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Wave-Dependent Phenotypic Plasticity in Feeding Legs of Intertidal Barnacles

by

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" I must confess, I took learning and philosophy and science — all the activities that are magniloquently lumped under the title of 'The Search for Truth' — very seriously, I regarded the Search for Truth as the highest of human tasks and the Searchers as the noblest of men. But in the last year or so I have begun to see that this famous Search for Truth is just an amusement, a distraction like any other, a rather refined and elaborate substitute for genuine living; and that Truth Searchers become just as silly, infantile, and corrupt in their way as the boozers, the pure aesthetes, the business men, the Good-Timers in theirs."

— *Aldous Huxley, Point Counter Point* —

ABSTRACT

In four intertidal barnacles from the northeastern Pacific — *Balanus glandula, Semibalanus balanoides, Chathamalus dalli, and Pollicipes polymerus* — feeding leg form varied significantly with wave-exposure. Barnacles from protected shores had feeding legs that were significantly longer (up to two-fold) and thinner than those from wave-exposed shores. These differences are likely adaptive: long, thin legs would yield a larger feeding area in low flow, whereas short, stout legs would be less vulnerable to damage in high flow. In the most variable species, *B. glandula*, this relationship was incredibly precise: water velocity explained 95.6-99.5% of leg length variation over a three fold range in wave-exposure. Common-garden experiments revealed that feeding leg variation in *B. glandula*: 1) is largely ecophenotypic, 2) can occur quickly (<1 month), and 3) can arise at any time during a barnacle's life. Spatial and temporal variation of flow in the wave-swept environment appear to have selected for extreme and rapid ecophenotypic responses in barnacle feeding structures.

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CHAPTER 1

GENERAL INTRODUCTION

1.0 EVOLUTIONARY PROBLEM: Selection in heterogeneous environments

Changes in environmental conditions (or increased environmental heterogeneity) are often accompanied by changes in the body form, physiology, and behaviour (i.e., the phenotype) of many animals (Stearns 1989, Okamura 1990, Travis 1994, Schlichting and Pigliucci 1998). Despite being a widespread phenomenon, the causes of such phenotypic differences remain hotly debated among evolutionary ecologists (Schlichting and Pigliucci 1998). Phenotypic differences may arise via two mechanisms: 1) genetic, where population divergence in a morphological character arises from selection for different genotypes under different conditions, or 2) environmental, where a single genotype produces different phenotypes under different conditions (phenotypic plasticity). Consequently, the phenotype observed at a particular time may be a product of either mechanism, or the interaction between them (Travis 1994, Schlichting and Pigliucci 1998). Determining which mechanism accounts for observed phenotypic differences requires manipulative experiments. The clearest examples of this can be seen in polyphenisms — discrete phenotypic differences produced under discrete conditions — usually in response to predation (Travis 1994). But phenotypic variation may also be continuous along an environmental gradient. Such continuous phenotypic responses are often predictable in magnitude and direction under lab conditions. However, such predictability is often blurred under natural conditions because of the confounding effects of many environmental variables. To determine the origin and nature of continuous phenotypic differences, and before testing theories about their evolution in heterogeneous environments, it is important to identify a study system where phenotypic differences are large, predictable, and precise.

1.1 OVERVIEW OF STUDY SYSTEM

1.1.1 Habitat: The wave-swept environment

The wave-swept environment is well known for its spatial and temporal heterogeneity in many physical and biological variables such as temperature, salinity, predation, and wave-exposure (Denny 1988). Wave-exposure is extremely heterogeneous, with velocities varying from essentially zero to over 14 m/s (Denny 1994). Although the velocity associated with wave-exposure is readily quantifiable using a number of techniques (Denny 1988) some studies still rely on qualitative descriptions of wave-exposure (Akester and Martel 2000, McQuaid and Lindsay 2000). Many species including snails, barnacles, mussels, bryozoans, anemones, and intertidal kelps (Denny 1988, Pentcheff 1991, Denny 1994, Wildish and Kristmanson 1997, Trussell 1997, Okamura and Partridge 1999, McQuaid and Lindsay 2000) exhibit different morphologies on shores experiencing different degrees of wave-exposure or flow. Most authors suggest this variation is ecophenotypic; however, fewer studies (relative to the number of descriptions) test this hypothesis.

1.1.2 Focal structure: Suspension feeding limbs

Because feeding effects growth and fitness (Meyer 1987, Okamura 1992) and because selection on feeding morphology appears particularly strong (Boag and Grant 1981, Travis 1994), changes in flow over spatial (along shorelines) and temporal scales, may be of special concern for invertebrates suspension feeding with fan-like structures. Temporal and spatial variation appears to affect the form of suspension feeding structures in bryozoans (Okamura and Partridge 1999), larval blackfies (Zhang and Malmqvist 1997), and an intertidal barnacle (Pentcheff 1995). Populations in low flow tend to possess feeding fans with longer and / or fewer limbs (or rays), whereas those in high flow tend to possess shorter and often stouter limbs (or rays).

1.1.3 Study Organism: Intertidal barnacles

Barnacles are ideal organisms for studying the effects of flow on suspension feeding morphology. First, they are common marine crustaceans that colonize almost any hard substrate throughout the world and many intertidal species inhabit coastal shorelines where wave conditions vary from quiet bays to extremely wave-exposed headlands. Second, all feed with a similar feeding fan (the cirral net; fig. 1-1) and therefore the flow-induced forces may act similarly among species. Third, they are well known to alter shell form in response to many environmental variables (Crisp and Bourget 1985, Lively 1986) and an unpublished study (Pentcheff 1995) reported that cirrus length in one species from the north eastern Pacific, *Balanus glandula*, varies with wave-exposure. Fourth, adults are sessile, so environmental effects on feeding form are not obscured by movement among habitats. Finally, barnacle larvae spend a number of weeks dispersing in the plankton before settling (Strathmann 1987). Thus, among-population differences in barnacle feeding form may arise from two broad causes: 1) genetic based differences — due to differential settlement (based on environmental cues such as water motion or biofilms; (Crisp 1955, Neal et al. 1996) and/or differential mortality of alternate genotypes (Schmidt and Rand 2001), or 2) different environmental conditions (differences in flow) simply induce barnacles to produce different feeding forms (phenotypic plasticity).

1.2 THESIS OBJECTIVES

The objective of this thesis was to document among-population variation in barnacle cirrus form along a wave-exposure gradient, identify if cirrus variation is adaptive, and determine if these differences arise via environmental or genetic mechanisms. This project was prompted by unpublished results (Pentcheff 1995) indicating that limb length in *Balanus glandula* varies with wave-exposure in the San Juan Islands,Washington State, USA. The four specific objectives were: 1) determine if *B. glandula* exhibits similar variation in the length of all three captorial cirri (fig. 1-1) across a wave-exposure gradient in Barkley Sound on the west coast of Vancouver Island, British Columbia, Canada (fig. 1-2), 2) to determine whether other cirrus dimensions (ramus length and diameter, seta length and intersetal spacing; see fig. 3-1) vary similarly with wave exposure, 3) to determine whether this relationship occurred among all four broadly sympatric intertidal barnacles (*B. glandula*, *Chthamalus dalli*, *Semibalanus cariosus*, *Pollicipes polymerus*) in Barkley Sound, and 4) to determine whether cirrus variation arises from genetic (differential settlement or differential mortality) or environmental (phenotypic plasticity) causes.

1.3 STRUCTURE AND OVERVIEW OF CHAPTERS

This thesis is composed of five chapters: this general introductory chapter (chapter 1), three chapters reporting on three distinct studies into the plasticity of barnacle cirrus form (chapters 2, 3, and 4), and a general conclusions chapter (chapter 5).

Chapter 2. Based on the unpublished observations of Dean Pentcheff (1995) that *Balanus glandula* produce longer feeding legs in quiet water, we set out to document both the magnitude and precision of intraspecific leg-length variation in *B. glandula* along a wave-exposure gradient in Barkley Sound, British Columbia, Canada. The precision of this relationship in all three pairs of feeding limbs suggests a remarkably close coupling between phenotype and growth environment.

Chapter 3. Because the dependence of limb length on wave-exposure was both dramatic and extremely precise in *Balanus glandula*, we suspected other species feeding with similar structures might exhibit similar variation. To assess the generality of this dependence we examined cirrus form in all four co-occurring intertidal barnacle species in Barkley Sound (*B. glandula*, *C. dalli*, *S. cariosus*, and *P. polymerus*). In addition, because flow at low Reynolds numbers is strongly influenced by small changes in shape (Cheer and Koehl 1987), we examined the effect of flow on three additional cirrus characters (ramus diameter, setae length, and intersetal spacing) to document changes in overall cirrus form.

Chapter 4. In this chapter, I report on three separate experiments conducted to determine the mechanism, age-dependence and response time of cirrus variation in *Balanus glandula*. First, a two-way transplant of juvenile barnacles to low and high flow environments tested whether cirrus variation in *B. glandula* arises via genetic or environmental mechanisms. Second, a one-way transplant of juveniles and adults into quiet water tested whether cirrus variation is limited to early stages. Third, a one-way transplant of adults into quiet water tested how quickly changes in cirrus form are induced by flow environment.

In chapter 5, I present the overall significance of each study and this thesis, and suggest future, potentially productive avenues of research.

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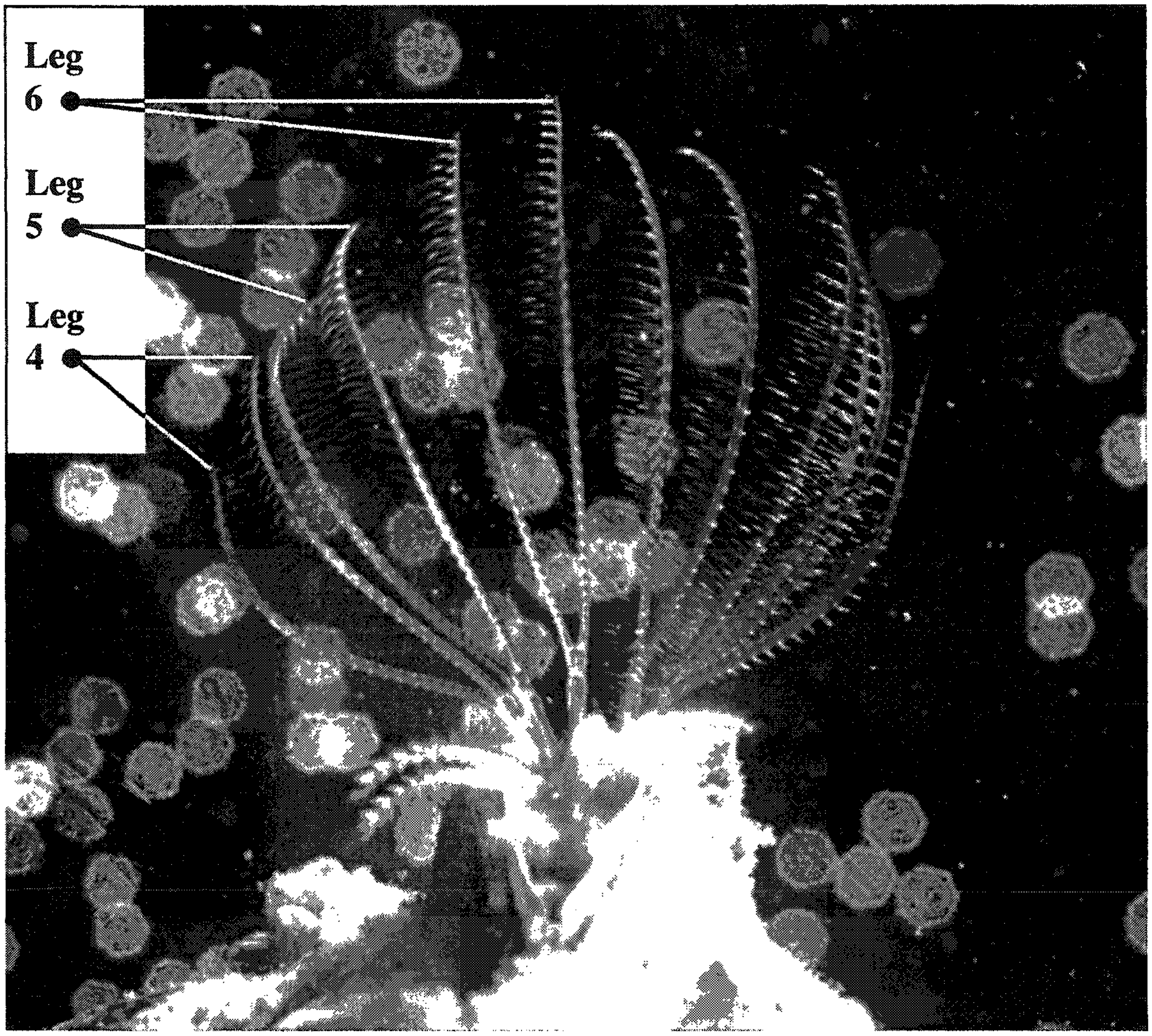

Figure 1-1. The fully extended cirral net of *Balanus glandula* displaying the posterior three pairs of captorial cirri (legs 6, 5 and 4) and rows of setae extending horizontally from the individual rami. The much shorter third pair of cirri are also present (but not labeled) below the fourth pair. The anterior three pairs of cirri (including the third pair seen here) are primarily used to transfer food from the captorial cirri towards the mouth (Anderson and Southward 1987). Photo by Dave Denning (2000).

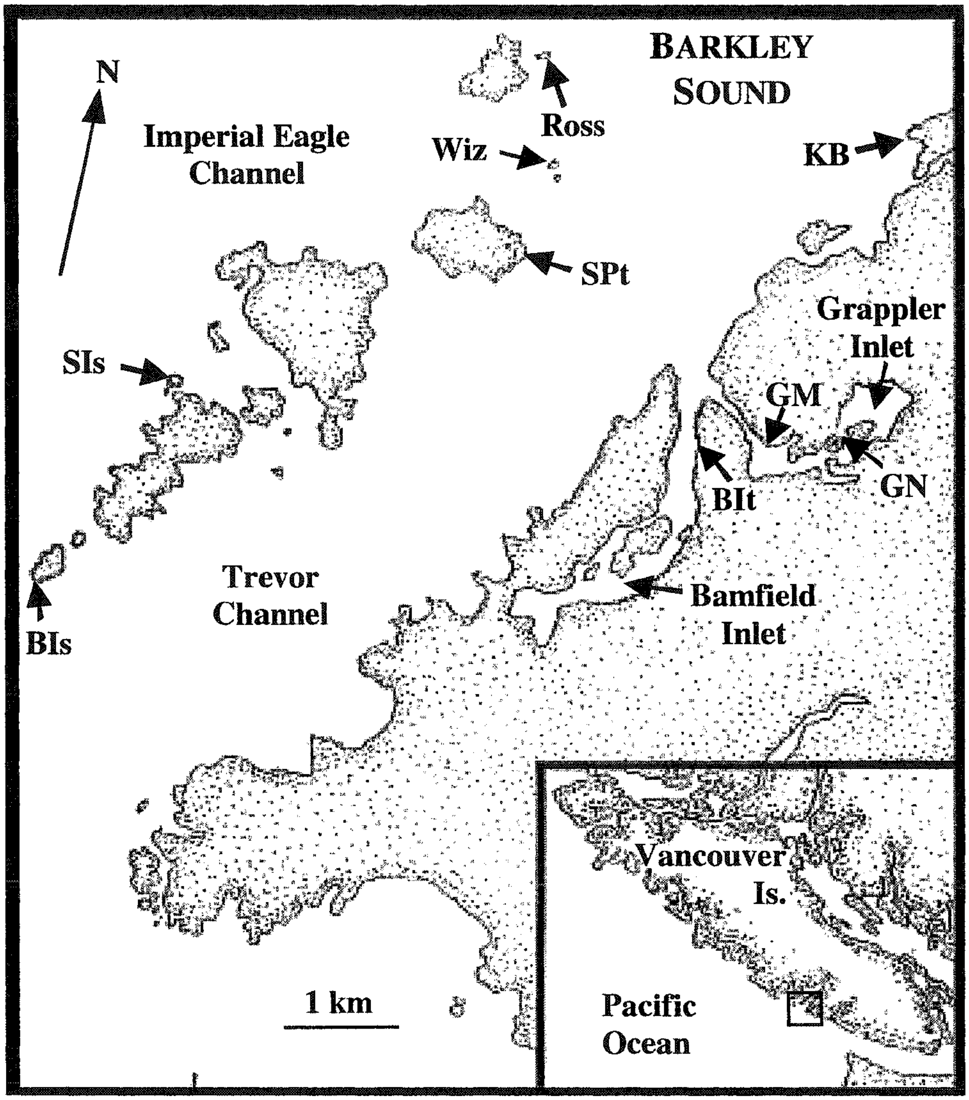

Figure 1-2. Map of study sites located in Barkley Sound, on the west coast of Vancouver Island, British Columbia, Canada (map modified from Akester and Martel, 2000). Sites in order of decreasing water velocity were: Sepping's Island (SIs), Bordelais Island (BIs), Kelp Bay (KB), Wizard Islet (Wiz), Self Point (SPt), Ross Islets (Ross), Bamfield (BIt), Grappler Mouth (GM) and Grappler Narrows (GN). See tables 2-1 and 3-1 for site descriptions.

CHAPTER 2

PRECISE TUNING OF BARNACLE LEG LENGTH TO COASTAL WAVE ACTION†

2.0 INTRODUCTION

Many animal species exhibit different forms in different environments (Schlichting and Pigliucci 1998, Stearns 1989, Travis1994). This intraspecific variation may be striking in some marine invertebrates. For example, encrusting sponges (Palumbi 1984), arborescent stony corals (Chamberlain and Graus 1975) and intertidal snails (Kitching *et al.* 1966, Vermeij 1973) produce more flow-tolerant forms under conditions of high wave-exposure; colonial soft corals produce larger colonies on shores with higher wave action (Sebens 1984); intertidal snails produce thicker shells where crab predation is more intense (Appleton and Palmer 1988, Kitching *et al.* 1966, Palmer 1990); food supply significantly affects skeletal form in herbivorous snails (Kemp and Bertness 1984), in mussels (Hickman 1979), and in both the larvae (Hart and Strathmann1994) and adults (Levitan 1991) of sea urchins. In addition, many animals exhibit conspicuous trophic polymorphisms (Travis1994) and predator-induced morphological differences (Tollrian and Harvell 1999). Clearly, phenotypic plasticity is widespread in animals (Stearns 1989). But how precisely does animal form track local environmental conditions?

Studies of conspicuous intraspecific variation have provided many insights about the costs and benefits of particular morphologies, about mechanisms of adaptation, and about the adaptive significance of plastic versus constitutive responses to environmental

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†A version of this chapter has been published. Arsenault, DJ, KB Marchinko, and AR Palmer. 2001. Proc. Roy. Soc. Lon. B. 268: 2149-2154.

*Order is alphabetical, all authors contributed equally to this project variation (reviewed in Schlichting and Pigliucci 1998, Tollrian and Harvell1999, Travis 1994). However, while induced differences are often predictable in magnitude and direction under controlled experimental conditions, the response to stimuli under natural conditions may differ because other factors also influence adult form. In addition, quantitative estimates of stimulus intensity (food supply, predation intensity, water flow conditions, etc.) are often difficult to obtain in the field, so robust conclusions about the precision of morphological responses to environmental stimuli under natural conditions have been elusive.

Barnacles are ideal animals for studying the precision of phenotypic plasticity. First, they are famous for being able to produce different forms under different conditions: crowding promotes elongate shells that under extreme conditions become trumpet-shaped (Barnes and Powell 1950, Bertness 1989); contact with predatory snails induces a radically bent form in *Chthamalus anisopoma* where the aperture is rotated nearly perpendicular to the substratum (Lively 1986); and differences associated with water motion induce differences in the length of feeding legs (Pentcheff 1995). Second, post-larval barnacles are sessile so local environmental effects are not blurred by animal movements. Third, barnacles are easy to manipulate in the field because they settle on a variety of substrata and may be transplanted readily to determine the causes of among-population variation (selective settlement, differential mortality, or phenotypic plasticity). Fourth, some intertidal species occur over a wide range of environmental conditions, setting the stage for many possible ecophenotypic responses. Finally, the extended period of larval dispersal (up to four weeks in *B. glandula*, Strathmann 1987) appears to yield low among-population genetic heterogeneity (< 4%, Hedgecock 1986), which suggests most observable morphological variation is ecophenotypic.

Because the feeding appendages of barnacles must be extended out of the shell to capture food, they must be able to function under a wide range of wave-exposure conditions. In addition, *Balanus glandula* appears able to produce legs of different length in response to different levels of water motion (Pentcheff 1995). We therefore set out to determine how precisely *Balanus glandula* adjusted the length of its limbs to local wave-exposure conditions.

2.1 METHODS

2.1.1 Field sites

Transducers for measuring wave exposure were deployed, and barnacles collected, from six sites of varying wave exposure in Barkley Sound, on the west side of Vancouver Island, British Columbia, Canada (Table 2-1). These sites ranged from very protected conditions, with minimal swell, to sites exposed to the full fury of northeastern Pacific Ocean swell during winter months.

2.1.2 Wave exposure measurement

Maximum water velocity was estimated at each site using two mechanical transducers (Carrington Bell and Denny 1994) fastened to the rock approx. 2 m apart. Each transducer consisted of a hollow plastic golf ball attached by 60 lb test monofilament line to a spring inside a PVC pipe housing. Drag on the ball extended the spring and a small rubber disk slid along the lubricated monofilament line inside the housing to record maximum extension. To maximize sensitivity of the transducers, we used springs of different compliance at the different sites: k= 92.1 N/m (range 83-110), 173.9 N/m (range 150-194) and 378.1 N/m (range 299-453). Spring extension was calibrated by suspending known masses to the balls. Force measurements were converted to velocity using a published calibration curve (Carrington Bell and Denny 1994). Transducers were deployed concurrently at all six sites on 16 days: July 7, 15 - 16, 18 - 21, July 30 - Aug. 4 and Aug. 14- 16, 2000, and re-zeroed after each 24 hr period, yielding 16 independent estimates of maximum velocity experienced during the preceding 24 hr. During this time period, average hourly maximum offshore wave height during high tide at the La Pérouse buoy maintained by Environment Canada (buoy #46206; 48° 50'06"N, 129° 59'54"W), approx. 50 km west of our nearest site, was 1.37 ± 0.453 m (mean ± SD; N= 16).

2.1.3 Collection and measurement of barnacles

We collected solitary, uncrowded barnacles from bare rock surfaces in the lower third of the *B. glandula* zone immediately up-shore from the location of the transducers on Aug. 3, 2000. Shell dimensions were recorded with Vernier calipers. The prosoma (fleshy part of the body, excluding the shell but including the legs) was removed, blotted dry for 20 seconds on dry Kimwipe® paper, and weighed to the nearest 0.1 mg to obtain body wet mass. The posterior three cirri (legs four, five and six) were dissected from the left side of the prosoma and splayed in seawater on a microscope slide. Lengths of the exo- and endopodite of each leg were measured from the tip to the base of the ramus (i.e., protopodite segments were not included), and the average of these two lengths was standardized to a common prosoma wet mass of 0.0219 g (approx. 3.8 mm opercular length) by ANCOVA. Ramus lengths were measured at magnifications of 40-50X using a *camera lucida* connected to a dissecting microscope, and digitizing tablet with a precision of 20 dots/mm.

2.1.4 Analyses

Least-squares linear regression analyses were conducted with Statview II (version 1.03) and analyses of covariance (ANCOVA) were conducted with SuperANOVA (ver. 1.11), both from Abacus Concepts. All regression and ANCOVA analyses were conducted on $\log_{10}$-transformed data. Least-square means of barnacle leg length for a standard body mass were computed assuming a common slope for all six sites.

2.2 RESULTS

2.2.1 Magnitude and form of variation

The lengths of the three primary pairs of feeding legs of the intertidal barnacle *Balanus glandula* increased with increasing barnacle size at the six sites we studied (Table 2-2a), as seen in other barnacles (Crisp and Maclean 1990). This increase was not significant statistically for some sites because the size range we chose was too narrow, but ANCOVA confirmed that this increase was highly significant for each of the three legs when all sites were analysed concurrently ($P < 0.001$, Table 2-3a-c). In addition, the slope of this relationship did not differ among sites ($P > 0.6$ for all three legs; Table 2-3a-c), so we could therefore compute a leg length for a standard-sized barnacle at each site. For a common prosoma wet mass of 0.0219 g (approx. 3.8 mm opercular length), legs from the quiet-water site were from 55% to 77% longer than those from the most wave-exposed site (Table 2-2a, b). More dramatically, at a very protected site in Grappler Inlet, exposed only to weak tidal currents and no ocean swell, feeding legs were 63%, 71%and 90% larger than those from the most wave-exposed site (legs 4, 5 and 6 respectively). For similar sized barnacles, legs differed not only in length, but also in stoutness, and the length and spacing of setae (fig. 2-1).

2.2.2 Precision of variation

The lengths of the barnacles' feeding legs exhibited surprisingly precise relationships with the maximum water velocities under breaking waves recorded on the adjacent rock surface (fig. 2-2). From 95.6% to 99.5% of the variation in average leg length among the six sites was explained statistically by variation in water velocity (Table 2-2b). This effect was most pronounced for the posterior-most two legs (legs 5 and 6), but was highly significant for all three: barnacle leg length varied in proportion to velocity $^{-0.32 \text{ to } -0.43}$ depending on the leg (Table 2-2b). Similar declines in leg length with increasing wave exposure have been observed in other northeastern Pacific acorn barnacles (*Chthamalus dalli*, and to a lesser extent in *Semibalanus cariosus*) and in the goose-neck barnacle *Pollicipes polymerus* (Chapter 3). J.Truscott (pers. comm.) has also confirmed that exposed-shore *Semibalanus balanoides* have shorter feeding legs than protected-shore individuals from the coast of Wales (UK).

2.3 DISCUSSION

2.3.1 Adaptive significance and predicted pattern

The highly precise dependence of barnacle leg form on local environmental conditions suggests some rather strict tradeoffs between the costs and benefits of different forms (Travis 1994). Presumably, leg length directly affects feeding performance. Longer legs would be able to sieve a larger volume of water per sweep than shorter ones (Crisp and Maclean 1990), and therefore be beneficial under quiet-water conditions. However, longer legs would be less able to remain erect in higher velocity flow because the higher drag would cause them to bend downstream or buckle. Because the outcome of competitive interactions between barnacles, and the ultimate fecundity of barnacles, depends on growth rate, natural selection should favor those individuals whose leg form is the most effective for feeding under local conditions of water motion.

Because barnacle legs probably experience free-stream velocities in the turbulence of breaking waves (Denny 1988), the expected decline in leg length with increasing wave exposure can be predicted from simple hydrodynamics, assuming geometric similarity of the legs. Fluid-dynamics theory predicts that, as water velocity increases under conditions of high Reynolds Number (Re $>10^3$), structure length perpendicular to flow should a vary in proportion to velocity $^{-1}$ for drag to remain the same. This prediction obtains because the force experienced due to pressure drag is $F_d = 0.5\ \rho\ u^2\ S_f C_d$, where ρ is seawater density, u is water velocity, S_f is the frontal or projected area in the direction of flow, and C_d is the coefficient of drag (Denny 1988). Because ρ is constant and C_d lacks units, $F_d \propto u^2\ S_f \propto u^2 l^2$, or $F_d^{0.5} \propto ul$, where l is structure length and structure shape is assumed to remain constant. For drag to remain constant, an increase in u must be matched by a proportional decrease in l. In our study, the observed decline in barnacle leg length, (proportional to velocity $^{-0.3}$ to $^{-0.4}$ Table 2c), was considerably less extreme than the exponent of -1 predicted due to simple scaling.

Three factors might account for why exposed-shore barnacles have proportionally longer legs than predicted from fluid-dynamics theory. First, exposed-shore barnacles may alter the timing of leg extension in oscillating flow (Barnes and Reese 1959, Trager *et al.* 1992) in a way that minimizes drag. Second, the sieving setae that extend perpendicular from the rami are shorter on more wave-exposed shores (fig. 2-1), which would affect the relation between total leg length and drag (i.e., the assumption of geometric similarity is violated). Finally, preliminary results revealed a thicker cuticle in the legs of exposed-shore *Balanus glandula* (K.B. Marchinko, unpublished obs.), so the legs of barnacles from wave-exposed shores are likely more resistant to buckling than those of quiet-water barnacles. However, the scaling of leg cuticle thickness to the velocity of breaking waves remains to be determined.

2.3.2 Precision of plasticity and implications

The precision with which barnacle leg length varied in response to wave exposure (fig. 2-2) seems remarkable: between 95 and 99% of the variation in their average leg length appears to be explained by wave action. Because individual *Balanus glandula* can alter the form of the feeding legs in response to water motion (Pentcheff 1995), this may be the most precise example of ecophenotypic fine-tuning of animal form to local environmental conditions yet reported. Leg length in *B. glandula* exhibits noticeable change within one or two molts following transfer to different flow conditions (D. Pentcheff, pers. comm.; Chapter 4).

The decline in barnacle leg length as the velocity of breaking waves increases (fig. 2-2) has three significant implications. First, the striking linearity of this relationship (on log axes) implies that drag forces — even in the complex, oscillating flow of breaking waves (Denny 1994) — and material and structural changes in the feeding legs, all vary as simple power functions of wave exposure. Second, since the variation in leg length is largely ecophenotypic (Pentcheff 1995), the close fit of the data to a simple power function implies that the cueing mechanisms and the architectural responses of the barnacles are surprisingly precise.

Third, the precision of this relationship suggests that barnacle leg length may provide a simple and reliable index of relative wave exposure in studies of coastal ecology. Direct measurements of wave exposure are cumbersome or time-consuming to obtain over a wide range of field sites (Denny and Gaines 1990). Indirect correlates have been proposed as indexes of wave exposure (Ballantine 1961, Denny 1994), but biological indicators are limited to the geographic area of the original study and abrasion/dissolution indicators cannot be translated into actual wave forces or velocities. *Balanus glandula*, on the other hand, ranges from the Aleutian Islands of Alaska to southern California (Cornwall 1969), and therefore potentially provides a wave-exposure meter for most shores of the northeastern Pacific. The circumboreal *Semibalanus balanoides* appears to exhibit a similar variation in leg length with wave exposure (J. Truscott, pers. comm.), therefore it could provide the same tool for North Atlantic and Arctic shores.

2.4 LITERATURE CITED

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Table 2-1. Field study sites. (Sites where exposure meters were deployed and barnacles collected, in order of increasing wave action.)*

Name	Sampling location	North latitude	West longitude	Shore height (m)† sampled	
				transducers	barnacles
Ross Islets	south-facing side of southern-most, fully treed island in the Ross Islets	48° 52'24"	125° 09'37"	2	3.1
Self Point	south-facing side of point, east side of Helby Is.	48° 50'54"	125° 09'42"	2.1	3.2
Wizard Islet	west-facing side of western islet, below light	48° 51'30"	125° 09'45"	2	3.4
Kelp Bay	south-facing side of point at north end of bay	48° 51'49"	125° 06'21"	2.3	3.4
Bordelais Island	south-facing side of southwestern point	48° 49'02"	125°13'54"	4	5.1
Seppings Island	western-most rocks on southwest-facing side	48° 50'30"	125°12'30"	2.8	3.6

*Barkley Sound, Vancouver Island, British Columbia, Canada
† relative to Canadian Hydrographic Service datum

Table 2-2. Barnacle body sizes, and least-squares linear regression equations for relations discussed in the text.

		Regression equations (±SE)				Mean body size (±SE)	
Site / Trait	N	Slope	Intercept	r^2	P	Operculum length (mm)	Prosoma wet mass (g)
a) Log(ramus length, mm) of the sixth thoracic leg as a function of log(body wet mass, g) of *Balanus glandula* from six sites*							
Ross It.	10	0.137 (0.2033)	0.942 (0.0188)	0.054	0.519	3.67 (0.079)	0.021 (001)
Self Pt.	10	0.202 (0.0972)	0.920 (0.0138)	0.350	0.072	3.75 (0.139)	0.023 (002)
Wizard It.	10	0.178 (0.0911)	0.856 (0.0147)	0.323	0.087	3.53 (0.074)	0.028 (003)
Kelp Bay	10	0.288 (0.0872)	1.032 (0.0136)	0.577	0.011	3.87 (0.187)	0.028 (002)
Bordelais Is.	10	0.330 (0.0443)	1.063 (0.0086)	0.874	<0.001	4.21 (0.173)	0.016 (002)
Seppings It.	10	0.334 (0.1091)	1.055 (0.0161)	0.539	0.016	3.75 (0.176)	0.022 (002)
b) Average log(leg length, mm) of *B. glandula* as a function of average log(water velocity, m/s) under breaking waves (data in fig. 2-2)**							
Leg 4	6	-0.324 (0.0349)	0.623 (0.0081)	0.956	<0.001		
Leg 5	6	-0.414 (0.0187)	0.748 (0.0043)	0.992	<0.001		
Leg 6	6	-0.433 (0.0161)	0.775 (0.0037)	0.994	<0.001		

* wet mass- mass of the prosoma only (i.e., body exclusive of the shell and mantle); ramus length- average of exopodite and endopodite (exclusive of protopodite) of the left leg. Similar slopes, but different intercepts were observed for legs 4 and 5, but are not reported here.

** water velocity- average maximum daily water velocity recorded over 16 days; leg length- least-square mean ramus length from ANCOVA at a common prosoma wet mass of 0.0219 g (approx. 3.8 mm operculum length)

Table 2-3. Results from ANCOVA tests for equality of slopes and the statistical significance of differences of intercepts among sites.

Source of variation	df	Mean square	F	*P*	
a) log(ramus length of fourth thoracic leg)					
Field site	5	0.03638	22.65	<0.001	***
Covariate [log(prosoma wet mass)]	1	0.08813	54.88	<0.001	***
Residual	53	0.00161			
Equality of slopes*	5	0.00038	0.22	0.95	
b) log(ramus length of fifth thoracic leg)					
Field site	5	0.05309	34.50	<0.001	***
Covariate [log(prosoma wet mass)]	1	0.08409	54.65	<0.001	***
Residual	52	0.00154			
Equality of slopes*	5	0.00044	0.27	0.93	
c) log(ramus length of sixth thoracic leg)					
Field site	5	0.06236	40.22	<0.001	***
Covariate [log(prosoma wet mass)]	1	0.07240	46.70	<0.001	***
Residual	53	0.00155			
Equality of slopes*	5	0.00104	0.65	0.66	

* when testing equality of slopes, the residual df was 48, and the residual mean squares was 3.5 - 7% larger than tabled above.

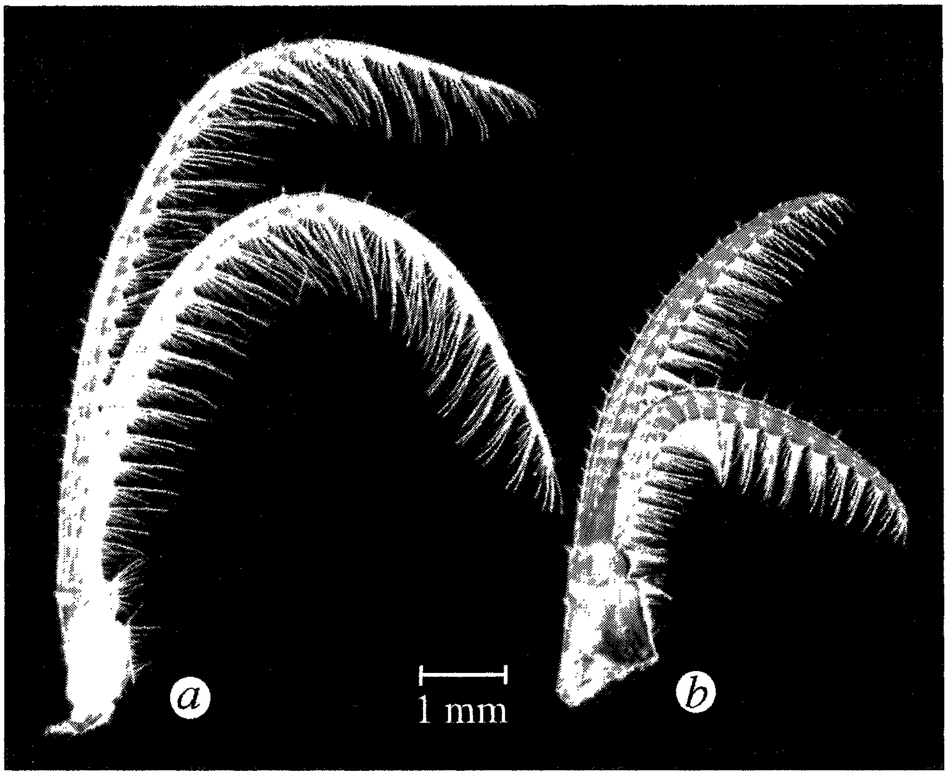

Figure 2-1. Feeding legs of similar sized barnacles (*Balanus glandula*) from two sites: a) protected harbor (Bamfield Inlet; prosomal wet mass = 0.059 g, operculum length 6.7 mm), and b) exposed outer coast (Seppings Island; prosomal wet mass = 0.069 g, operculum length = 6.9 mm), both in Barkley Sound, British Columbia, Canada. The sixth (posterior most) thoracic leg from the left side is shown for each barnacle.

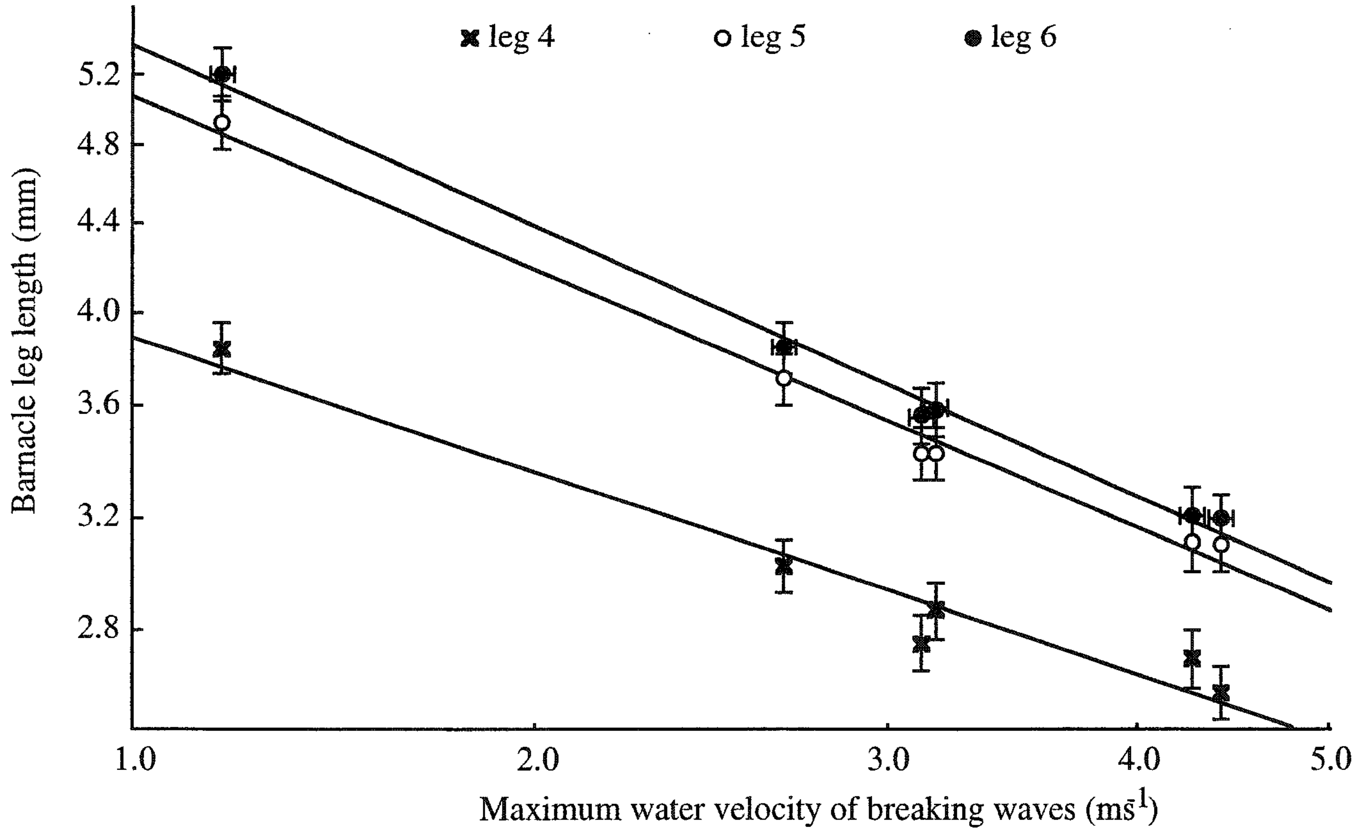

Figure 2-2. Average length of three feeding legs of *Balanus glandula* from six sites of differing wave exposure in Barkley Sound, Vancouver Island, British Columbia, Canada (both axes are log scale). Legs 4, 5 and 6 refer to the fourth, fifth and posterior-most (sixth) pair of thoracic legs of balanomorph barnacles. Leg lengths (N= 10 per site) were standardized to a common prosoma wet mass of 0.0219 g (approx. 3.8 mm opercular length) by ANCOVA. Daily maximum water velocities adjacent to the rock surface were obtained at high tide concurrently for all six sites on 16 days in July and August, 2000, and averaged. All points are mean± SE (error bars for velocity are shown only for leg 6 for clarity). Sites are (left to right): Ross It., Self Pt., Wizard It., Kelp Bay, Bordelais Is., Seppings Is. See Table 2-2b for regression statistics.

CHAPTER 3

FEEDING IN FLOW EXTREMES: DEPENDENCE OF CIRRUS FORM ON WAVE-EXPOSURE IN FOUR BARNACLE SPECIES*

3.0 INTRODUCTION

Flow can have a significant impact on the form, behavior and distribution of aquatic organisms (Allan 1995, Wildish and Kristmanson 1997, Zhang and Malmqvist 1997, Leonard et al. 1998). In wave swept environments, flow variation is extreme, with water velocities ranging from several centimeters per second to well over 10 m/s (Denny 1988). In response to these extremes, many organisms exhibit conspicuous phenotypic plasticity of overall body size and shape. Intertidal snails produce flow-tolerant shells and a larger foot to increase tenacity on wave-exposed shores (Kitching et al. 1966, Trussell 1997*a, b*). On wave-exposed shores barnacles produce shorter feeding legs (Pentcheff 1995, Arsenault et al. 2001), and shorter, thicker shells that are more resistance to impact (Pentcheff 1991). Mussels from wave-exposed shores have thicker shells and a lower height/width ratio (Seed 1968, Raubenheimer and Cook 1990, Akester and Martel 2000). Sponges, hydrozoans, corals and octocorals all exhibit a more compact body shape under conditions of high flow (Patterson 1980, Kaandorp 1999). Plasticity of body form may be common under the extreme flow conditions of the wave swept environment, but how do these extremes affect the form of feeding structures?

Many intertidal invertebrates rely on ambient flow to bring them food, creating two problems. First, a feeding structure must be strong enough to withstand extreme flow conditions, yet fine enough to capture the next meal effectively. Alternatively, feeding structures may withdraw and sacrifice feeding during periods of high flow.

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Second, flow conditions may affect growth rate by influencing the rate of food supply or feeding efficiency (Okamura 1992). On wave-exposed shores, barnacles grow more rapidly (Crisp and Bourget 1985, Sanford et al. 1994, Sanford and Menge 2001), whereas bryozoans (Okamura 1992) and sponges (Palumbi 1986) grow more slowly. In mussels, the effects of wave-exposure appear species-dependent (Seed 1968, McQuaid and Lindsay 2000). Higher growth rates on wave-exposed shores likely result from an increased flux of food particles, due to higher velocities and to greater turbulent mixing (Fréchette et al. 1989). However, an 'optimal' flow for feeding and growth likely exists for many species; when flow becomes too fast or too slow, feeding and growth may be suppressed (Eckman and Duggins 1993, Okamura and Partridge 1999). Curiously, although wave-exposure affects body size, shape, and growth rate in many species, as noted above, surprisingly little is known about how flow affects the form of suspension feeding structures (Okamura and Partridge 1999).

Maintaining feeding performance under different flow conditions can be a real problem for suspension feeders. Both flow velocity, and the size and shape of a feeding apparatus, greatly affect feeding performance (Cheer and Koehl 1987). At low Reynolds numbers, small changes in shape can transform a feeding structure from a sieve to a paddle that effectively pushes water and food away (Cheer and Koehl 1987). Different mechanisms have evolved to maintain feeding performance under different flow conditions. First, barnacles, anemones, phoronids, and articulate brachiopods modify either body position, or position of feeding structures in response to changes in velocity or direction of flow (Barnes and Reese 1960, Emig and Becherini 1970, Koehl 1977, LaBarbera 1977, Trager et al. 1990). Second, changes in the colony form of gorgonian sea fans and scleractinian corals in response to differences in the direction and velocity of water flow affects the rate of prey capture (Leversee 1976, Helmuth and Sebens 1993). By re-orienting the body and feeding structures, these animals likely enhance the rate of feeding while reducing hydrodynamic forces (Wildish and Kristmanson 1997).

The form of feeding structures also varies with flow conditions. Bryozoan lophophores are smaller in diameter and have fewer tentacles in colonies experiencing greater flow (Okamura and Partridge 1999). The primary labral fan of black flies has fewer rays and smaller feeding area in streams experiencing high water velocity (Zhang and Malmqvist 1997). Although overall leg length varies with wave-exposure in one barnacle species (Pentcheff 1995, Arsenault et al. 2001), the response of other features of the feeding fan, and the generality of this response in other species, is unknown.

Barnacles feed by extending feather-like legs (cirri) from the shell into ambient flow to capture small particles and plankton, although a few species capture larger prey (Howard and Scott 1959, Jones 1968). In low flow, they may switch to an active sweep-feeding mode (Trager et al. 1990). Six pairs of biramous cirri (fig. 3-1) make up the feeding net. Each ramus, or branch, of a cirrus has many segments and each segment bears four to six pairs of evenly spaced setae. Because changes in flow regime, and the size and spacing of filtering elements, affect feeding performance of suspension feeders (Cheer and Koehl 1987), both the size of a ramus, and the length and spacing of setae, may affect the ability of barnacles to feed under different flow conditions.

Barnacles are ideal organisms for studying how flow environment influences the form of feeding structures. They are sessile, and they settle and grow under a wide range of environmental conditions. As a consequence, they exhibit considerable lability of form. Crowding induces longer, narrower shells in many species (Crisp and Bourget 1985), contact with a predatory snail induces 'bent' shells in *Chthamalus anisopoma* (Lively 1986*a, b*), and higher salinity promotes a lower height to basal diameter ratio in *Semibalanus balanoides* (Barnes and Barnes 1962). A long period of larval dispersal (Strathmann 1987) and low among-population genetic heterogeneity (Hedgecock 1986) suggest morphological changes with wave exposure are not likely complicated by local genetic differentiation (Arsenault et al. 2001). Finally, all barnacles must grow from a newly settled cyprid into an adult at the spot where they attach initially, so the ontogeny of form may be monitored in individual barnacles under different flow regimes.

We examined three aspects of wave-exposure dependence of barnacle cirral form: 1) variation in the relative size and shape of feeding legs (ramus length, ramus diameter, seta length, intersetal spacing), 2) body-size dependence (allometry) of leg size and shape, and 3) magnitude and similarity of cirral form variation among four co-occurring species.

3.1 METHODS

3.1.1 Study sites

Barnacles were collected from three exposure regimes along a wave-exposure gradient in Barkley Sound, Vancouver Island, British Columbia, Canada (Table 3–1). These sites ranged from sheltered inlets that experienced only weak tidal currents to shores fully exposed to open Pacific swell. Two replicate populations of each species were sampled under each exposure regime. A third population was sampled from the sheltered regime for a total of seven populations.

3.1.2 Water velocity measurements

The maximum water velocities under breaking waves for the four wave-exposed sites (Kelp Bay, Self Point, Bordelais Island and Sepping's Island; see Table 3–1) were from Arsenault et al. (2001). Velocities were recorded with two mechanical transducers (Carington Bell and Denny 1994) per site and deployed concurrently over 16 days in July and August 2000. These velocities were averaged over 16 days to yield a single 'average maximum velocity' for each of these sites (see Arsenault et. al 2001 for details). Maximum water velocities at the three sheltered sites were too slow to be recorded using mechanical transducers and were obtained using an Acoustic Doppler Velocimeter (SonTek / YSI Inc. 10-MHz ADV). Velocities were recorded in the lower section of the *B. glandula* zone on six days between May 9th and June 5th 2001. Five of the six sampling days fell within two days of the two spring tides during that period and sampling times were between one and two hours before or after slack tide, so these measurements provided reasonable estimates of maximum tidal-current velocity. The ADV probe was placed 5 cm above the substrate and instantaneous velocity was recorded at a sampling rate of 25 Hz for five minutes in the x, y, and z dimensions. Each sampling day a single value of velocity for each site was calculated as the mean of individual velocities $(u^2 + v^2 + w^2)^{1/2}$, where u, v, and w represent velocity in the x, y, and z direction (Okamura and Partridge 1999). These single velocities were then averaged over the six sampling days to obtain the single value of water velocity for each sheltered site reported in Table 3–1.

Although the type of velocity measurement differed between the sheltered regime and the two wave-exposed regimes, both provide reasonable estimates of the maximum velocities barnacles were likely to experience during the summer months at these sites. However, even if flow was around 1 m/s at the sheltered sites, conclusions below about the relationship between water velocity and cirral form would not be altered significantly. For example, increasing flow by 1 m/s in all sheltered sites increased the slope of the relationship between ramus length and water velocity for *B. glandula* by only 23%. The difference between this slope and the original slope was non-significant (one-way ANCOVA, *P* (equality of slopes) = 0.25).

3.1.3 Study species

We examined four sympatric species of intertidal barnacles: three balanomorph (acorn) barnacles, *Balanus glandula* Darwin, *Chthamalus dalli* (Pilsbry), *Semibalanus cariosus* (Pallas), and one lepadomorph (goose-neck) barnacle, *Pollicipes polymerus* (Sowerby). Although *Semibalanus balanoides* may occur in British Columbia and may sometimes be confused with *B. glandula*, no *S. balanoides* were encountered in this study. We focused on the morphology of the posterior three cirri because these extend furthest from the shell and are used primarily for food capture. The three anterior cirri are shorter and scrape food off the posterior cirri towards the mouth (Anderson and Southward 1987). All three acorn barnacles were found along the entire wave-exposure gradient we sampled. The goose neck barnacle, however, was absent from shores without sufficient wave action.

3.1.4 Collection and measurement of barnacles

For each species, ten solitary individuals, representing at least a four-fold range in body size (Table 3–2), were collected in the lower one-third of their respective vertical zone at each site. Barnacles from Kelp Bay, Self Point, Bordelais Is. and Sepping's Is.

were collected in the area immediately adjacent to the location of wave transducers. Wave transducers were located in the upper region of the *S. cariosus* zone. Barnacles were collected in August and early September 2000, within 18 days of the last wave-force measurement at the exposed sites (Arsenault et al. 2001).

To measure body size, the soft tissue (prosoma), excluding egg masses and opercular plates, was removed from the shell, blotted dry for 20 seconds on a dry Kimwipe®, and weighed to the nearest 0.1 mg (prosomal wet mass). Although solitary individuals should not exhibit the effects of crowding, shell measurements were not used to describe body size because shell form is so plastic (Crisp and Bourget 1985, Lively 1986*a, b*). The posterior three cirri (legs 4, 5 and 6) were dissected from the left side of the prosoma and mounted in seawater on a microscope slide. As barnacles are bilaterally symmetrical, we assumed measurements from the left side were representative of both sides. We measured four traits on each cirrus: ramus length, ramus diameter, maximum seta length, and intersetal space. Ramus length (fig. 3-1a) was the curvilinear distance traced from base to tip on the dorsal side of the ramus. Ramus diameter (fig. 3-1b) was the distance between the dorsal and ventral side of the first (basal) segment of each ramus. To obtain a single value of ramus length and ramus diameter for each individual, values from both the endo- and exopodite were averaged for each cirrus. Seta length (fig. 3-1c) was the length of the longest seta on the middle three segments of the endopodite. Intersetal space (fig. 3-1d) was the length of the space between the two longest adjacent setae on the middle three segments of the endopodite. To obtain a single value of seta length and intersetal space for each individual, we took the average value from the middle three segments. All traits were measured at 25-50X magnification using a dissecting scope mounted with a *camera lucida*, and a digitizing tablet with a precision of 20 dots/mm.

3.1.5 Statistical analyses

Where error exists in both X and Y variables, the reduced-major-axis (RMA) regression is often recommended over ordinary-least-squares (OLS) regression when describing scaling relationships, because error associated with the X-axis consistently biases the OLS slope downwards (McArdle 1988, LaBarbera 1989). However, this study examined the slopes of four characters, each regressed on body mass. Because the covariate was the same for all four traits, and because Analysis of Covariance (ANCOVA) methods for model II regression are not well developed, OLS regression and ANCOVA (StatView 5.0, 1998 SAS Institute Inc., Cary, NC; SuperANOVA 1.11, Abacus Concepts, Inc., Berkeley, CA) were used to describe and test for differences in the relationships of cirral dimensions to body mass and water velocity.

3.1.5a Allometry

Cirral traits and body size were $\log_{10}$ transformed to meet assumptions of ANCOVA. Body size (prosomal wet mass) was used as the covariate in all allometric analyses. A one-factor ANCOVA (main effect= population) tested for differences in the dependence of cirral characters on body mass among populations from habitats of different wave-exposure. Two-factor ANCOVA (main effects= species, population) was used to test for differences in these relationships among species. Only the sites where all four species co-occur (Self Pt., Kelp Bay, Bordelais Is., and Sepping's Is.) were used in among-species comparisons. Departures from isometry (expected slope of 0.333) were tested with a one-sample *t*-test. Where conclusions about statistical significance might have been confounded by conducting multiple tests, significance levels were also assessed by a sequential Bonferroni test (Rice 1989).

3.1.5b Wave-exposure and cirrus form

To compare the relationship of cirral traits and water velocity for a standard-sized individual, the least-squares mean of each cirral trait for a standard body mass was calculated for each site and each species using ANCOVA (slopes were assumed to be equal if slope differences were not significant) and then regressed on water velocity. The standard prosomal wet mass used for each species reflected the average body size of that species: 0.0096 g for *B. glandula*, 0.0018 g for *C. dalli*, 0.084 g for *S. cariosus*, and 0.23 g for *P. polymerus*. A one-factor ANCOVA (main effect= leg number) test whether the relation between water velocity and cirral dimensions varied among the three cirri. Again, cirral traits were $\log_{10}$ transformed to meet assumptions of ANCOVA; however, the covariate, water velocity, was not transformed. A one-factor ANCOVA (main effect= species) was also used to test for differences in the dependence of cirral dimensions on water velocity among species.

3.2 RESULTS

3.2.1 Scaling of cirral traits with body mass

3.2.1a Within-species comparisons

All four cirral dimensions increased with increasing body mass in all four species (figs. 3-2 and 3-3). This relationship held true for all three posterior feeding legs (legs 4, 5 and 6) at all sites sampled, as observed previously for ramus length (Crisp and MacLean 1990, Arsenault et al. 2001). This relationship was significant for each cirral trait ($P < 0.05$) in 245 of 278 OLS regressions (Table 3–2). Only setae length in one population of *C. dalli* and the intersetal space in four populations of *C. dalli* and *S. cariosus* were consistently non-significant. However, ANCOVA revealed body mass as a significant covariate ($P < 0.01$) for ramus length (Table 3–4), and all remaining cirral traits (Appendix 3–1), when analyzing populations of each species concurrently.

Within each species, the slopes of cirral dimensions versus body mass did not differ significantly among populations ($P > 0.11$) for ramus length (Table 3–4), seta length, intersetal space, or ramus diameter (Appendix 3–1) when corrected for multiple tests. So for each species, a common scaling coefficient was calculated for each trait using ANCOVA (Table 3–5). The dimensions of the cirral net showed negative allometry for each species in all but one case ($P < 0.01$, Table 3–5). However, the extent of negative allometry differed among cirral characters. Seta length and intersetal space tended to exhibit the strongest negative allometry whereas ramus length and diameter tended to exhibit weaker negative allometries (the principal exception to this pattern was *C. dalli*).

Bonferroni-Dunn tests for multiple comparisons revealed that for a standard wet mass, sheltered populations of each species had cirri with consistently and significantly longer rami ($P < 0.002$), longer setae ($P < 0.003$), and narrower ramus diameters ($P < 0.003$) than populations from intermediate and wave exposed habitats (Table 3–4; fig. 3-2; Appendix 3–1). Ramus diameter of *P. polymerus* did not vary significantly among populations, however, because only intermediate and exposed sites were sampled for this species.

3.2.1b Among-species comparisons

All four species showed similar allometries in ramus length and seta length (fig. 3-3, Table 3–5), and the differences that were present were not significant ($P > 0.11$, Table 3–6). The allometry of both intersetal space and ramus diameter, however, did differ significantly among species ($P < 0.013$, Table 3–6). The large, low-shore *S. cariosus* exhibited the strongest negative allometry in intersetal spacing whereas the diminutive, high shore *C. dalli* exhibited the strongest negative allometry in ramus diameter. Nonetheless, regardless of species or site, all cirral traits exhibited a consistent negative allometry with body mass (Table 3–2; Table 3–5).

3.2.2 Dependence cirral form on wave-exposure

The cirral trait allometries did not differ significantly among populations (Table 3–4; Appendix 3–1). Therefore, predicted cirral dimensions were calculated for a standard sized barnacle of each species (see methods) to determine how they varied as a function of wave-exposure. Percentage differences were computed as the total difference in trait dimension between the site of lowest water velocity (0.0066 m/s) and the site of highest velocity (4.41 m/s, Table 3–3); according to the regression equation of each trait to water velocity for each species (Table 3–3a, b).

3.2.2a Ramus length

In each species, populations experiencing lower water velocities exhibited longer rami than populations from higher velocity habitats (figs. 3-4 and 3-5a), as reported previously for *B. glandula* (Pentcheff 1995, Arsenault et al. 2001). Ramus lengths of legs 4, 5 and 6 of *B. glandula* were 75-80% longer in sheltered habitat compared to the most wave-exposed habitat (fig. 3-4). Significant, though less dramatic, differences in ramus length were observed between these extreme sites in both *C. dalli* (47-68% longer) and *S. cariosus* (29-37% longer; fig. 3-4), and between habitats of intermediate (2.66 m/s) and high wave-exposure (4.41 m/s) in the gooseneck barnacle *P. polymerus* (7-12% longer). OLS regressions revealed a highly significant dependence of ramus length on water velocity ($P < 0.005$) that was also quite precise (r^2 varied from 0.89-0.92) for legs 4, 5 and 6 of *B. glandula* and *S. cariosus* (Table 3–3a). For *C. dalli* and *P. polymerus* this relationship was only significant statistically for leg 6; however, the slopes did not differ significantly among legs 4, 5 and 6 for any species (Table 3–7).

3.2.2b Seta length

Barnacles from quiet-water habitats tended to posses longer setae compared to those from wave-exposed shores (fig. 3-5b). Between the two most extreme sites (Grappler Inlet, Sepping's Is.), setae of leg 6 were 50% longer in *B. glandula*, 41% longer for *C. dalli*, and 36% longer for *S. cariosus*. *Pollicipes polymerus* from intermediate wave-exposure had setae 11% longer than those from highly wave-exposed shores. The increase in seta length with decreasing water velocity was significant for *B. glandula* ($P = 0.005$), *S. cariosus* ($P = 0.001$) and *C. dalli* ($P = 0.048$), but not for *P. polymerus* ($P = 0.14$; Table 3–3b).

3.2.2c Intersetal space

The intersetal space only increased significantly with decreasing water velocity in *B. glandula* ($P = 0.006$; Table 3–3b). On leg 6, it was 21% greater at the most protected compared to the most exposed site (fig. 3-5c). *S. cariosus* exhibited a similar difference between these two sites (25%), whereas the intersetal space of *C. dalli* increased by only 2%, neither increase was significant ($P > 0.19$). In contrast, the intersetal space declined by 8% from the most exposed to intermediately exposed site in *P. polymerus*, however this decline was also not statistically significant ($P > 0.08$; Table 3–3b).

3.2.2d Ramus diameter

Ramus diameter declined with decreasing water velocity in all four species, and this decline was significant for the three acorn barnacles (*B. glandula*, *C. dalli*, and *S. cariosus*; $P < 0.03$), but not for *P. polymerus* ($P = 0.20$; Table 3–3b). For leg 6, ramus diameter was 25% smaller for *B. glandula* from the most protected site compared to the most wave-exposed site (fig. 3-5d). This difference was similar, though less pronounced, for *C. dalli* (18%), *S. cariosus* (21%) and *P. polymerus* (6%).

3.2.3 Among-species variation in cirral form with wave-exposure

All four species exhibited strikingly similar changes in cirral form (except the intersetal space; fig. 3-5c; Table 3–3b): as water velocity increased among sites, ramus length and seta length decreased, whereas ramus diameter increased (fig. 3-5). The slopes of these relationships did not differ significantly among species ($P > 0.33$, Table 3–8).

3.3 DISCUSSION

3.3.1 Allometry of cirral dimensions

In all four species examined, larger barnacles exhibited proportionally shorter cirri using either OLS or RMA techniques (Table 3–9). This same pattern was also apparent for two of three additional species for which data were available (Table 3–9). Hydrodynamic theory suggests such negative allometry is adaptive. As barnacles grow larger their feeding cirri extend farther away from the substratum and therefore, as in other animals (Thomason et al. 1998), they are more likely to experience free-stream velocities. Consequently, smaller barnacles may benefit from having proportionally longer cirri because they can feed further out of the boundary layer where particle flux is greater. However, size-dependent differences in metabolic and feeding rates (Sebens 1982, Denny 1988) might also influence this pattern even in the absence of boundary-layer effects.

Curiously, the degree of negative allometry in feeding leg length did not appear to differ among populations from very different wave-exposure regimes. Because boundary layers are thinner at higher velocities (Vogel 1988), the benefits to smaller barnacles of having proportionally longer cirri should be least under high-velocity conditions. Yet coefficients of allometry did not differ among exposure conditions for any of the four cirral characters examined (total ramus length, ramus diameter, seta length and intersetal spacing; Table 3–2). Therefore, contrary to expectation, barnacles appear to respond to differences in wave exposure not with changes in the allometry of limb growth but rather with changes in limb size and shape specific to a particular wave-exposure regime (i.e., the intercepts of the size-dependence relationships change but the slopes do not).

3.3.2 Cirrus form and wave action

Feeding legs were shorter and stouter on wave-exposed shores and up to 80% longer and 25% thinner in sheltered bays. The high precision of these relationships in all four barnacle species (r^2 varied from 0.65 to 0.94 depending on trait and species; Table 3–3b) suggests that strict trade-offs govern the form of feeding structures to ensure maximal feeding effectiveness under different hydrodynamic conditions.

Although striking, our quantitative results must be interpreted with caution, because of qualitative differences in the type of maximal flows experienced by barnacles and because of the way barnacles respond behaviorally to flow. At the intermediate and most exposed sites, maximal water velocities were experienced immediately after a wave breaks followed by lower velocities during the backwash in an oscillatory manner. At the most sheltered sites maximal water velocities arose from sustained tidal currents (see Methods). These flow regimes are associated with different feeding behaviors. In oscillating flow, leg-extension is precisely timed to the frequency of oscillation (Trager et al. 1990), but legs may or may not be maximally extended at the maximum velocity. In contrast, in slow, sustained currents barnacles may actively sweep-feed to increase the volume of water cleared per unit time. Therefore, in oscillating flow drag on feeding legs may vary and be quite high, whereas in weak tidal currents drag on feeding legs may be negligible. As a consequence, the differences in leg form observed over the wide range of velocities we examined likely reflect a complex response to mechanical forces induced by drag and to ingestion rates that depend on the form of the feeding leg. Nonetheless, the qualitative differences we observed seem likely to be adaptive.

3.3.3 Feeding in slow moving currents

Barnacles from sheltered bays experience weaker currents and thicker boundary layers that may limit the rate of food supply (Fréchette et al. 1989). Those with longer rami and setae would clear a greater volume of water per sweep and presumably have higher capture rates (Crisp and MacLean 1990). In barnacles, as in larval black flies (Zhang and Malmqvist 1997), a wider spacing of sieve elements in slow-moving water may permit higher capture rates because fluid transmission rates are higher (Cheer and Koehl 1987). However, the Reynolds number for flow through *B. glandula* cirri from Bamfield Inlet was approximately 0.5 (based on a seta diameter of 35 μm, which was roughly equal to the distance between adjacent setae, and a water velocity of 0.01 m/s), suggesting that the intersetal space may be too large for spacing differences to affect fluid transmission (Cheer and Koehl 1987). Furthermore, active sweep-feeding would also increase the feeding rate of barnacles at low water velocity (Crisp and Southward 1961, Trager et al. 1990).

3.3.4 Feeding under breaking waves

On wave-exposed shores barnacles possess shorter, stouter cirri, which should reduce drag (Denny 1988) and hence the risk of damage. In addition, shorter, stouter cirri should allow barnacles to hold their cirri erect at higher velocities and feed more effectively. Flume experiments bear this out. *Balanus glandula* from a wave-exposed site maintained full cirral extension at velocities up to one m/s, whereas sheltered-shore individuals fully retracted their cirri around 20 cm/s (KB Marchinko, unpublished data). This compares favorably to observations on *Balanus hameri* from areas of high current, where cirri were held erect at velocities up to 1 m/s (Crisp and Southward 1961).

The stouter rami of barnacles from wave-exposed shores may also be less likely to buckle when extended in high-velocity flow for two reasons. First, the cuticle of barnacle legs resembles a tube, and larger diameter tubes have material further from the neutral axis (Denny 1988). Second, stouter rami also have a thicker cuticle (KB Marchinko, unpublished). Both of these factors would reduce the extent to which rami deflect downstream due to drag. Studies of how cuticle thickness and second moment of area vary with increasing water velocity are currently underway.

3.3.5 Relation of feeding to growth and fitness

Feeding rate has a large impact on growth rate and fitness (Ritchie 1990, Okamura 1992), so variation in traits that effect feeding rate should experience strong selection. The differences in limb form between exposed and protected-shore barnacles likely reflect a trade-off between feeding effectiveness and structural integrity. On the one hand, longer feeding limbs and filtering setae would allow a barnacle to sieve a larger volume of water per sweep, or to clear a larger volume of water during passive feeding. However, the longer the limb and setae, the greater the drag at a given velocity. If a limb is too long to be held erect at a given velocity, the volume of water cleared will decrease. Similarly, a stouter ramus with thicker cuticle could be held erect at higher velocities, but it would also be more costly to maintain because more cuticle would have to be replaced each molt.

If the differences in limb form reported here allow equivalent rates of feeding under widely different wave-exposure regimes, then growth rates should be similar in sheltered- and exposed-shore barnacles. However, many studies have reported higher rates of growth or reproduction in barnacles from more wave-exposed shores (Lewis and Chia 1981, Crisp and Bourget 1985, Bertness et al. 1991, Sanford et al. 1994, Sanford and Menge 2001). Each of these studies suggests the higher flux of food in wave-exposed sites promotes higher growth rate. If the feeding limbs of the barnacles in these studies vary with wave exposure as they do in the species reported here, then the costs of producing shorter, stouter cirri with thicker cuticle appear to be more than offset by the increased flux of food. In addition, barnacles feeding under low flow conditions must resort to active sweep-feeding (Trager et al. 1990), which is energetically more costly

(Jørgensen 1966). Higher water temperatures in sheltered bays would also yield higher metabolic rates, and thus greater rates of energy loss to maintenance metabolism. All of these factors may contribute to lower rates of growth in slower moving water.

3.3.6 Confounding effects of growth and form in studies of suspension feeders

Care should be taken when measuring growth rates of suspension feeders to ensure morphological changes that may affect feeding performance do not confound the environmental variables being studied. For example, Eckman and Duggins (1993) collected juvenile barnacles from a single site and measured growth rate under different laboratory flow regimes over 54 days. They reported that *B. glandula, S. cariosus*, and *P. polymerus* all grew at similar rates over a 2-15 cm/s range of velocities. However, the consistent growth rates they reported might have reflected changes in cirrus morphology reported here. Because *B. glandula* appears able to transform the form of its feeding limbs to suit ambient flow conditions after a single molt (i.e., within 18 - 25 days, KB Marchinko, unpublished), the barnacles in Eckman and Duggins' (1993) study may have achieved similar rates of growth by altering cirral form to suit ambient velocities.

3.3.7 Differences among species in relative variability

Although the difference in leg form for a given difference in water velocity was similar for all four species studies (i.e., slopes were statistically indistinguishable; fig. 3-5, Table 3–8), some species exhibited proportionally more variation over the same range of water velocities than others, relative to leg size. The lowest-shore species, *S. cariosus*, exhibited the lowest proportional difference in leg length between extreme sites (29-37%) whereas the higher shore species, *B. glandula* and *C. dalli,* proportionally much greater variation (75-80% an d 47-68% respectively).

Three possible explanations might account for these differences among species. First, shore height may influence the maximal velocities experienced because, as water depth increases, the maximum water velocities at the substratum due to passing waves decreases (Denny 1988). Therefore, wave-exposed populations of *S. cariosus* would not only have a greater opportunity to feed at lower velocities, but they would also have more opportunity to feed when waves were not breaking directly over them. As a consequence, the range of velocities they experience while feeding might be less than that reported here. More accurate measures of velocities at different heights on the shore, and of the hydrodynamic conditions under which *S. cariosu*s will feed, would be required to confirm this.

Second, body size may influence the extent of cirral variation. Adult body size of the three balanomorph barnacles differed (*S. cariosus* > *B. glandula* > *C. dalli*; see methods), and the lowest proportional differences in leg length between extreme sites for a species was observed in the largest species. However, the proportionally greater variation in the larger bodied *B. glandula*, compared to the smaller bodied *C. dalli*, suggests that differences in variability among species are not simply due to body size differences. Nonetheless, surface rugosity may allow small individuals to hide deeper in the boundary layer. The smaller-bodied *C. dalli* may therefore exhibit less variability than the larger-bodied *B. glandula* because it experiences lower local velocities on the same shore. This sensitivity to surface rugosity may also explain why the cirri of *C. dalli* varied less predictably with water velocity at intermediate and exposed sites (figs. 3-4 & 3-5).

Third, relative variability may relate to the range of conditions typically experienced by each species. Our data suggest that species inhabiting a wider range of wave-exposure conditions exhibit proportionally greater variability of feeding cirri. *Balanus glandula* occurs at high density throughout the wave-exposure gradient we studied and exhibited the greatest proportional variation in leg length. In contrast, *C. dalli* and *S. cariosus* are less common in protected bays, and exhibited intermediate variability in leg length. Finally, the leg lengths of *P. polymerus*, which can not survive on high-energy shores, were least variable. If different degrees of variability in leg form reflect different degrees of phenotypic plasticity — as seems likely based on experiments with *B. glandula* (Chapter 4) — then the capacity to alter the form of feeding appendages to suit local fluid-dynamic conditions may significantly affect the range of exposure conditions under which a species may feed and grow effectively. In other words, more phenotypically plastic species may be able to survive and grow under a wider range of hydrodynamic conditions.

3.3.8 Variability of different cirral traits

Within species, some traits exhibited greater variation over the wave-exposure gradient than others. However the rank order of proportional variability of the three significant traits was consistent for all four species: ramus length > seta length > ramus diameter. This rank order also parallels the rank order of trait size: the larger the trait, the more variable it was. Presumably, these differences in variability reflect the impact each trait has on feeding efficiency, or risk of damage, at different water velocities.

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Table 3–1. Collection site descriptions.

Wave Exposure	Site Name	Location description and species sampled	North Latitude	West Longitude	Water Velocity m/s*
Sheltered	Grappler Narrows	East-facing shore of Grappler Inlet narrows *Balanus glandula, Semibalanus cariosus*	48°49'94"	125°07'07"	0.0052
	Grappler Mouth	West-facing shore at mouth of Grappler Inlet *B. glandula, Chthamalus dalli*	48°49'91"	125°07'65"	0.0066
	Bamfield Inlet	West-facing shore of Bamfield Inlet near mouth *B. glandula, C. dalli, S. cariosus*	48°50'06"	125°08'20"	0.0123
Intermediate	Self Point[†]	South-facing shore on southeastern point of Helby Is. *B. glandula, C. dalli, S. cariosus, Pollicipes polymerus*	48°50'90"	125°09'70"	2.66
	Kelp Bay[†]	South-facing shore on north end of Kelp Bay *B. glandula, C. dalli, S. cariosus, P. polymerus*	48°51'82"	125°06'35"	3.17
Fully Exposed	Bordelais Island[†]	South-facing shore on southwestern point *B. glandula, C. dalli, S. cariosus, P. polymerus*	48°49'03"	125°13'90"	4.26
	Seppings Island[†]	Southwest -facing shore, gentlest slope of sites *B. glandula, C. dalli, S. cariosus, P. polymerus*	48°50'50"	125°12'50"	4.41

† Site descriptions from Arsenault et al 2001.

*Average maximum tidal current velocity from six days (sheltered), or average maximum velocity of breaking waves from 16 days (intermediate and fully exposed).

Table 3–2. OLS regression equations for the relationship between the four cirral dimensions (ramus length, seta length, intersetal space and ramus diameter) and body mass for cirrus 6.

		OLS Regression Equations				Body Size (prosomal wet mass, g)*		
Species/Trait/Site	n	Slope (SE)	Intercept (SE)	r2	*P* (slope)	Mean (SE)	Min	Max
Balanus glandula								
Ramus length								
Grappler Narrows	10	0.293 (0.017)	1.297 (0.037)	0.974	<0.0001	0.0133 (0.0035)	0.0006	0.0318
Grappler Mouth	10	0.247 (0.017)	1.189 (0.034)	0.964	<0.0001	0.0241 (0.0079)	0.0019	0.0758
Bamfield Inlet	10	0.337 (0.033)	1.292 (0.067)	0.929	<0.0001	0.0205 (0.0059)	0.0014	0.0544
Self Pt.	10	0.287 (0.021)	1.066 (0.043)	0.961	<0.0001	0.0161 (0.0048)	0.0011	0.0451
Kelp Bay	10	0.324 (0.038)	1.096 (0.077)	0.898	<0.0001	0.0175 (0.0044)	0.0016	0.0409
Seppings Is.	10	0.296 (0.028)	1.028 (0.060)	0.932	<0.0001	0.0165 (0.0047)	0.0012	0.0376
Bordelais Is.	10	0.215 (0.024)	0.861 (0.052)	0.910	<0.0001	0.0125 (0.0035)	0.0008	0.0324
Seta length								
Grappler Narrows	10	0.216 (0.015)	0.211 (0.033)	0.963	<0.0001	–	–	–
Grappler Mouth	10	0.189 (0.022)	0.145 (0.044)	0.901	<0.0001	–	–	–
Bamfield Inlet	10	0.225 (0.025)	0.137 (0.051)	0.911	<0.0001	–	–	–
Self Pt.	10	0.179 (0.023)	-0.034 (0.048)	0.886	<0.0001	–	–	–
Kelp Bay	10	0.233 (0.026)	0.005 (0.052)	0.904	<0.0001	–	–	–
Seppings Is.	10	0.231 (0.017)	0.049 (0.036)	0.958	<0.0001	–	–	–
Bordelais Is.	10	0.159 (0.026)	-0.099 (0.057)	0.820	0.0003	–	–	–
Intersetal space								
Grappler Narrows	10	0.206 (0.033)	-1.094 (0.073)	0.826	0.0003	–	–	–
Grappler Mouth	10	0.102 (0.030)	-1.414 (0.058)	0.599	0.0086	–	–	–
Bamfield Inlet	10	0.161 (0.033)	-1.314 (0.066)	0.755	0.0011	–	–	–
Self Pt.	10	0.187 (0.026)	-1.327 (0.055)	0.866	<0.0001	–	–	–
Kelp Bay	10	0.201 (0.041)	-1.317 (0.082)	0.749	0.0012	–	–	–
Seppings Is.	10	0.072 (0.024)	-1.558 (0.050)	0.536	0.0161	–	–	–
Bordelais Is.	10	0.090 (0.045)	-1.517 (0.097)	0.334	0.0802	–	–	–

Ramus diameter								
Grappler Narrows	10	0.194 (0.017)	-0.381 (0.038)	0.940	<0.0001	–	–	–
Grappler Mouth	10	0.241 (0.023)	-0.284 (0.045)	0.935	<0.0001	–	–	–
Bamfield Inlet	10	0.243 (0.025)	-0.267 (0.051)	0.923	<0.0001	–	–	–
Self Pt.	10	0.207 (0.029)	-0.228 (0.060)	0.867	<0.0001	–	–	–
Kelp Bay	10	0.265 (0.024)	-0.157 (0.047)	0.940	<0.0001	–	–	–
Seppings Is.	10	0.248 (0.031)	-0.155 (0.065)	0.889	<0.0001	–	–	–
Bordelais Is.	10	0.226 (0.017)	-0.184 (0.037)	0.957	<0.0001	–	–	–
Chthamalus dalli								
Ramus length								
Grappler Mouth	10	0.265 (0.071)	1.139 (0.200)	0.636	0.0057	0.0017 (0.0003)	0.0008	0.0030
Bamfield Inlet	10	0.253 (0.057)	0.983 (0.163)	0.709	0.0023	0.0017 (0.0003)	0.0007	0.0031
Self Pt.	10	0.350 (0.121)	1.068 (0.340)	0.511	0.0202	0.0018 (0.0003)	0.0008	0.0039
Kelp Bay	10	0.220 (0.058)	0.729 (0.156)	0.644	0.0052	0.0023 (0.0004)	0.0013	0.0054
Seppings Is.	10	0.256 (0.040)	0.893 (0.111)	0.834	0.0002	0.0021 (0.0004)	0.0009	0.0044
Bordelais Is.	10	0.221 (0.052)	0.714 (0.134)	0.690	0.0029	0.0031 (0.0005)	0.0014	0.0059
Seta length								
Grappler Mouth	10	0.145 (0.034)	-0.145 (0.095)	0.699	0.0026	–	–	–
Bamfield Inlet	10	0.206 (0.055)	-0.066 (0.158)	0.634	0.0059	–	–	–
Self Pt.	10	0.217 (0.112)	-0.146 (0.313)	0.322	0.0873	–	–	–
Kelp Bay	10	0.166 (0.068)	-0.268 (0.185)	0.424	0.0413	–	–	–
Seppings Is.	10	0.161 (0.051)	-0.250 (0.141)	0.555	0.0134	–	–	–
Bordelais Is.	10	0.261 (0.071)	-0.046 (0.183)	0.628	0.0062	–	–	–
Intersetal space								
Grappler Mouth	10	0.277 (0.096)	-0.979 (0.270)	0.512	0.0199	–	–	–
Bamfield Inlet	10	0.314 (0.075)	-0.919 (0.212)	0.689	0.0030	–	–	–
Self Pt.	10	0.100 (0.159)	-1.572 (0.448)	0.047	0.5480	–	–	–
Kelp Bay	10	0.147 (0.070)	-1.400 (0.189)	0.354	0.0694	–	–	–
Seppings Is.	10	0.050 (0.066)	-1.597 (0.182)	0.066	0.4721	–	–	–
Bordelais Is.	10	0.182 (0.128)	-1.301 (0.328)	0.203	0.1913	–	–	–

Ramus diameter								
Grappler Mouth	10	0.149 (0.056)	-0.546 (0.158)	0.469	0.0288	–	–	–
Bamfield Inlet	10	0.104 (0.027)	-0.708 (0.077)	0.650	0.0048	–	–	–
Self Pt.	10	0.302 (0.095)	-0.118 (0.266)	0.560	0.0127	–	–	–
Kelp Bay	10	0.178 (0.073)	-0.455 (0.197)	0.426	0.0408	–	–	–
Seppings Is.	10	0.186 (0.039)	-0.365 (0.107)	0.743	0.0013	–	–	–
Bordelais Is.	10	0.142 (0.051)	-0.475 (0.130)	0.495	0.0233	–	–	–
Semibalanus cariosus								
Ramus length								
Grappler Narrows	10	0.180 (0.035)	1.045 (0.039)	0.764	0.0009	0.1556 (0.0457)	0.0186	0.4137
Bamfield Inlet	10	0.204 (0.061)	1.101 (0.073)	0.585	0.0100	0.1152 (0.0315)	0.0141	0.3134
Self Pt.	10	0.243 (0.036)	1.025 (0.040)	0.850	0.0001	0.1169 (0.0217)	0.0134	0.2361
Kelp Bay	10	0.283 (0.026)	1.092 (0.031)	0.934	<0.0001	0.1504 (0.0485)	0.0123	0.4269
Seppings Is.	10	0.203 (0.027)	0.958 (0.030)	0.877	<0.0001	0.1558 (0.0418)	0.0117	0.4027
Bordelais Is.	10	0.277 (0.051)	1.017 (0.064)	0.788	0.0006	0.0908 (0.0244)	0.0135	0.2308
Seta length								
Grappler Narrows	10	0.139 (0.032)	-0.005 (0.035)	0.707	0.0023	–	–	–
Bamfield Inlet	10	0.124 (0.042)	-0.018 (0.050)	0.518	0.0190	–	–	–
Self Pt.	10	0.166 (0.033)	-0.061 (0.036)	0.757	0.0011	–	–	–
Kelp Bay	10	0.142 (0.027)	-0.100 (0.031)	0.777	0.0007	–	–	–
Seppings Is.	10	0.135 (0.023)	-0.115 (0.026)	0.807	0.0004	–	–	–
Bordelais Is.	10	0.185 (0.031)	-0.100 (0.039)	0.816	0.0003	–	–	–
Interseta space								
Grappler Narrows	10	0.118 (0.044)	-1.329 (0.049)	0.474	0.0277	–	–	–
Bamfield Inlet	10	0.046 (0.039)	-1.461 (0.046)	0.152	0.2656	–	–	–
Self Pt.	10	0.218 (0.038)	-1.282 (0.042)	0.801	0.0005	–	–	–
Kelp Bay	10	0.057 (0.049)	-1.497 (0.057)	0.144	0.2791	–	–	–
Seppings Is.	10	0.016 (0.031)	-1.480 (0.034)	0.034	0.6095	–	–	–
Bordelais Is.	10	0.021 (0.045)	-1.645 (0.056)	0.026	0.6558	–	–	–

Ramus diameter								
Grappler Narrows	10	0.255 (0.056)	-0.242 (0.062)	0.720	0.0019	–	–	–
Bamfield Inlet	10	0.211 (0.039)	-0.305 (0.047)	0.786	0.0006	–	–	–
Self Pt.	10	0.254 (-0.211)	-0.211 (0.022)	0.951	<0.0001	–	–	–
Kelp Bay	10	0.250 (0.013)	-0.154 (0.015)	0.980	<0.0001	–	–	–
Seppings Is.	10	0.210 (0.029)	-0.211 (0.032)	0.871	<0.0001	–	–	–
Bordelais Is.	10	0.226 (0.042)	-0.173 (0.052)	0.786	0.0006	–	–	–
Pollicipes polymerus								
Ramus length								
Self Pt.	10	0.251 (0.026)	1.206 (0.018)	0.919	<0.0001	0.3197 (0.0660)	0.0676	0.6139
Kelp Bay	10	0.258 (0.018)	1.208 (0.014)	0.961	<0.0001	0.3566 (0.0886)	0.0401	0.8923
Seppings Is.	10	0.259 (0.023)	1.169 (0.018)	0.939	<0.0001	0.2847 (0.0675)	0.0381	0.6150
Bordelais Is.	10	0.290 (0.018)	1.183 (0.014)	0.970	<0.0001	0.3197 (0.0742)	0.0551	0.6988
Seta length								
Self Pt.	10	0.172 (0.022)	0.219 (0.015)	0.888	<0.0001	–	–	–
Kelp Bay	10	0.170 (0.017)	0.209 (0.012)	0.927	<0.0001	–	–	–
Seppings Is.	10	0.131 (0.024)	0.162 (0.018)	0.791	0.0006	–	–	–
Bordelais Is.	10	0.160 (0.040)	0.152 (0.031)	0.663	0.0041	–	–	–
Intersetal space								
Self Pt.	10	0.211 (0.036)	-0.962 (0.024)	0.813	0.0004	–	–	–
Kelp Bay	10	0.183 (0.021)	-0.981 (0.016)	0.901	<0.0001	–	–	–
Seppings Is.	10	0.167 (0.031)	-0.963 (0.024)	0.781	0.0007	–	–	–
Bordelais Is.	10	0.177 (0.025)	-0.947 (0.019)	0.861	0.0001	–	–	–
Ramus diameter								
Self Pt.	10	0.317 (0.030)	0.084 (0.020)	0.935	<0.0001	–	–	–
Kelp Bay	10	0.277 (0.025)	0.058 (0.019)	0.938	<0.0001	–	–	–
Seppings Is.	10	0.291 (0.018)	0.104 (0.014)	0.970	<0.0001	–	–	–
Bordelais Is.	10	0.312 (0.026)	0.093 (0.020)	0.947	<0.0001	–	–	–

* Mean prosomal wet mass was the same for all traits within a species.

Table 3–3a. OLS regression equations of the relationship of log(ramus length) to water velocity for legs 4, 5, and 6 (see fig. 3-3).

	OLS Regression Equations				
Species/Leg	n	Slope (SE)	Intercept (SE)	r^2	*P* (slope)
Balanus glandula					
Leg 6	7	-0.058 (0.008)	0.663 (0.021)	0.921	0.0006
Leg 5	7	-0.055 (0.010)	0.635 (0.027)	0.866	0.0023
Leg 4	7	-0.055 (0.008)	0.559 (0.023)	0.903	0.0010
Chthamalus dalli					
Leg 6	6	-0.051 (0.018)	0.329 (0.055)	0.665	0.0480
Leg 5	6	-0.045 (0.016)	0.301 (0.049)	0.655	0.0509
Leg 4	6	-0.038 (0.014)	0.226 (0.043)	0.640	0.0560
Semibalanus cariosus					
Leg 6	6	-0.031 (0.005)	0.866 (0.015)	0.913	0.0029
Leg 5	6	-0.029 (0.005)	0.846 (0.015)	0.895	0.0043
Leg 4	6	-0.025 (0.004)	0.754 (0.013)	0.893	0.0045
Pollicipes polymerus					
Leg 6	4	-0.029 (0.006)	1.127 (0.022)	0.921	0.0402
Leg 5	4	-0.022 (0.008)	1.089 (0.031)	0.770	0.1221
Leg 4	4	-0.016 (0.007)	1.025 (0.027)	0.694	0.1670

Table 3–3b. OLS regression equations for the log-linear relationship of cirral dimensions of leg 6 to water velocity (see fig. 3-4)

Cirral trait / Species	n	Slope (SE)	Intercept (SE)	r^2	*P* (slope)
Log(ramus length)					
Balanus glandula	7	-0.058 (0.008)	0.663 (0.021)	0.921	0.0006
Chthamalus dalli	6	-0.051 (0.018)	0.329 (0.055)	0.665	0.0480
Semibalanus cariosus	6	-0.031 (0.005)	0.866 (0.015)	0.913	0.0029
Pollicipes polymerus	4	-0.029 (0.006)	1.127 (0.022)	0.921	0.0402
Log(seta length)					
Balanus glandula	7	-0.040 (0.009)	-0.267 (0.024)	0.816	0.0053
Chthamalus dalli	6	-0.034 (0.012)	-0.599 (0.036)	0.665	0.0480
Semibalanus cariosus	6	-0.030 (0.004)	-0.154 (0.011)	0.940	0.0014
Pollicipes polymerus	4	-0.027 (0.011)	0.183 (0.042)	0.736	0.1423
Log(intersetal space)					
Balanus glandula	7	-0.019 (0.004)	-1.631 (0.012)	0.802	0.0064
Chthamalus dalli	6	-0.002 (0.010)	-1.782 (0.030)	0.006	0.8832
Semibalanus cariosus	6	-0.022 (0.014)	-1.478 (0.043)	0.381	0.1920
Pollicipes polymerus	4	0.021 (0.006)	-1.155 (0.023)	0.853	0.0765
Log(ramus diameter)					
Balanus glandula	7	0.028 (0.005)	-0.761 (0.013)	0.880	0.0018
Chthamalus dalli	6	0.020 (0.006)	-0.979 (0.019)	0.718	0.0333
Semibalanus cariosus	6	0.023 (0.005)	-0.523 (0.014)	0.855	0.0083
Pollicipes polymerus	4	0.016 (0.008)	-0.164 (0.031)	0.645	0.1968

Table 3–4. Results from ANCOVA testing for differences in trait means and equality of slopes of the relationship between length of leg 6 and prosomal wet mass among populations of four barnacle species, *Balanus glandula*, *Chthamalus dalli*, *Semibalanus cariosus*, and *Pollicipes polymerus*. Populations of each species were from habitats differing in wave exposure (see Table 3–3). All data were log-transformed.

	Balanus glandula			*Chthamalus dalli*			*Semibalanus cariosus*			*Pollicipes polymerus*		
Source of variation	df	MS	*P*	df	MS	*P*	df	MS	*P*	df	MS	*P*
Population	6	0.149	<0.001	5	0.147	<0.001	5	0.042	<0.001	3	0.006	<0.001
Log(wet mass)	1	1.584	<0.001	1	0.192	<0.001	1	0.597	<0.001	1	0.400	<0.001
Error	62	0.002		53	0.002		53	0.003		35	0.001	
Equality of slopes	6	0.005	0.036†	5	0.001	0.83	5	0.004	0.32	3	0.0004	0.58

When testing for equality of slopes, the error degrees of freedom were 56 for *B. glandula*, 48 for *C. dalli* and *S. cariosus*, and 32 for *P. polymerus*, and the error MS were 1.7-13.8% larger than tabled here.

† Interaction became non-significant ($P = 0.11$) after Sequential Bonferroni Correction (3 legs = 3 tests for each species)

Table 3–5. Common slopes of the dimensions of cirrus 6 with prosomal wet mass for each species. Slopes for each species were obtained from ANCOVA on log-transformed data (Tables 3–4 and 3–6). Regressions for each population are found in Table 3–2. OLS Slopes were tested against an isometric slope of 0.333 using a one-sample Student's t-test (t_{allom}).

Species	Slope (SE)	Intercept (SE)	r	df	t_{allom}
log (ramus length) vs. log (prosomal wet mass) (Fig. 1 and 2)					
Balanus glandula	0.287 (0.011)	1.123 (0.022)	0.975	69	4.152 ***
Chthamalus dalli	0.261 (0.028)	0.919 (0.077)	0.935	59	2.550 NS
Semibalanus cariosus	0.230 (0.016)	1.036 (0.019)	0.916	59	6.385 ***
Pollicipes polymerus	0.266 (0.010)	1.192 (0.008)	0.976	39	6.618 ***
log (seta length) vs. log (prosomal wet mass) (Fig. 2)					
B. glandula	0.204 (0.009)	0.062 (0.018)	0.969	69	14.232 ***
C. dalli	0.193 (0.027)	-0.152 (0.074)	0.875	59	5.143 ***
S. cariosus	0.145 (0.012)	-0.070 (0.014)	0.912	59	15.538 ***
P. polymerus	0.159 (0.013)	0.186 (0.010)	0.918	39	13.220 ***
log (intersetal space) vs. log (prosomal wet mass) (Fig. 2)					
B. glandula	0.146 (0.014)	-1.377 (0.028)	0.842	69	13.263 ***
C. dalli	0.181 (0.042)	-1.289 (0.116)	0.637	59	3.589 ***
S. cariosus	0.071 (0.018)	-1.456 (0.021)	0.797	59	14.436 ***
P. polymerus	0.183 (0.014)	-0.964 (0.010)	0.918	39	10.583 ***
log (ramus diameter) vs. log (prosomal wet mass) (Fig. 2)					
B. glandula	0.231 (0.009)	-0.237 (0.019)	0.961	69	11.253 ***
C. dalli	0.174 (0.024)	-0.454 (0.067)	0.869	59	6.570 ***
S. cariosus	0.233 (0.014)	-0.216 (0.014)	0.926	59	7.084 ***
P. polymerus	0.297 (0.012)	0.084 (0.009)	0.972	39	2.963 **

**P < 0.01, ***P < 0.001, NS = non-significant.

Table 3–6. Results from 2-factor ANCOVA testing for equality of slopes of the relationship of the cirral dimensions of leg 6 to prosomal wet mass among the barnacle species: *Balanus glandula, Chthamalus dalli, Semibalanus cariosus*, and *Pollicipes polymerus* (see fig. 3-3). Analyses were performed on log-transformed data and included only the four most wave exposed sites, Self Point, Kelp Bay, Bordelais Island and Sepping's Island.

		Ramus length		Seta length		Intersetal space		Ramus diameter	
Source of variation †	df	MS	*P*	MS	*P*	MS	*P*	MS	*P*
Species (spp)	3	0.0476	<0.001 ***	0.1015	<0.001 ***	0.3616	<0.001 ***	0.1120	<0.001 ***
Population (pop)	3	0.0030	0.182	0.0002	0.948	0.0023	0.536	0.0007	0.702
Log(wet mass, g) (wm)	1	1.1298	<0.001 ***	0.5147	<0.001 ***	0.2724	<0.001 ***	0.9549	<0.001 ***
spp x pop	9	0.0024	0.211	0.0012	0.662	0.0084	0.008 **	0.0014	0.465
spp x wm	3	0.0012	0.583	0.0034	0.106	0.0119	0.013 *	0.0069	0.004 **
pop x wm	3	0.0009	0.687	0.0006	0.796	0.0080	0.064	0.0014	0.430
spp x pop x wm	9	0.0027	0.171	0.0018	0.380	0.0033	0.428	0.0016	0.385
Error	128	0.0018		0.0016		0.0032		0.0015	

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

†Main effects are not considered meaningful in these analyses. Differences among species reflect differences in body size and differences among populations are tested within species (Table 3–4, Appendix 3–1).

Table 3–7. Results from ANCOVA testing for equality of slopes and differences in intercept between legs 4, 5 and 6 for the relationship of leg length at a standard body mass to water velocity in *Balanus glandula, Chthamalus dalli, Semibalanus cariosus*, and *Pollicipes polymerus* (see fig. 3-4 for sample means)

	Balanus glandula			*Chthamalus dalli*			*Semibalanus cariosus*			*Pollicipes polymerus*		
Source of variation	df	MS	*P*	df	MS	*P*	df	MS	*P*	df	MS	*P*
Leg number	2	0.019	<0.001	2	0.008	0.202	2	0.017	<0.001	2	0.003	<0.001
Water velocity	1	0.231	<0.001	1	0.118	<0.001	1	0.048	<0.001	1	0.003	<0.001
Error	17	0.002		14	0.005		14	0.0004		8	0.0001	
Equality of slopes	2	0.00007	0.962	2	0.001	0.846	2	0.0002	0.671	2	0.00007	0.501

When testing for equality of slopes, the error degrees of freedom were 15 for *B. glandula*, 12 for *C. dalli*, 12 for *S. cariosus*, and 6 for *P. polymerus*. The error mean squares were 1.3-13.4% larger than tabled here.

Table 3–8. Results from ANCOVA testing for equality of slopes and differences in intercept among barnacle species for the log-linear relationship of the dimensions of leg 6 to water velocity.

		Ramus length		Seta length		Ramus diameter	
Source of variation	df	MS	*P*	MS	*P*	MS	*P*
Species	3	0.677	<0.001	0.525	<0.001	0.536	<0.001
Water Velocity	1	0.147	<0.001	0.080	<0.001	0.037	<0.001
Error	18	0.002		0.001		0.0005	
Equality of Slopes	3	0.003	0.329	0.0004	0.828	0.0003	0.682

When testing for equality of slopes, the error degrees of freedom were 15 and the error mean squares were 4.3-13.3% larger than tabled here.

Table 3-9. Slopes of the length of leg 6 with wet mass for all barnacle species for which data are available.

Species	OLS Slope	r	RMA Slope
Balanus glandula	0.287	0.975	0.294
Chthamalus dalli	0.261	0.935	0.279
Semibalanus cariosus	0.230	0.916	0.251
Pollicipes polymerus	0.266	0.976	0.273
*Semibalanus balanoides**	0.233	0.953	0.244
*Eliminius modestus**	0.249	0.930	0.268
Tetraclita squamosa rubescens †	0.319	0.888	0.363

* data from Crisp and Mclean 1990.

† KB Marchinko, unpublished data.

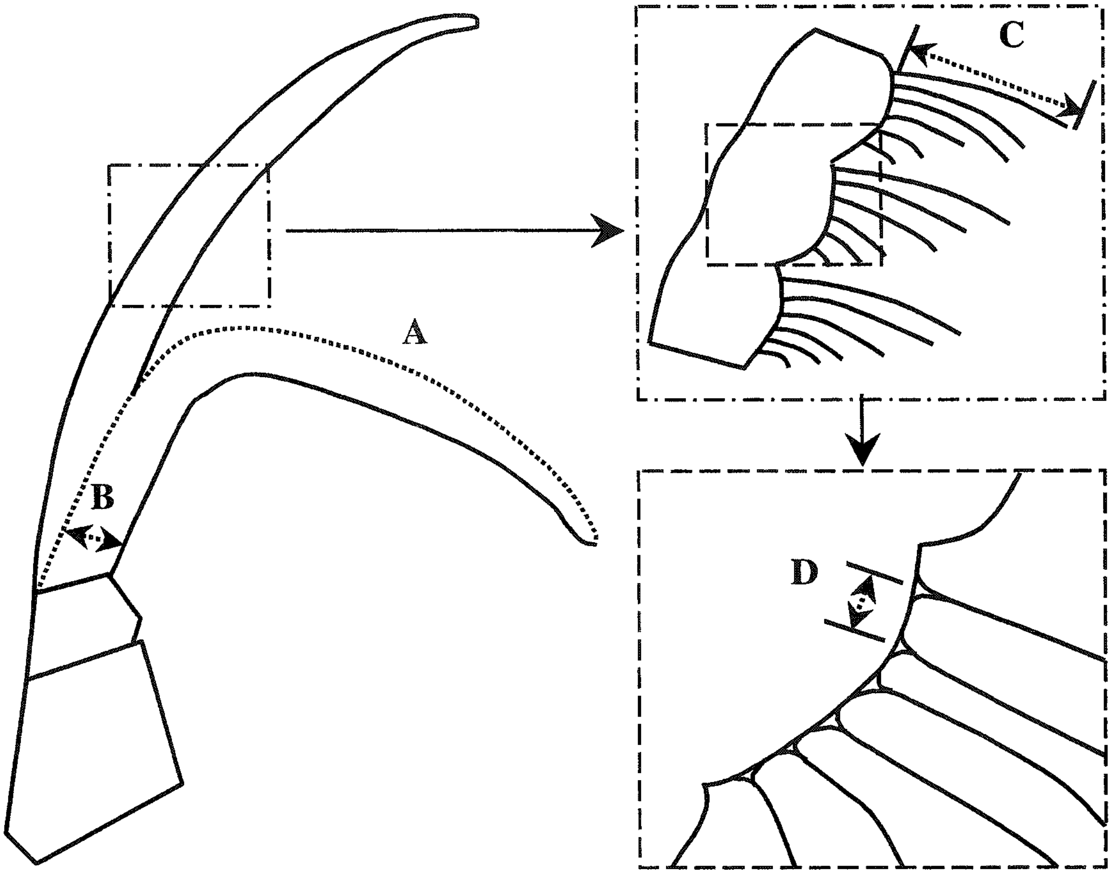

Figure 3-1. Diagram of a single, bi-ramus cirrus showing the four traits measured. A) ramus length (perimeter of the dorsal edge along the dotted line), B) diameter of the ramus between the first and second segments, C) length of the longest seta, and D) distance between the two longest setae (intersetal space).

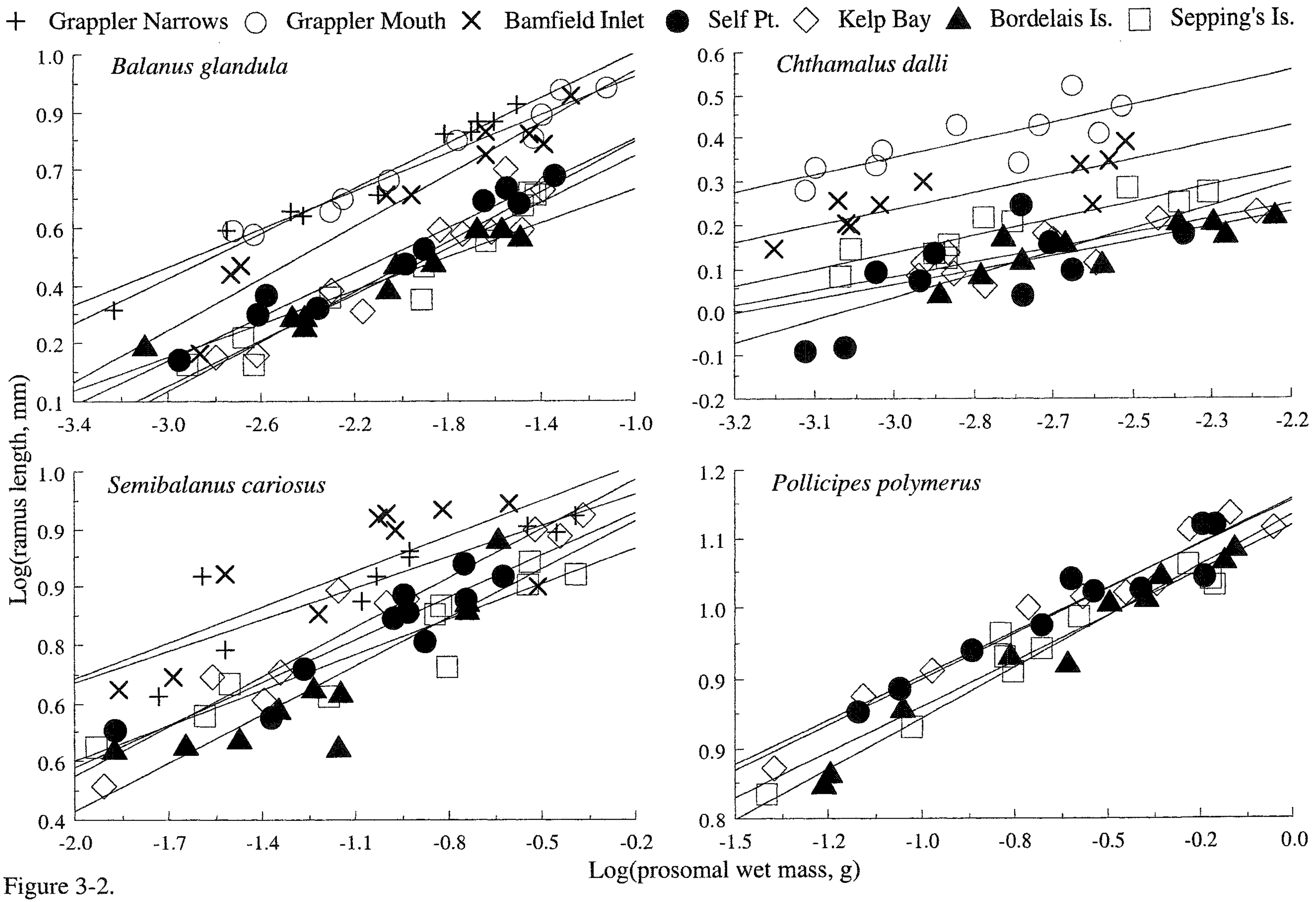
+ Grappler Narrows ○ Grappler Mouth × Bamfield Inlet ● Self Pt. ◇ Kelp Bay ▲ Bordelais Is. □ Sepping's Is.
Balanus glandula
Chthamalus dalli
Semibalanus cariosus
Pollicipes polymerus
Log(ramus length, mm)
Log(prosomal wet mass, g)
1.0
0.9
0.7
0.6
0.4
0.2
0.1
-3.4
-3.0
-2.6
-2.2
-1.8
-1.4
-1.0
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-3.2
-3.1
-3.0
-2.8
-2.7
-2.5
-2.3
-2.2
1.0
0.9
0.9
0.8
0.6
0.6
0.4
-2.0
-1.7
-1.4
-1.1
-0.8
-0.5
-0.2
1.2
1.1
1.0
0.9
0.9
0.8
-1.5
-1.2
-1.0
-0.8
-0.5
-0.2
0.0

Figure 3-2.

Figure 3-2. Log-log relationships of length of the sixth thoracic leg to body mass for four species of intertidal barnacle from different sites in Barkley Sound, British Columbia, Canada. Sites are listed, from left to right, in order of increasing wave exposure. Each point represents an individual barnacle (n = 10 for each species). Differences in slope among populations within species were non-significant ($P > 0.11$) when corrected for multiple tests (Table 3–4). The relationships of seta length, intersetal space, and ramus diameter to prosomal wet mass exhibit similar patterns for each species and for the fourth and fifth thoracic legs (see Tables 3–1 and 3–2).

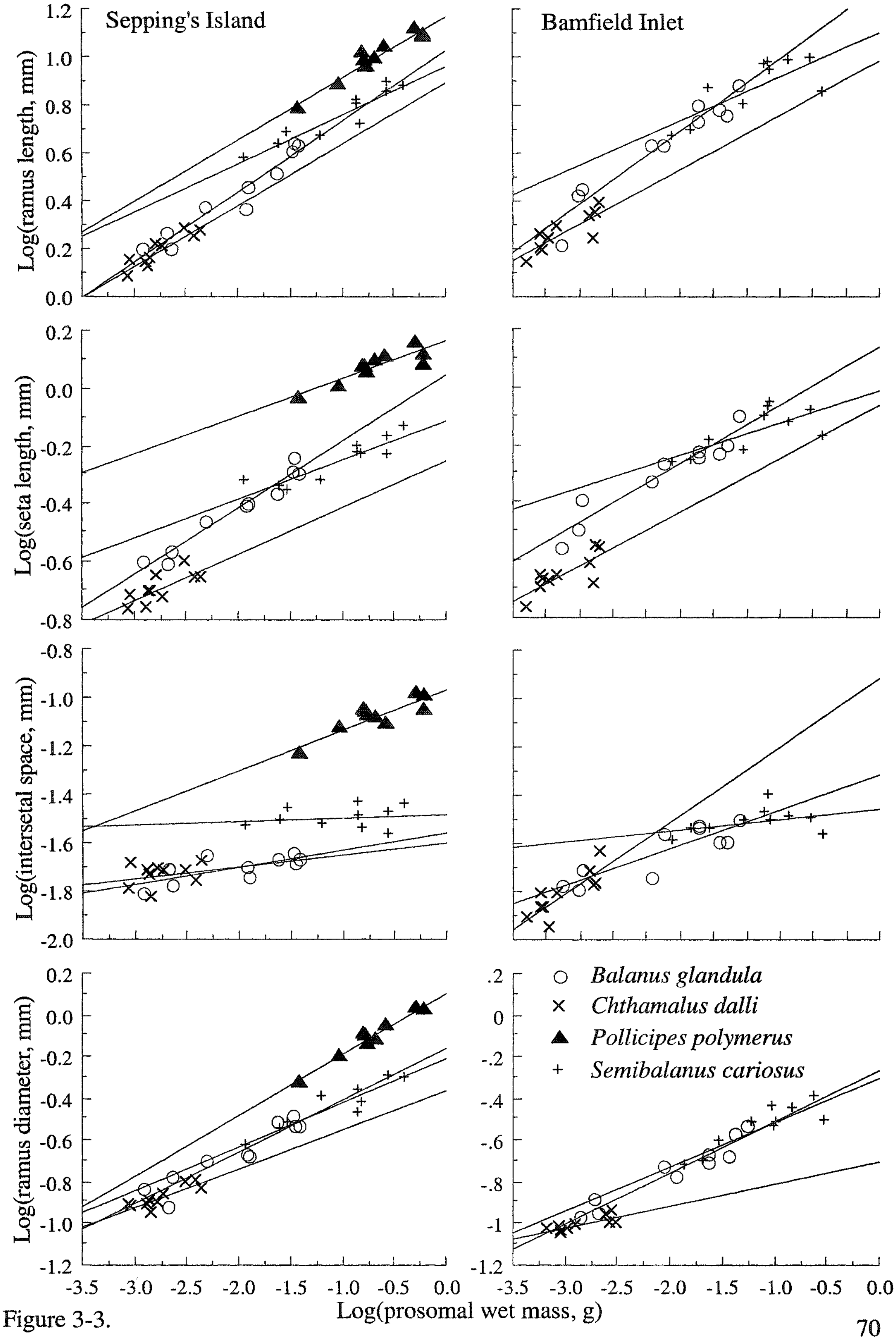
Sepping's Island
Bamfield Inlet
Log(ramus length, mm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Log(seta length, mm)
0.2
0.0
-0.2
-0.4
-0.6
-0.8
Log(interseta1 space, mm)
-0.8
-1.0
-1.2
-1.4
-1.6
-1.8
-2.0
Log(ramus diameter, mm)
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
.2
0
-.2
-.4
-.6
-.8
-1
-1.2
○ Balanus glandula
× Chthamalus dalli
▲ Pollicipes polymerus
+ Semibalanus cariosus
-3.5
-3.0
-2.5
-2.0
-1.5
-1.0
-0.5
0.0
Log(prosomal wet mass, g)

Figure 3-3.

Figure 3-3. Cirral trait variation of the sixth thoracic leg as a function of body mass for four barnacle species: *Balanus glandula*, *Chthamalus dalli*, *Semibalanus cariosus*, and *Pollicipes polymerus*. Each point represents a single individual (n = 10 for each species). Only one wave exposed (Sepping's Island) and one sheltered site (Bamfield Inlet) is shown for clarity. Differences in slopes among species were non-significant ($P > 0.11$) for ramus and seta length, while the slope of ramus diameter and the intersetal space to body mass differed significantly among species ($P < 0.013$, Table 3–6). OLS statistics for each trait in each population are in Table 3–2.

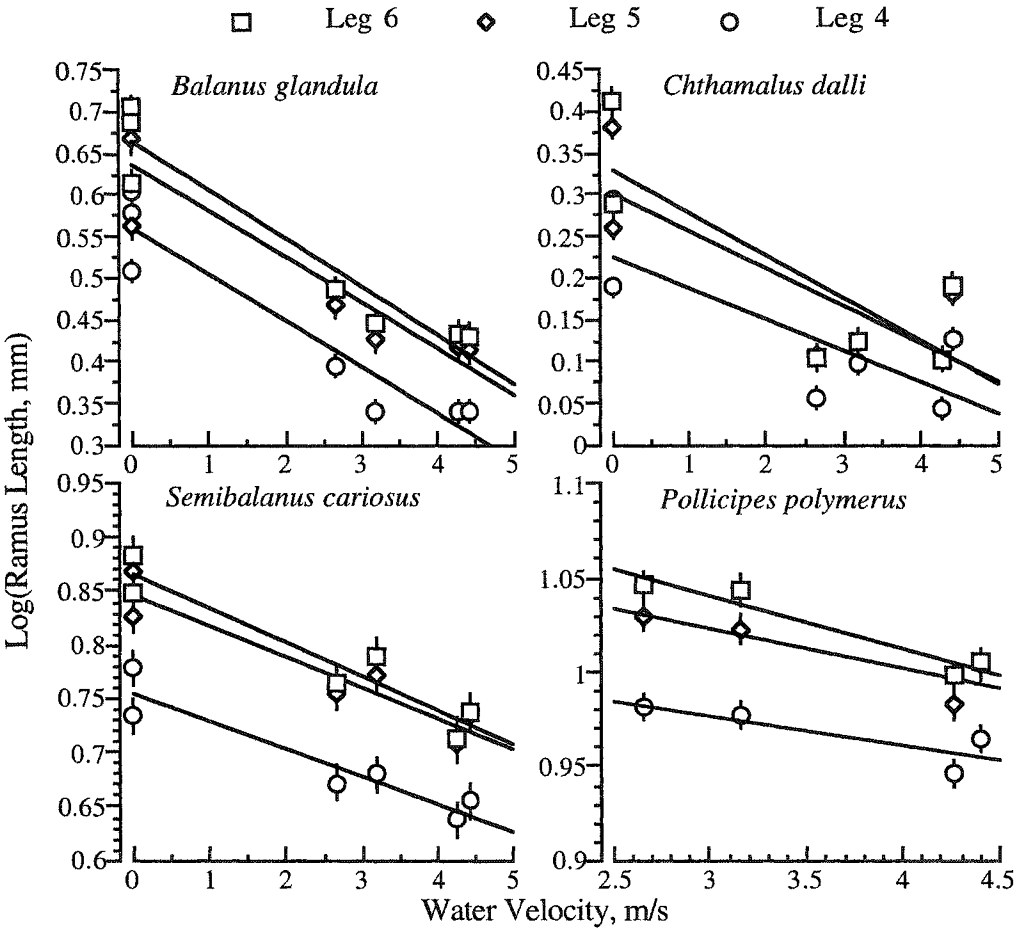

Figure 3-4. Average length of the posterior three feeding legs (legs 4, 5, 6) for a standard sized barnacle as a function of water velocity (Table 3–3). Each point represents the least-squares mean leg length (±SE) of 10 individuals from one of seven sites differing in Barkley Sound, British Columbia, Canada. Standard log(prosomal wet mass, g) for *B. glandula* was -2.017 (SE, 0.064), *C. dalli* -2.741 (0.031), *S. cariosus* -1.074 (0.057), *P. polymerus* -0.635 (0.060). The slopes of these relationships did not differ significantly among legs 4, 5 and 6 in any species ($P > 0.5$, Table 3–7). OLS statistics for these relationships are in Table 3–3a.

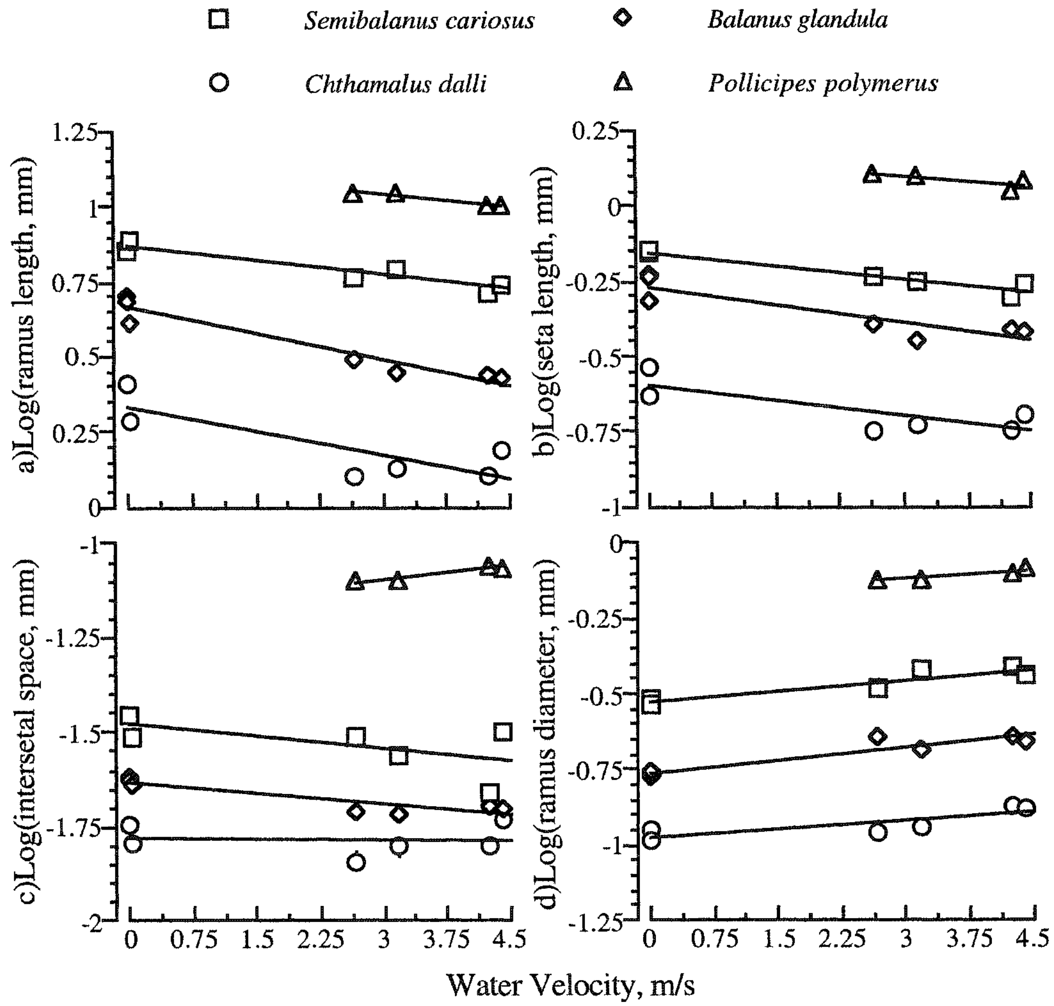

Figure 3-5. Least-squares mean trait dimension of leg 6 for a standard sized barnacle (see legend of 3-4) as a function of water velocity (Table 3–3). Each point represents the least-squares mean (±SE) for 10 individuals. Where error bars are absent they are less than the symbol size. The slope of these relationships did not differ significantly among species ($P > 0.33$, Table 3–8). OLS statistics for these relationships are in Table 3–3b.

CHAPTER 4

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

4.0 INTRODUCTION

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 vary 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 (Okamura and Partridge 1999, Arsenault et al. 2001, Chapter 3) and bryozoans (Okamura and Partridge 1999), the cause of these differences — ecophenotypic, or genetic differentiation — is unclear. However, phenotypic plasticity is strongly suspected (Pentcheff 1995).

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). Because water flow is spatially variable (horizontally) along shorelines, larvae of many suspension feeders may settle under a wide range of conditions, from virtually still water in protected bays to over 14 m/s on very exposed shores (Denny 1994). As a consequence, variation in suspension feeding structures with water velocity (Okamura and Partridge 1999, Arsenault et al. 2001, Chapter 3) 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 (differential 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 may also arise via phenotypic plasticity or behavioral modifications after differential settlement or differential mortality have 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 (Chapter 3): 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 surface area of the feeding fan in low flow where particle flux is limited, while possibly reducing the damage due to greater drag under high flow (Chapter 3). In one species, *Balanus glandula*, water velocity accounts for a surprising 95-99% of leg length variation among sites of different wave exposure, suggesting these trade-offs between environments are quite severe (Arsenault et al. 2001). Although preliminary results suggest the variation is ecophenotypic (Pentcheff 1995), the magnitude, age- or size-dependence, and the time-course of the response remains unknown.

Because barnacles, 1) are well known to be morphologically plastic in response to environmental conditions (Barnes and Barnes 1962, Crisp and Bourget 1985, Lively 1986*a,b*), 2) possess a long larval dispersal period (Strathmann 1987), 3) are well known to settle based on environmental cues (Crisp 1955, Neal and Yule 1994, Neal et al. 1996), and 4) 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 differential mortality), phenotypic plasticity, or some combination of both mechanisms. Therefore, in this study, I address four questions using the most variable barnacle 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 ecophenotypic response?

4.1 METHODS

4.1.1 Study species

Balanus glandula ranges from the Aleutian Is. in Alaska to Baja California and is commonly found in the mid-intertidal from quiet bays to extremely wave-exposed shores (Barnes and Barnes 1956). Like all balanomorph barnacles, *B. glandula* feeds using a cirral net, which is 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): protected shore populations possess legs up to 80% longer than wave-exposed ones (Chapter 3), and up to 99% of the variation in leg length is explained statistically by wave action (Chapter 2).

4.1.2 Collection sites and experimental location

For each experiment, I collected barnacles from two populations in Barkley Sound, Vancouver Is., British Columbia, Canada: one protected shore (Bamfield It.), and one wave-exposed shore (Sepping's Is.) were chosen for the extreme differences in leg length documented in Chapter 3. 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 It. 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 0.0096g (Chapter 3). The exposed shore site (48°50'50"N, 125°12'50"W) was located on the southwest-facing shore of Sepping's Is., and characterized by high flow (4.41 x 10^2 cm/s, see Arsenault et al. 2001 for details) produced by breaking waves. Leg length of the exposed shore population was 2.7 mm at a body mass of 0.0096g (Chapter 3).

Three separate common-garden experiments ran from April 14 to September 16, 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 (May 9 to June 5, 2001) using an Acoustic Doppler Velocimeter (SonTek / YSI Inc. 10-MHz ADV), operating at 25 Hz for five minutes each day. Five of the six sampling days coincided (± 2 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 tidal-current velocity 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/s.

4.1.3 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 April 9 (experiment one) and May 30, 2001 (experiment two). Adults were also collected on May 30 (experiment two) and on August 6, 2001 (experiment three). After collection, mussel shells were cut with a variable-speed MultiPro® Rotary tool (Dremel®) 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) 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 It. according to each experiment (see details below). The time from collection to submersion did not exceed 48 hours.

4.1.4 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, Chapter 3). The sixth cirrus was dissected from the prosoma and measured at 25-50X magnification using a dissecting scope mounted with a *camera lucida*, and a digitizing tablet with a precision of 20 dots/mm. I measured three traits on each cirrus (see fig 3.1) 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, data for ramus length only 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.

To avoid the confounding effects of environmental variation on shell shape common among barnacles (Crisp and Bourget 1985, Lively 1986*b*), body size was estimated as prosomal wet mass. To measure prosomal wet mass, the soft tissue (prosoma) was separated from all shell plates (including the opercular plates) and from any egg mass that was present, blotted dry for 20 seconds on a dry Kimwipe®, then 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.

4.1.5 Experiment one: Genetic vs. environmental control of cirrus form

To test whether cirrus variation arises via phenotypic plasticity, or genetic differences, I transplanted juvenile *B. glandula* from the exposed shore and protected shore sites into high (35.4 cm/s; see below) and low flow (0.96 cm/s; see above) growth environments at the experimental Bamfield It. location. To simulate low flow conditions, mussel fragments containing a single barnacle were glued (see above) to the underside of a 15 X 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 the water surface in ambient flow (0.96 cm/s), 1.5 m from the high flow setup, for 35 days (April 11 to May 16, 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 It. 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 in one of four quadrants of a stationary disk (two quadrants for each population; fig. 4-1A). 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 of 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 barnacles in each group were not measured (fig. 4-1A). Thus, measurements were only taken on barnacles that had 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 due to 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 Plexiglass 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. 4-1B). The disk was rotated clockwise at 32 RPM by a shaft attached to a 1.5V DC motor (fig. 4-1B). The rotating disk and paddle generated a mean ± SE water velocity of 35.4 ± 0.62 cm/s (N = 19), with a periodic increase (54.1 ± 0.70 cm/s, N = 19) and subsequent decrease (14.1 ± 0.77 cm/s, N = 19) in water velocity each time the paddle passed (every 1.9s; fig. 4-1C). 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 probe 15 mm in diameter (Novonic Instruments, Gloucester, England). Flow measurements were read once every minute for 19 minutes (separate trials were run for mean, maximum and minimum velocities) in a tank of fresh water 2m in diameter. Velocity measured in fresh water should approximate velocity in salt water, as hydrodynamic forces are proportional to density (Denny 88), and salt water is only 2.3% denser than fresh water.

Leg dimensions were analysed using a two-factor 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 form is under environmental control, whereas significant differences between populations would suggest cirrus form 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 if cirrus dimensions produced under high and low treatments were significantly different from pre-treatment populations (collected on April 11). 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 population (100 m away) in Bamfield It. If significant differences exist between these two groups, any change in cirrus form may be confounded by some step in the experimental procedure.

4.1.6 Experiment two: 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 It. Juvenile and adult barnacles from the protected shore population in Bamfield It. were also transplanted back to Bamfield It. to test for artifacts due to experimental conditions. Mussel fragments containing a single barnacle were glued (see above) on the underside of a 15 X 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 (June 1 to July 22, 2001), after which barnacles were collected, weighed, and ramus length was measured as described above. Only five adults from each population survived the experiment.

Data were analysed 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, while differences in the protected shore population would suggest experimental procedure influenced cirrus form. One-factor ANCOVA was employed separately for each population to test if ramus length differed between transplanted and original, pre-treatment populations (tested against barnacles collected on August 6, 2001 for experiment three). Significant differences between pre-treatment and transplanted exposed shore barnacles would re-confirm that ramus length is influenced by water flow, whereas significant differences between pre-treatment and transplanted barnacles from the protected shore population would suggest that changes in cirrus form in either population were confounded by experimental procedure.

4.1.7 Experiment three: response time

To examine how quickly barnacles from the exposed shore population modify ramus length, I transplanted exposed shore adults into low flow conditions in Bamfield It. Protected shore adults were also transplanted back to Bamfield It. to test for experimental artifacts. Barnacles were collected on August 6, 2001, glued and arranged on two 15 X 30 cm Plexiglas plates in the same manner as experiment two, 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 August 8 to September 12, 2001. Ramus lengths of six untreated barnacles from both populations (collected on August 6) were included as pre-treatment values (0 days). 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.

As I was no longer concerned with source-population differences, data were analysed 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 $\propto = 0.0125$).

4.2 RESULTS

4.2.1 Experiment one: Two-way transplant (high vs. 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. 4-2). Differences in ramus and seta length between growth environments were highly significant ($P < 0.001$; Table 4-1). 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 ($P = 0.016$; Table 4-1).

Ramus and seta length of pre-treatment 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 pre-treatment protected shore population (fig. 4-2). 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. Surprisingly, those grown experimentally under high flow possessed limbs significantly shorter ($P < 0.001$), than the pre-treatment, exposed shore individuals (fig. 4-2).

Growth environment and source population had no effect on ramus diameter ($P = 0.384$ and $P = 0.107$ respectively, Table 4-1; fig. 4-2), unlike the pattern observed for ramus and seta length. Surprisingly, ramus diameter of the transplanted exposed shore barnacles was consistently, though 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. 4-2) and in previous work (Chapter 3). Ramus diameters of transplanted barnacles from both exposed and protected shores were significantly different from their respective pre-treatment 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, pre-treatment population, while protected shore barnacles transplanted to low flow had significantly thicker rami than protected shore barnacles before treatment.

4.2.2 Experiment two: One-way transplant to low flow (juveniles vs. 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 ($P = 0.67$; Table 4-2; fig. 4-3). Population differences were significant ($P = 0.0012$; Table 4-2), and similar to experiment one: exposed shore barnacles had consistently shorter rami than protected shore barnacles. Within-population comparisons of treated to pre-treatment barnacles showed ramus length of transplanted exposed shore barnacles was significantly longer than pre-treatment ($P < 0.001$), while rami of treated and pre-treatment protected shore barnacles were statistically indistinguishable ($P = 0.59$).

4.2.3 Experiment three: 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 It. (figs. 4-4, 4-5). Treatment (number of days after transplant) had a significant effect on exposed shore barnacles ($P = 0.0016$: Table 4-3) and multiple comparisons (Bonferroni adjusted $\alpha = 0.0125$) revealed that barnacles sampled 18, 25, and 35 days after transplant possessed rami significantly longer than pre-treatment barnacles ($P < 0.007$; fig. 4-4). Moreover, the homogeneity of error among mean ramus length in Figure 4 suggests that changes in ramus length of all barnacles occurred within a similar time period. Treatment had no significant effect on ramus length of protected shore barnacles ($P = 0.284$; Table 4-3; fig. 4-4).

Because the transplant of Bamfield It. (protected shore) barnacles back into ambient (low) flow in Bamfield It. 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.

4.3 DISCUSSION

4.3.1 Genotypic or ecophenotypic response?

The extreme modification of ramus and seta length in response to changes in water velocity (figs. 4-1 to 4-5) is clearly ecophenotypic. Both protected shore and wave-exposed populations of *B. glandula* grew longer limbs (up to 84%) when raised in low vs. 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% Chapter 3); 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 (Chapter 3), suggests that spatial and temporal variation in flow along coast-lines played a large role in selection for phenotypically plastic feeding fans in barnacles.

Although the adjustment of cirrus form to different flow 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 current 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). Pentcheff (1995) 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 ($P <$ 0.016, Tables 1, 2) difference in ramus and seta length between populations (exposed shore < protected shore; figs. 4-2, 4-3). Of the two possible mechanisms for generating such genetic differences (see introduction to this chapter), differential mortality is likely responsible for two reasons. First, in *Semibalanus balanoides*, differential mortality in response to environmental conditions occurs sometime within two weeks after settlement (Schmidt and Rand 2001) and, therefore, may have taken place before juveniles *B. glandula* were collected during this study. Second, settlement discrimination does not appear to occur 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, Chapter 3). 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 environmental variables that correlate with wave-exposure.

Alternatively, insufficient time may have passed for complete convergence of cirrus form. Because cirrus form should only change during growth at each molt, and juvenile molting rate is 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 began to change within 18 days, and since ramus length of juveniles and adults were statistically indistinguishable ($P = 0.67$; Table 4-2) after 51 days (fig. 4-3), experiment length appears not to have prevented convergence in cirrus form.

4.3.2 Range of phenotypic plasticity

Surprisingly, exposed shore barnacles transplanted to high flow had even shorter limbs ($P < 0.001$; fig. 4-2) than individuals experiencing extreme flow under breaking waves in their original habitat (Sepping's Is.). This has two significant implications.

First, by possessing the shortest limbs under experimental high flow (fig. 4-2), 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 Is. (4.6 m/s, Arsenault et al., 2001) was much greater than maximum experimental high flow (0.54 m/s), production of shorter limbs in experimental high flow suggests that barnacles choose to feed in considerably lower velocities after waves break, or in their backwash. Alternatively, differences between pre-treatment and high-flow transplants from the exposed shore might be an artifact of differences in velocity generated by a rotating disk and paddle vs. flow under breaking waves. For example, the shorter period of experimental high flow, 1.9s vs. 7-10s of average ocean swell (Denny 1988) may have provided 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 effect on ramus diameter ($P = 0.107$; Table 4-1). Moreover, ramus diameter of transplanted juveniles from both populations was similar to pre-treatment, protected shore individuals (fig. 4-2). Unfortunately, explanation of this phenomenon is difficult, as 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 vs. 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. The presence of significant differences between protected shore barnacles transplanted to low flow and pre-treatment individuals does suggest experimental procedure confounded results on ramus diameter (see methods).

4.3.3 Age-dependence of phenotypic plasticity

Remarkably, exposed shore adults increased leg length by an extreme 110% (fig. 4-3) after transplant to low flow in Bamfield It. 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. Second, 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 (DFO 2001), and temporal changes in surface relief due to the development bluff bodies, such as barnacle hummocks

(Thomason et al. 1998) and settlement of intertidal organisms, may generate both 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.

4.3.4 Significance of response time

Selection pressure to transform wave-exposed into protected-shore phenotypes must be strong to illicit the quick response observed in adult barnacles: around 18 days (figs. 4-4, 4-5), likely within one to two molts (Crisp and Patel 1961, Wu and Levings 1978). However, the direction of selection is 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 due to 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 quicker 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 vs. still flow (Barnes and Barnes 1982), individuals transplanted from low to high flow may respond even quicker 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. 4-1).

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Table 4-1. Results from a two-factor ANCOVA testing for differences in cirrus form of *Balanus glandula* from two populations (one wave-exposed and one protected) 31 days after transplant to high (35.4 cm/s) and low (0.96 cm/s) flow conditions in Bamfield It. (fig. 4-2). All variables were $\log_{10}$ transformed before analysis.

		Ramus length		Seta length		Ramus diameter	
Source of variation	df	MS	*P*	MS	*P*	MS	*P*
Source population	1	0.019	< 0.001	0.01	0.016	0.003	0.107
Growth environment (high vs. low flow)	1	0.198	< 0.001	0.103	< 0.001	0.001	0.384
Log(prosomal wet mass, g)	1	0.025	< 0.001	0.015	0.004	0.02	< 0.001
Error	20	0.001		0.001		0.001	
Source pop X Growth environment	1	9.5×10^{-7}	0.975	0.0002	0.725	0.003	0.099

Interactions with the covariate were all non-significant (i.e., slopes were equal); and are not included in this table.
*When testing source population by growth environment interaction, the error degrees of freedom were 19 and mean squares were 7.1-11.1% larger than tabled here (mean square ramus diameter was 8.3% smaller than tabled here).

Table 4-2. Results from two-factor ANCOVA 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 It. (fig. 4-3). All variables were $\log_{10}$ transformed before analysis.

Source of variation	df	Mean Square	*P*
Source population	1	0.012	0.001
Transplant age (juveniles vs. adults)	1	0.0002	0.668
Log(prosomal wet mass, g)	1	0.034	< 0.001
Error	18	0.001	
Source pop X Transplant age*	1	0.002	0.168

Interactions with the covariate were non-significant (i.e., slopes were equal); and are not included in this table.

*When testing source population by transplant age interaction, the degrees of freedom were 17 and mean squares were 5.7% smaller than tabled here.

Table 4-3. Results from one-factor ANCOVA 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 It. (figs. 4-4, 4-5). 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 MS were 6.7 and 9.1% larger than tabled here.

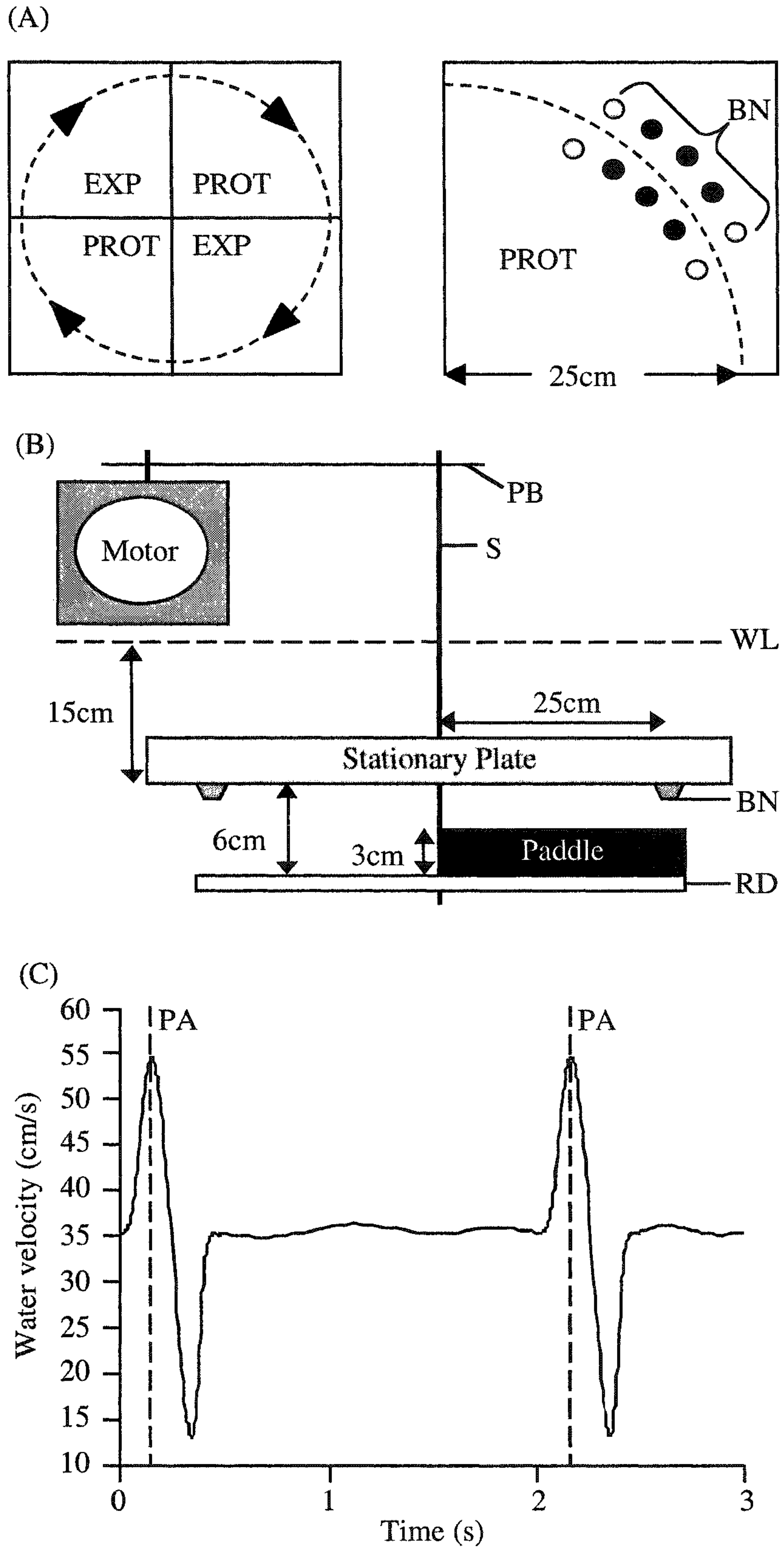
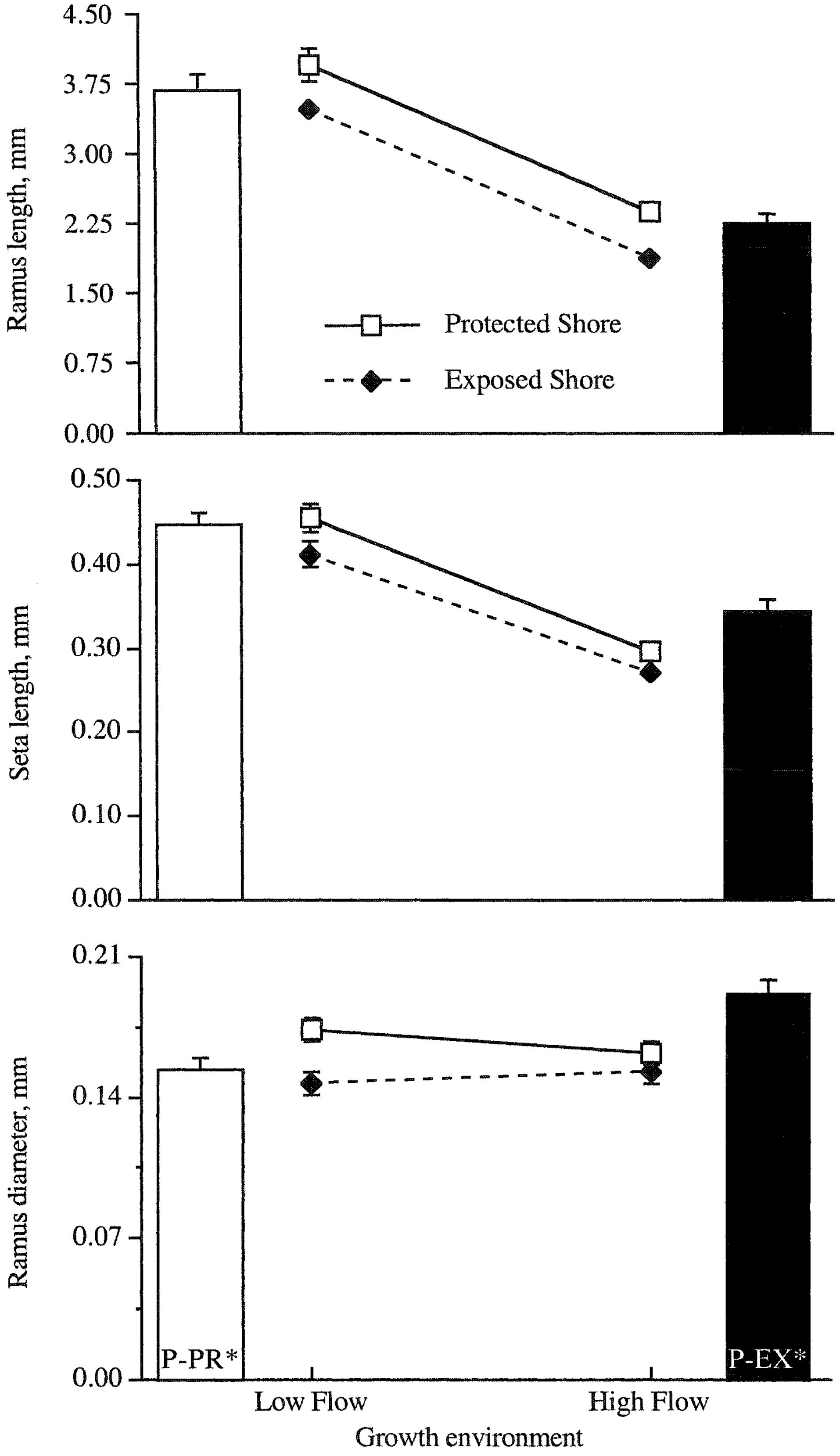

(A)
EXP
PROT
PROT
EXP
BN
PROT
25cm
(B)
PB
Motor
S
WL
15cm
25cm
Stationary Plate
BN
6cm
3cm
Paddle
RD
(C)
PA
PA
60
55
50
45
40
35
30
25
20
15
10
Water velocity (cm/s)
0
1
2
3
Time (s)

Figure 4-1.

Figure 4-1. 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 plexiglass 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 were not (see methods). B) Barnacles (BN) 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/s with a periodic increase (54.1 cm/s) and subsequent decrease (14.1 cm/s) in velocity each time the paddle passed (every 1.9 s; PA in fig. 4-1C). PB= Pulley and Belt, S= Stainless Steel Shaft.



Ramus length, mm
4.50
3.75
3.00
2.25
1.50
0.75
0.00
Protected Shore
Exposed Shore
Seta length, mm
0.50
0.40
0.30
0.20
0.10
0.00
Ramus diameter, mm
0.21
0.14
0.07
0.00
P-PR*
P-EX*
Low Flow
High Flow
Growth environment

Figure 4-2

Figure 4-2. 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/s) and high-flow (35.7 cm/s) growth environments. Each point or bar represents the mean (± SE) of six individuals adjusted to a common prosomal wet mass of 0.0062g by ANCOVA (Table 4-1). 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 pre-treatment individuals (P-PR= pre-treatment, protected, P-EX= pre-treatment, exposed)

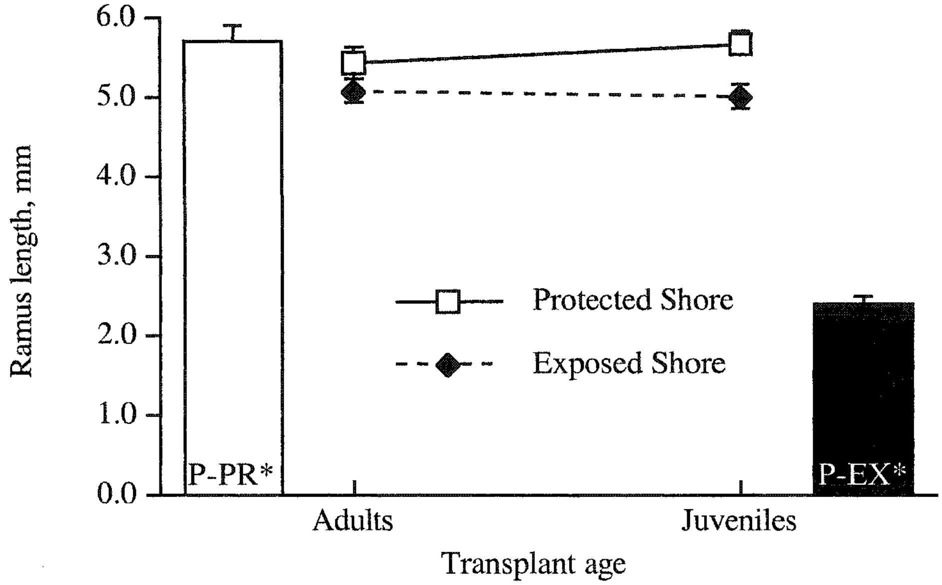


Figure 4-3. Feeding leg length of adult and juvenile *Balanus glandula* from two populations (one wave-exposed and one protected) before (bars) and 51 days after (points and lines) transplant to low-flow conditions (0.96 cm/s) in Bamfield Inlet. Each point or bar represents mean ramus length (± SE) adjusted to a common prosomal wet mass of 0.0262g by ANCOVA (Table 4-2). Standard errors were less than the symbol size where absent. Sample size (n) equaled 6 for original populations and juveniles, while n = 5 for adults. Points connected by lines represent barnacles grown experimentally under different flow conditions. Bars represent cirrus dimensions measured in pre-treatment individuals (P-PR= pre-treatment, protected, P-EX= pre-treatment, exposed)

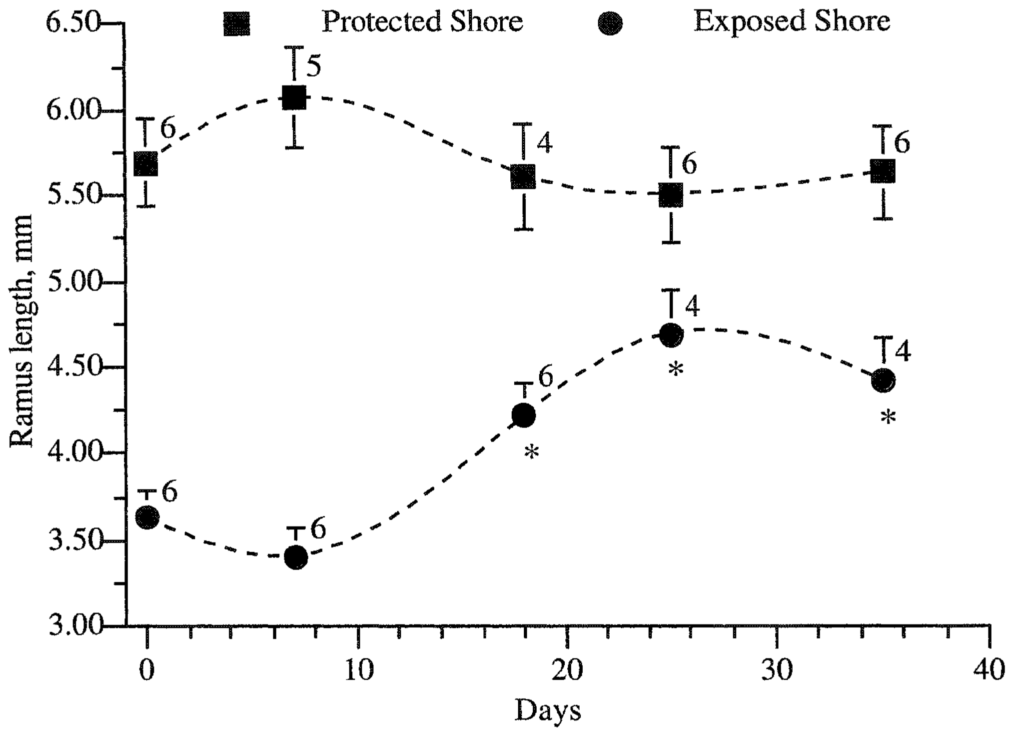


Figure 4-4. Feeding leg length of exposed and protected shore *Balanus glandula* 0, 7, 18, 25, and 35 days after transplant to low flow conditions (0.96 cm/s) in Bamfield Inlet. Each point represents mean ramus length (± SE) of barnacles adjusted to a common prosomal wet mass of 0.0259g by ANCOVA (Table 4-3). Points with the * symbol underneath (exposed shore only) were significantly different from the pre-treatment (0 days) population ($P < 0.007$; Bonferroni adjusted $\propto = 0.0125$; see methods). Differences in ramus length of protected shore barnacles were non-significant ($P = 0.206$; Table 4-3). Numbers above each point represent sample size for each group.

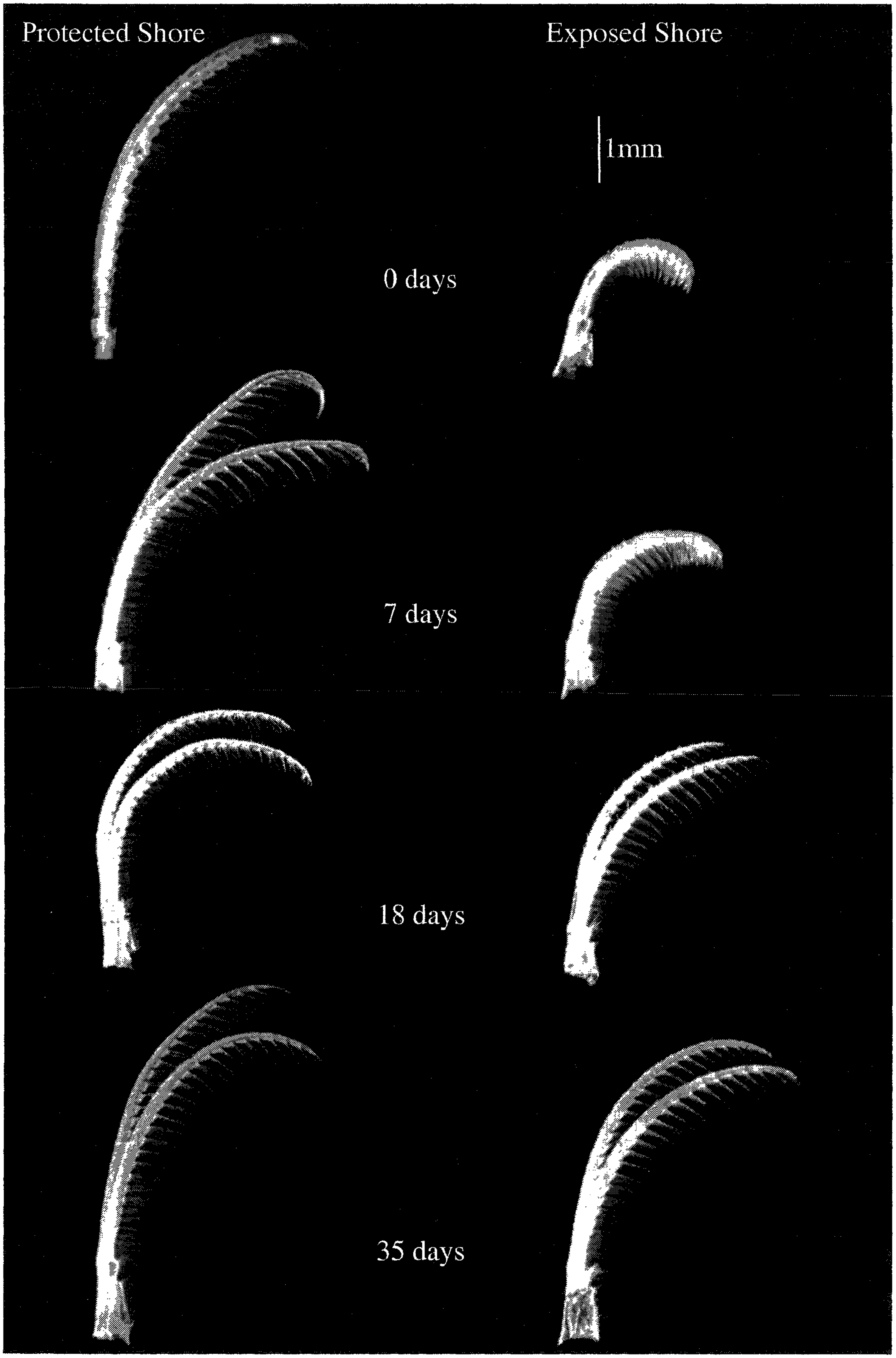

Protected Shore
Exposed Shore
1mm
0 days
7 days
18 days
35 days

Figure 4-5

Figure 4-5. 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/s) 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 (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, and exposed shore, 0.023, 0.026, 0.026 and 0.032. Each photograph was taken at 90X magnification.

CHAPTER 5

GENERAL CONCLUSIONS

5.0 THE INFLUENCE OF FLOW ON SUSPENSION FEEDING MORPHOLOGY

Wave-swept organisms face extreme differences in numerous environmental variables; differences that are correlated with morphological variation in many plants and animals (Denny 1988, 1994, Wildish and Kristmanson 1997). Variation in cirrus form of *Balanus glandula* described in chapters 2 and 3, is a clear example of this pattern, and, because this variation is largely ecophenotypic (chapter 4), possibly the most precise example of morphological fine tuning to growth environment ever recorded in animals. Surprisingly, amidst vast differences in physical and biological conditions between wave-exposures (Denny 1988, 1994), and even in the complex hydrodynamic forces of oscillating flow, 95-99% of this variation — at least in moderate to exposed conditions (chapter 2) — is explained by a single variable: average maximum velocity of breaking waves. This, coupled with the similar dependence of cirrus form on wave-exposure in all four sympatric intertidal barnacles inhabiting Barkley Sound, illustrates the profound influence hydrodynamic forces have on the shape of suspension feeding limbs.

The influence of flow on feeding morphology; however, is not limited to the extreme magnitude of flow differences. The quick generation of longer limbs in quiet water and the extreme flexibility of feeding form throughout a barnacle's life, suggest both spatial and temporal heterogeneity of flow have also played a significant role in the selection of plastic feeding limbs. Furthermore, velocity-dependent feeding form in these four barnacle species, coupled with similar variation in marine bryozoans (Okamura 1992, Okamura and Partridge 1999) and fresh water larval blackflies (Zhang and

Malmqvist 1997, Zhang 1998), suggest that the selective force arising from flow variation may be similar among other groups of suspension feeders feeding with extended limbs (phoronids, entoprocts, polychaetes, pterobranch hemichordates) in both marine and freshwater systems.

5.1 THE ADAPTIVE NATURE OF CIRRUS PLASTICITY

The clear dependence of cirrus form on wave-exposure, and quick transformation of cirrus form when exposed to different flows, suggests strict trade-offs result from differences in cirrus form among wave environments. The adaptive nature of barnacles possessing different cirrus form under different wave-exposures was identified in three modes. First, the functional morphology of barnacle limbs suggests that proportionally longer cirri and setae yield a larger feeding area. Larger surface area of the feeding net would enable barnacles to capture more food in lower particle flux associated with low velocities (Fréchette et al. 1989, Crisp and MacLean 1990). Proportionally shorter, stouter cirri with shorter setae would be less vulnerable to damage and may increase the ability to feed with cirri fully extended at very high velocities. Second, qualitative observation that the most variable species, *B. glandula*, inhabits the broadest range of wave conditions in Barkley Sound yields further evidence that cirrus variation is adaptive. If different cirrus morphologies are adapted to maintain performance and fitness under different wave-conditions, a wider range of possible phenotypes should result in a wider range of inhabited wave-conditions. Thus, *B. glandula* may have a wider niche space due to a greater plasticity of cirrus form. Lastly, experiments in chapter 4 showed protected shore barnacles possessed slightly (and significantly) longer limbs than exposed shore populations, regardless of transplant to high and low flow.

Because these differences are in the direction of environmentally induced differences among populations, it appears selection for longer limbs in quiet water (or shorter limbs on wave-exposed shores) has, or is taking place. For selection to take place in the direction of plasticity we must assume that longer limbs in quiet water yield some fitness advantage over other cirrus phenotypes, and thus are better adapted to protected shore environments (Travis 1994). Although each of these suppositions provides only indirect evidence that cirrus plasticity is adaptive, the arguments taken as a whole are quite compelling.

5.2 FUTURE DIRECTIONS

The identification of such dramatic ecophenotypic variation in barnacle cirri has opened a door for novel research on an organism that has been well studied since Darwin's time (see Darwin 1854). This thesis also reveals a new system to explore the nature and origin of continuous phenotypic plasticity and for testing our theories on its evolution. Moreover, many authors must now be wary of the extreme plasticity of feeding limbs when studying the effects of flow on feeding behaviours and barnacle growth (Trager et al. 1990, Trager et al. 1992, Eckman and Duggins 1993).

Research included in this thesis illustrates the requirement for work in at least four areas. First, quantitative evidence of the fitness or performance consequences of different cirrus form in different flow is necessary to show indisputably that cirrus dependence on wave exposure is indeed adaptive. Preliminary results show that exposed shore phenotypes are able to maintain fully extended cirri in 1 m/s flow, while protected shore phenotypes stop cirral extension around 20 cm/s. Second, since both velocity and food concentration can influence feeding structures (Zhang and Malmqvist 1997, Lucas and

Hunter 1999), experiments separating the effects of water velocity and food availability on cirrus form will be quite useful in identifying which is the primary stimulus for cirrus modification. Pentcheff's (1995) attempt to do this suggested that food supply, rather than water velocity, was the proximate cue, but these experiments need repeating. Third, using biomechanical theory we may be able to interpret the effect of flow on ramus diameter by accounting for how ramus diameter increases with wave-exposure. Increased ramus diameter may occur via ultra-structural changes in thickness or materials of the cuticle, or via an increase in area of the fluid filled cavity of the ramus (second moment of area) so that more material exists further from the neutral axis of the ramus. Understanding how ramus diameter changes may also help tie together why the dimensions of the cirral net did not respond similarly in experiments of chapter 4. Finally, further research into the precision of cirrus dependence on wave-exposure in *B. glandula* along the entire Eastern Pacific and *Semibalanus balanoides* on theWestern Atlantic would be useful to assess the universality of using barnacles as biological indicators of wave exposure.

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Appendix 3–1. Results from ANCOVA testing for differences in trait means and equality of slopes (prosomal wet mass = covariate) of four cirral traits among populations of *Balanus glandula, Chthamalus dalli, Semibalanus carious* and *Pollicipes polymerus.* Populations of each species were from habitats differing in wave exposure. Cirral traits and prosomal wet mass were log-transformed.

		Mean Square			
Source of variation	df	Ramus length	Setae length	Intersetal space	Ramus diameter
Balanus glandula					
Leg 6					
Population (POP)	6	0.149 ***	0.081 ***	0.019 ***	0.036 ***
Log[wet mass] (WM)	1	1.584 ***	0.802 ***	0.407 ***	1.025 ***
Error	62	0.002	0.001	0.004	0.002
Equality of slopes	6	0.005 *†	0.002	0.009 *†	0.002
Leg 5					
POP	6	0.142 ***	0.088 ***	0.023 ***	0.045 ***
WM	1	1.664 ***	0.796 ***	0.384 ***	1.059 ***
Error	62	0.003	0.001	0.003	0.003
Equality of slopes	6	0.006	0.003 *†	0.006 *†	0.004
Leg 4					
POP	6	0.138 ***	0.103 ***	0.007	0.028 ***
WM	1	1.780 ***	0.989 ***	0.409 ***	0.997 ***
Error	62	0.002	0.002	0.003	0.002
Equality of slopes	6	0.022 *†	0.004 *†	0.010 *†	0.001
Chthamalus dalli					
Leg 6					
POP	5	0.147 ***	0.063 ***	0.016 *	0.020 ***
WM	1	0.192 ***	0.105 ***	0.093 ***	0.085 ***
Error	53	0.002	0.002	0.005	0.002
Equality of slopes	5	0.001	0.001	0.005	0.002
Leg 5					
POP	5	0.113 ***	0.058 ***	0.025 ***	0.017 ***
WM	1	0.176 ***	0.104 ***	0.033 **	0.106 ***
Error	53	0.002	0.001	0.003	0.001
Equality of slopes	5	0.001	0.001	0.002	0.002

Leg 4					
POP	5	0.083 ***	0.040 ***	0.012 *	0.015 ***
WM	1	0.198 ***	0.104 ***	0.037 **	0.123 ***
Error	53	0.001	0.002	0.004	0.001
Equality of slopes	5	0.001	0.001	0.002	0.002
Semibalanus cariosus					
Leg 6					
POP	5	0.042 ***	0.038 ***	0.050 ***	0.024 ***
WM	1	0.597 ***	0.238 ***	0.057 ***	0.616 ***
Error	53	0.003	0.002	0.004	0.002
Equality of slopes	5	0.004	0.001	0.009 *†	0.001
Leg 5					
POP	5	0.036 ***	0.042 ***	0.036 ***	0.031 ***
WM	1	0.683 ***	0.288 ***	0.124 ***	0.755 ***
Error	53	0.003	0.002	0.004	0.002
Equality of slopes	5	0.003	0.001	0.007	0.0005
Leg 4					
POP	5	0.028 ***	0.032 ***	0.035 ***	0.030 ***
WM	1	0.813 ***	0.512 ***	0.114 ***	0.727 ***
Error	53	0.002	0.002	0.002	0.002
Equality of slopes	5	0.002	0.004	0.001	0.002
Pollicipes polymerus					
Leg 6					
POP	3	0.006 ***	0.007 **	0.004	0.003
WM	1	0.400 ***	0.142 ***	0.190 ***	0.501 ***
Error	35	0.001	0.001	0.001	0.001
Equality of slopes	3	0.0004	0.0005	0.0004	0.001
Leg 5					
POP	3	0.005 **	0.006 **	0.003	0.005
WM	1	0.442 ***	0.146 ***	0.231 ***	0.520 ***
Error	35	0.001	0.001	0.002	0.001
Equality of slopes	3	0.001	0.0002	0.0004	0.001
Leg 4					
POP	3	0.003 *	0.006 **	0.007 *	0.004 *
WM	1	0.479 ***	0.192 ***	0.294 ***	0.531 ***
Error	35	0.001	0.001	0.002	0.0005
Equality of slopes	3	0.0004	0.0001	0.002	0.001

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. Main effects and error df and MS exclude non-significant interaction terms.

When testing for equality of slopes, the error degrees of freedom were 56 for *B. glandula*, 48 for *C. dalli* and *S. cariosus*, and 32 for *P. polymerus*.

† Interaction became non-significant after Sequential Bonferroni Correction (three legs = three tests for each species) and original main effects and error df and MS are used in the full analysis.