thinner rami regardless of growth environment, as seen here. Finally, this pattern may be an artifact of continuous submersion (not usually experienced by intertidal barnacles) during the experiment. Significant differences between protected-shore barnacles transplanted to low flow and pretreatment individuals suggest experimental procedure may have confounded results on ramus diameter (see Materials and Methods).

Age Dependence of Phenotypic Plasticity

Remarkably, exposed shore adults increased leg length by an extreme 110% (Fig. 3) after transplant to low flow in Bamfield Inlet. This increase, coupled with a similar 108% increase in juvenile leg length, has two important implications. First, cirrus form does not appear to be fixed at any time during development; thus, cirri can be modified throughout an individual's lifetime. Secondly, because feeding rate strongly influences growth and fitness (Meyer 1987; Ritchie 1990; Okamura 1992), flow heterogeneity likely imposes strong selection on cirrus form in two ways: spatially, in the range of larval distribution; and temporally, within a single location throughout a sessile individual's lifetime. Four weeks of larvae dispersal (Strathmann 1987), predictable increase in swell height during winter months (Canadian Department of Fisheries and Oceans 2001), and temporal changes in surface relief because of the development of bluff bodies, such as barnacle hummocks (Thomason et al. 1998) and settlement of intertidal organisms, may generate spatial and temporal flow heterogeneity (Vogel 1988) sufficient for this type of selection. Alternatively, if little cost is associated with maintaining cirrus plasticity throughout life, selection may simply not favor its loss.

Significance of Response Time

Selection pressure to transform exposed-shore into protected-shore phenotypes appears strong to illicit the quick response observed in adult barnacles: around 18 days (Figs. 4 and 5), likely within one to two molts (Crisp and Patel 1961; Wu and Levings 1978). Alternately, if little cost is associated with cirrus plasticity, there might be little reason for it not to occur rapidly. The direction of selection on feeding limbs, however, remains unclear and production of longer limbs in low flow may arise in two ways: (1) In low flow conditions, selection may favor longer cirri and greater fan area to feed in thicker boundary layers with lower particle flux (Fréchette et al. 1989), or (2) in high flow, selection may favor smaller limbs that reduce damage as a result of drag; thus, in low flow limbs grow longer after being released from selection pressure. Knowing how quickly protected-shore barnacles switch from low to high flow phenotypes—the opposite of the time-course experiment reported here—may provide some insight into this problem. Intertidal snails (Trussell 1997) and sponges (Palumbi 1984) produce wave-tolerant phenotypes more quickly when transplanted from low to high flow. Both Trussell (1997) and Palumbi (1984) suggest stronger selection for flow-tolerant morphologies was responsible for this asymmetry in response time. Since molting rate in *Semibalanus balanoides* is about 28% faster in turbulent versus still flow (Barnes and Barnes 1982), individuals transplanted from low to high flow may respond even more quickly than documented here. Thus, wave-exposed and protected-shore populations of *B. glandula* may be asymmetric in response time, although equally plastic in form (Fig. 1).

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Literature Cited

Anderson, D. T., and A. J. Southward. 1987. Cirral activity of barnacles. Pp. 135–174 *in* A. J. Southward, ed. Barnacle biology. A. A. Balkema, Rotterdam.

Arsenault, D. J., K. B. Marchinko, and A. R. Palmer. 2001. Precise tuning of barnacle leg length to coastal wave action. Proc. R. Soc. Lond. B 268:2149–2154.

Barnes, H. 1959. Stomach contents and microfeeding of some common cirripeds. Can. J. Zool. 37:231–236.

Barnes, H., and M. Barnes. 1956. The general biology of *Balanus glandula* Darwin. Pac. Sci. 10:415–422.

———. 1962. The distribution and general ecology of *Balanus balanoides* together with some observations on *Balanus improvisus* in the waters around the coasts of Denmark, southern Sweden and northeast Germany. Lund Universitets Arskrift N. F. AVD. Vol. 58.

Barnes, M., and H. Barnes. 1982. Effect of turbulence on the feeding and molting of the cirripede *Balanus balanoides* (L) given an algal diet. J. Exp. Mar. Biol. Ecol. 65:163–172.

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

Cheer, A. Y. L., and M. A. R. Koehl. 1987. Paddles and rakes: fluid flow through bristled appendages of small organisms. J. Theor. Biol. 129:17–39.

Crisp, D. J. 1955. The behaviour of barnacle cyprids in relation to water movement over a surface. J. Exp. Biol. 32:569–590.

Crisp, D. J., and E. Bourget. 1985. Growth in barnacles. Adv. Mar. Biol. 22:199–244.

Crisp, D. J., and B. S. Patel. 1961. The moulting cycle in *Balanus balanoides* L. Biol. Bull. 118:31–47.

Delafontaine, M. T., and B. W. Flemming. 1989. Physical factors in barnacle community structure: A conceptual model. Sci. Mar. 53:405–410.

Denny, M. W. 1988. Biology and the mechanics of the wave-swept environment. Princeton Univ. Press, Princeton, NJ.

———. 1994. Roles of hydrodynamics in the study of life on wave-swept shores. Pp. 169–204 *in* P. C. Wainwright and S. M. Reilly, eds. Ecological morphology: integrative organismal biology. Univ. of Chicago Press, Chicago, IL.

Canadian Department of Fisheries and Oceans. 2001. 2000 Pacific region state of the ocean. DFO science ocean status report 2000–01. Canadian Department of Fisheries and Oceans, Nanaimo, British Columbia.

Fréchette, M., C. A. Butman, and W. R. Geyer. 1989. The importance of boundary-layer flows in supplying phytoplankton to the benthic suspension feeder, *Mytilus edulis* L. Limnol. Oceanogr. 34:19–36.

LaBarbera, M. 1984. Feeding currents and particle capture mechanisms in suspension feeding animals. Am. Zool. 24:71–84.

Lively, C. M. 1986a. Competition, comparative life histories, and maintenance of shell dimorphism in a barnacle. Ecology 67: 858–864.

———. 1986b. Predator-induced shell dimorphism in the acorn barnacle *Chthamalus anispoma*. Evolution 40:232–242.

Lucas, P., and F. F. Hunter. 1999. Phenotypic plasticity in the labral fan of simuliid larvae (Diptera): Effect of seston load on primary ray number. Can. J. Zool. 77:1843–1849.

Marchinko, K. B., and A. R. Palmer. 2003. Feeding in flow extremes: dependence of cirrus form on wave-exposure in four barnacle species. Zoology 106: *in press*.

Meyer, A. 1987. Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. Evolution 41:1357–1369.

Neal, A. L., and A. B. Yule. 1994. The tenacity of *Elminius modestus* and *Balanus perforatus* cyprids to bacterial films grown under different shear regimes. J. Mar. Biol. Assoc. UK 74:251–257.

Neal, A. L., F. N. Simões, and A. B. Yule. 1996. Interactions between shear rates and biofilms affecting exploratory behaviour by cyprids of *Elminius modestus* (Cirripedia). Mar. Biol. 127: 241–246.

Okamura, B. 1992. Microhabitat variation and patterns of colony growth and feeding in a marine bryozoan. Ecology 73: 1502–1513.

Okamura, B., and J. C. Partridge. 1999. Suspension feeding adaptations to extreme flow environments in a marine bryozoan. Biol. Bull. 196:205–215.

Palumbi, S. R. 1984. Tactics of acclimation: morphological changes of sponges in an unpredictable environment. Science 225: 1478–1480.

Ritchie, M. E. 1990. Optimal foraging and fitness in Columbian ground squirrels. Oecologia 82:56–67.

Schlichting, C. D., and M. Pigliucci. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer, Sunderland, MA.

Schmidt, P. S., and D. M. Rand. 2001. Adaptive maintenance of genetic polymorphism in an intertidal barnacle: habitat- and life-

stage-specific survivorship of *MPI* genotypes. Evolution 55: 1336–1344.
Shimeta, J., and P. A. Jumars. 1991. Physical mechanisms and rates of particle capture by suspension feeders. Oceanogr. Mar. Biol. Annu. Rev. 29:191–257.
Stearns, S. C. 1989. The evolutionary significance of phenotypic plasticity. Bioscience 39:436–445.
Strathmann, M. F. 1987. Reproduction and development of marine invertebrates of the northern Pacific Coast. Univ. of Washington Press, Seattle, WA.
Thomason, J. C., J. M. Hills, A. S. Clare, A. Neville, and M. Richardson. 1998. Hydrodynamic consequences of barnacle colonization. Hydrobiologia 375/376:191–201.
Travis, J. 1994. Evaluating the adaptive role of morphological plasticity. Pp. 99–122 *in* P. C. Wainwright and S. M. Reilly, eds. Ecological morphology. Univ. of Chicago Press, Chicago, IL.
Trussell, G. C. 1997. Phenotypic plasticity in the foot size of an intertidal snail. Ecology 78:1033–1048.
———. 2000. Predator induced plasticity and morphological tradeoffs in latitudinally separated populations of *Littorina obtusata*. Evol. Ecol. Res. 2:803–822.
Vogel, S. 1988. Life's devices. Princeton Univ. Press, Princeton, NJ.
Walton-Smith, F. G. 1974. CRC handbook of marine science. CRC Press, Cleveland, OH.
Wu, R. S. S., and C. D. Levings. 1978. An energy budget for individual barnacles (*Balanus glandula*). Mar. Biol. 45:225–235.
Zhang, Y., and B. Malmqvist. 1997. Phenotypic plasticity in a suspension-feeding insect, *Simulium lundstromi* (Diptera: Simuliidae), in response to current velocity. Oikos 78:503–510.

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