Sex allocation, reproductive behavior and mating success in simultaneously hermaphroditic barnacles

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Abstract of the Dissertation

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Acorn barnacles are simultaneous hermaphrodites, functioning as both males and females at once. The species from this dissertation, *Semibalanus balanoides* and *Balanus glandula*, mate with long penises during brief mating seasons in the fall and winter. Barnacles are ideal organisms for studies of reproductive investment, function and success. They are sessile, allowing for manipulation of populations and transplant experiments. Their anatomy is well suited for comparisons of allocation to the sex roles. Potential mates and levels of mating competition can be assessed because they are limited by the reach of the penis. Fertilized eggs are brooded for several weeks until the development of free-swimming larvae; during this time mating success can be measured. I used field observations and experiments to answer several questions about barnacle reproductive dynamics. I investigated variation in the functional morphology of the penis of the barnacle. I observed that barnacles from dense patches had on average shorter penises than those from sparse aggregations. I also observed that in sites exposed to ocean waves barnacles had thicker penises than those in protected, calm sites. Barnacles in these calm sites were consistently able to reach more distant mates than those from wavy sites. I performed transplant experiments that showed that this difference was the result of phenotypic plasticity and that these morphologies increased ability to successfully mate at the sites in which they develop. I performed experiments that demonstrate that observed differences in penis length are a result of the settlement density of the barnacles and not mate distance alone. Sex allocation theory predicts that as mating group size increases, barnacles should make greater investments into the male role to remain competitive and that this should tradeoff against investments to female function. My experiments show that greater settlement density does lead to increases in allocation to male function, but that increases in mate numbers do not. The assumption of a tradeoff with female function was not supported. My results indicate that the major factor underlying allocation to female function is body size and brood space.

Dedication

This dissertation is dedicated to my friends and colleagues in the Department of Ecology and Evolution at Stony Brook University, who have been like a second family. Special recognition must be made paid to Jeffrey S. Levinton, who has been an excellent mentor.

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Chapter 1 - Introduction

Acorn barnacles are simultaneous hermaphrodites that reproduce by copulating (actually pseudo-copulating). Owing to their sessile lifestyle, they accomplish this with a long, flexible penis (Darwin 1854); they are obligate-outcrossers. For most of the year barnacles are not reproductive, the penis is degenerate and non-functional and barnacles do not have reproductive tissues (Barnes 1992). In the period before the reproductive season eggs, testes and penises are rapidly developed (Klepal et al. 1975, reviewed in Barnes 1992). The penis has an annulated exoskeleton (with accordion-like folds), allowing it to stretch to many times its relaxed length (Klepal et al. 1972). Mating occurs when an individual becomes receptive as a functional female (Barnes et al. 1977). Neighbors acting as males search for it using the sensory penis (Munn et al. 1974). The penis is inserted into the mantle cavity of the functional female, where sperm is deposited and fertilization occurs (Barnes et al. 1977, Klepal et al. 1977, Klepal 1990). Copulation may last for up to several minutes (Murata et al. 2001). After fertilization, embryos are brooded in the mantle cavity until they are released as nauplius larvae. The North Atlantic acorn barnacle, *Semibalanus balanoides*, mates in the fall, broods its larvae until their release in mid-winter, which settle as cyprids in the spring (Crisp and Patel 1958, Stubbings 1975, Hoch 2008). The Pacific acorn barnacle, *Balanus glandula*, has its major reproductive period in the winter, and releases its larvae in the spring, although it has minor periods of reproductive activity year round.

A major goal of sex allocation theory has been to understand how sex ratios maximize fitness (Fisher 1930, Hamilton 1967, Charnov 1982). For organisms with separate sexes, sex ratio is the proportion of individuals of either sex in the population at some point in their life history. For simultaneous hermaphrodites this is the relative investment into male and female function in a single individual. Given competition between functional males, fitness is predicted to be optimized when the proportion of reproductive resources devoted to male function (q, allocation to male function divided by the sum of allocation to total reproduction) are allocated following Charnov (1980):

$$q = (k - 1) / (2k - 1)$$

Where k is the number of individuals competing with a focal individual for mates. The ratio allocated to male function (q) ranges from zero in the smallest mating groups (actually the minimum allocation that could fertilize all of a single mate's eggs) to an asymptote of 50% as k approaches infinity. As the number of competitors increases, the proportional allocation to male function must increase for functional males to remain competitive. This local mate competition model (LMC) assumes a tradeoff between male and female function and that allocation to the sex roles are energy limited. Bateman's principle states that male fecundity is limited by access to females and that fecundity for females tends to be limited by resources available for egg production (Bateman 1948, Arnold 1994, Morgan 1994). In simultaneous hermaphrodites this applies to male and female functional roles.

Charnov's LMC model was first developed for barnacles (Charnov 1980, see also Fischer 1980). The mating system of acorn barnacles makes them ideal animals for studies of sex allocation and reproductive success. Competitive interactions between functional males are likely mediated by sperm competition after multiple matings by a single functional female (Murata et al. 2001, David Rand personal communication). Barnacle anatomy is well suited for comparisons of allocation to either sex role. Eggs are packed discreetly within the shell and are easily removed. Testes and male-specific tissue can be dissected from the rest of the body. Embryos are brooded for several weeks, allowing one to collect offspring and measure mating success. Barnacles settle gregariously (Crisp and Barnes 1954), and mating group sizes range from small and sparse to large and dense (Barnes and Powell 1950, Bertness et al. 1998). This natural variation is useful for comparisons of sex allocation among mating groups with differing levels of mate competition.

In this dissertation, I answered several questions about sex allocation, reproductive behavior and penis functional morphology in acorn barnacles. First, I quantified variation in penis morphology and function at sites varying for wave exposure and aggregation density for the Atlantic barnacle *Semibalanus balanoides*. I performed experiments that identified variation over wave exposure levels as adaptive phenotypic plasticity. Next, I performed experiments that identified the cues to which barnacles respond when developing penises of different length. I performed experiments with the Pacific barnacle, *Balanus glandula*, showing that they too exhibit phenotypically plastic penis morphology in response to wave action and that ability to fertilize neighbors is affected by wave exposure. Finally, I performed experiments with both species of barnacle, measuring relative allocation to the sex roles (as well as allocation to each sex role individually) and identified some of the cues that barnacle use to gauge their future levels of mate competition.

References

Arnold, S. 1994. Bateman's Principle and the measurement of sexual selection in plants and animals. American Naturalist **144**:s126-s149.

Barnes, H., M. Barnes, and W. Klepal. 1977. Studies on the reproduction of cirripedes. I. Introduction: Copulation, release of the oocytes and formation of the egg lamellae. Journal of Experimental Marine Biology and Ecology **27**:195-218.

Barnes, H. and H. T. Powell. 1950. The development, general morphology, and subsequent elimination of barnacle populations after heavy initial settlement Journal of Animal Ecology **19**:175-179.

Barnes, M. 1992. The reproductive periods and condition of the penis in several species of common cirripedes. Oceanography and Marine Biology **30**:483-525.

Bateman, A. 1948. Intra-sexual selection in *Drosophila*. Heredity **2**:349-368.

Bertness, M., S. Gaines, and S. Yeh. 1998. Making mountains out of barnacles: the dynamics of acorn barnacle hummocking. Ecology **79**:1382-1394.

Charnov, E. 1980. Sex Allocation and local mate competition in barnacles. Marine Biology Letters **1**:269-272.

Charnov, E. L. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton NJ.

Connell, J. 1961. Effects of competition, predation by *Thais lapillis*, and other factors on natural populations of the barnacle, *Balanus balanoides*. Ecological monographs **31**:61-104.

Crisp, D. J. and H. Barnes. 1954. The orientation and distribution of barnacles at settlement with particular reference to surface contour. Journal of Animal Ecology **23**:142-162.

Crisp, D. J. and B. S. Patel. 1958. Relation between brooding and ecdysis in cirripedes. Nature **181**:1078-1079.

Darwin, C. 1854. *A monograph on the sub-class Cirripedia, with figures of all the species. The Balanidæ, (or sessile cirripedes); the Verrucidæ, etc. etc. etc.* The Ray Society, London.

Fischer, E. 1980. The relationship between mating system and simultaneous hermaphroditism in the coral reef fish, *Hypoplectrus nigricans* (Serranidae). Animal Behaviour **28**:620-633.

Fisher, R. A. 1930. *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science **156**:477-488.

Hoch, J. 2008. Variation in penis morphology and mating ability in the barnacle, *Semibalanus balanoides*. Journal of Experimental Marine Biology and Ecology **359**:126-130.

Klepal, W. 1990. The Fundamentals of Insemination in cirripedes. Oceanography and Marine Biology **28**:353-379.

Klepal, W., H. Barnes, and M. Barnes. 1975. Variability in the 'loss' of the penis in *Balanus balanoides* (L.) under natural and experimental conditions and histology of degeneration. Pages 275-286 *in* Proc. 9th Europ. mar. biol. symp. Aberdeen University Press.

Klepal, W., H. Barnes, and M. Barnes. 1977. Studies on reproduction in cirripedes. VI. Passage of spermatozoa into the oviducal sac and closure of pores. Journal of Experimental Marine Biology and Ecology **27**:289-304.

Klepal, W., H. Barnes, and E. A. Munn. 1972. The morphology and histology of the cirripede penis. Journal of Experimental Marine Biology and Ecology **10**:243-265.

Morgan, M. 1994. Models of sexual selection in hermaphrodites, especially plants. American Naturalist **144**:s100-s125.

Munn, E. A., W. Klepal, and H. Barnes. 1974. The fine structure and possible function of the sensory setae of the penis of *Balanus balanoides* (L.). Journal of Experimental Marine Biology and Ecology **14**:89-98.

Murata, A., M. Imafuku, and N. Abe. 2001. Copulation by the barnacle *Tetraclita japonica* under natural conditions. Journal of Zoology **253**:275-280.

Stubbings, H. 1975. *Balanus balanoides*. Liverpool University Press, Liverpool.

Chapter 2 - Variation in Penis Morphology and Mating Ability in the Acorn Barnacle, *Semibalanus balanoides*

Abstract

I examined variation in penis morphology of the acorn barnacle, *Semibalanus balanoides*, at different aggregation densities and at different levels of wave exposure. Barnacles in sparse, un-crowded aggregations had significantly longer penises than those from densely crowded groups, suggesting a response to increase the chance of reaching distant mating partners. Barnacles exposed to oceanic waves had penises with significantly greater basal diameter, possibly to strengthen the penis and retain function in turbulent conditions. I compared the percentage of individual barnacles with fertilized broods over a range of distances to their nearest possible mate in sites exposed to or protected from waves. As neighbor distance increased, the proportion of individuals with fertilized egg masses decreased in both wave-exposed and protected sites. However, at greater mate distances in the wave exposed sites, the proportion of individuals with fertilized eggs was significantly lower than the proportion in protected sites, indicating that exposure to waves hinders mating with neighbors at increasing distances. These results suggest that the intensity of mate competition may differ for barnacles between environments with different levels of wave exposure. These differences in male ability are predicted to alter relative sex allocation to male and female function.

Introduction

Frequency dependent selection is generally thought to drive the ratio of male to female offspring towards equality in sexually reproducing organisms (Fisher, 1930; Hamilton, 1967). Hamilton (1967) realized that some conditions, including local mate competition, may favor selection for sex ratios that differ from one-to-one. This concept was subsequently extended to simultaneously hermaphrodites, including barnacles (Charnov, 1980) and coral reef fish (Fischer, 1980), where sex ratio refers to the ratio of female to male tissue within a single individual, rather than to the ratio of offspring produced. Owing to the simplicity of their mating system and the ease with which mating group parameters can be measured, acorn barnacles are ideal model organisms for the study of sex allocation. They are sessile, simultaneous hermaphrodites that copulate with neighbors using a long penis. They brood their offspring, allowing measurement of mating success.

Charnov's (1980) model predicts the level of allocation to either sex role based on local mate competition. As competition amongst functional males for functional females increases, allocation to male function should increase towards an asymptote of 50%, following:

$$q = (k - 1) / (2k - 1)$$

Where $k + 1$ is the size of the mating group and k is the number of competitors for mates (Charnov, 1980). Empirical tests have supported this hypothesis for another species of acorn barnacle, *Catomerus polymerus* (Raimondi and Martin, 1991), which was shown to have greater egg masses in smaller mating groups. The model is also well supported for other hermaphroditic organisms (Brauer, Scharer and Michiels, 2007; Lorenzi, Sella, Schleicherova and Ramella, 2005; Scharer and Wedekind, 2001; Scharer and Ladurner, 2003; Tan, Govedich and Burd, 2004; Trouve, Jourdane, Renaud, Durand and Morand, 1999).

Variation in barnacle penis traits may be important when comparing sex allocation of barnacles for several reasons. As the number of individuals in the mating group increases, mate competition intensifies, which is predicted to lead to greater relative allocation to male function (Charnov, 1980; Charnov, 1982). The penis itself represents a significant investment into male function, in terms of construction, maintenance and performance costs. For example, the presence of the penis, located between the feeding cirri on the terminal body segment (Klepal, Barnes and Munn, 1972), may reduce feeding efficiency. As penises grow larger, interference with feeding is expected to increase.

A barnacle's mating group consists of all of the neighbors within reach of its penis and all of its potential competitors for mates. Variation in penis reach may have an important role in determining mating group size and local mate competition. The barnacle's penis is a long, muscular organ with an annulated exoskeleton allowing it to stretch to several times its resting length (Barnes, Barnes and Klepal, 1977). For *Semibalanus balanoides* (Linnaeus), variation in penis condition over the year is a well-documented phenomenon. The penis is degenerate for most of the year, grows rapidly in September and October, and reaches a maximum for the brief mating season in early November, after which it is cast off with the next molt (reviewed in Barnes, 1992; Crisp and Patel, 1958). The rapid growth and loss of the penis suggests that it is costly to maintain a fully expressed penis; the ability to molt allows barnacles to confine such costs to the period of the mating season.

Natural aggregations of barnacles vary greatly in population density. Sparsely settled barnacles may have several centimeters between themselves and potential mates. At high densities, the shells of neighboring barnacles grow together into “hummocks”, containing tall, trumpet-shaped individuals that are only a fraction of a centimeter from many neighbors (Bertness, Gaines and Yeh, 1998). The effects of crowding on fecundity and energy allocation have been reported, but not in the context of mate competition or sex allocation theory (Wethey, 1984; Wu, Levings and Randall, 1977). Raimondi and Martin (1991) showed that the available number of mates affects sex allocation; barnacles from smaller mating groups had larger egg masses than barnacles from larger groups.

Semibalanus balanoides is found over a wide range of wave exposures, from sites on the exposed Atlantic coast with constant wave action to protected bays and harbors, where extreme wave action is very infrequent. Recent observations of extreme phenotypic plasticity in cirri morphology between environments differing in wave exposure suggest that hydrodynamic forces may have large effects on scales relevant to copulating barnacles (Marchinko, 2003; Marchinko and Palmer, 2003). These same forces may reduce the number of mates available to a barnacle by decreasing the reach of the penis or restricting the time available for mating activities to periods of calm water.

In this study, I measured penis length, diameter and fertilization success rates of reproductive barnacles (*Semibalanus balanoides*) from sites in New York to test three hypotheses (1) that penis characteristics (i.e., length and basal diameter) vary between barnacles from different aggregation densities, (2) that barnacle penis characteristics vary between sites of different wave exposure, and (3) that fertilization ability is hindered by exposure to waves. I predicted that barnacle penises would differ morphologically between aggregation types and environments differing for wave exposure and that mating barnacles would be less successful reaching distant mates in sites of greater wave exposure. These data will allow predictions about the population and environmental parameters that are important for reproductive success in barnacles and will suggest conditions in which sex allocation may vary in simultaneous hermaphrodites.

Methods

(A) Penis morphology

In November 2004, I collected *Semibalanus balanoides* from 2 sites in Long Island, NY. The wave exposed site, Shinnecock Inlet Jetty (40^{o}50’23.19 N, 72^{o}28’24.80 W) faces the open Atlantic Ocean. The protected site, Shinnecock County Park (40^{o}50’35.64 N, 72^{o}28’21.39) is located in Shinnecock Bay, immediately within the bay off of the main channel of Shinnecock Inlet. It is protected from large, frequent, oceanic waves and is only hit by the less intense wind-waves of Shinnecock Bay. I chose these sites because they were geographically close to each other (approximately 1450 meters apart), supplied with the same mass of water (from strong tidal exchange), were similar for physical and biological parameters (temperature, productivity, salinity, etc.), but were unequivocally different in wave exposure. Barnacle aggregations were abundant at each site and had similar size distributions, reproductive conditions and population densities.

Collections were taken from the approximate center of the *Semibalanus* zone of the intertidal and barnacles in each site spent about the same proportion of their time submerged. Barnacles were identified as "crowded" when their shells touched the shells of neighbors (often as hummocks (Bertness, Gaines and Yeh, 1998)). Aggregations were characterized as "un-crowded" when their shells were not touching those of any neighbor. Un-crowded barnacles had unambiguously greater distances to the aperture of their nearest possible mate (always more than 0.5 cm) than did crowded barnacles (always less than 0.5 cm). Both crowded and un-crowded barnacles came from aggregations of approximately equal size and differed only in crowding.

To analyze potential differences in barnacle penis characteristics from crowded and un-crowded aggregations, I removed whole barnacles with a chisel and preserved them in 70% ethanol. I used digital calipers, with accuracy of 0.01mm, to measure the height of the shell (h), the diameter ($2r_1$) of the aperture (along the mid-line, from tergum to scutum) and the diameter ($2r_2$) of the base (from rostrum to carina). Body volume was estimated by calculating total shell volume as a truncated cone:

$$^1/_3\, \pi\, h\, (r_1^2 + r_1 r_2 + r_2^2).$$

I dissected the barnacles under a dissecting microscope and removed the penis. Penises were assigned an identification number, mounted on glass slides and photographed through a compound microscope (40x magnification) with an attached digital camera. The photographs were analyzed using the image analysis software, *Image J* (Rasband, 1997-2006). All measurements were carried out blindly with respect to sample origin. I measured penis length by counting the number of annulations in the cuticle. The number of annulations in the exoskeleton of the penis is a more consistent measure of length than linear measurements, as penises are capable of stretching several times their relaxed length, and may be twisted, broken or irregularly compressed during preservation and dissection (Barnes, 1992). I used a stage micrometer to calculate scale on the digital images, and used *Image J* to measure the diameter of each penis at its base.

All data were log-transformed prior to analysis to correct for heteroscedacity. I compared penis length of barnacles from crowded and un-crowded aggregations and differences in the penis length-shell volume relationship as a function of aggregation type with ANCOVA (SAS 9.1, SAS Institute 2003), using shell volume as the covariate (Cody and Smith, 1997). I used similar tests to compare penis length (annulation number) for barnacles from site exposed to waves and protected site, penis basal diameter between crowded and un-crowded aggregations and penis basal diameter from exposed and protected sites.

(B) Penis function

Observations on penis function were made in the Fall of 2006. I observed the first successfully mated barnacles on 25 October. By the end of November mating activity had ceased, and all populations had high percentages of individuals brooding fertilized egg masses. Between 5 December and 17 December 2006 I visited six sites: three on the Atlantic coast, exposed to waves: Shinnecock Inlet Jetty East (40^{o}50’23.19 N, 72^{o}28’24.80 W), Shinnecock Inlet Jetty West (40^{o}50’23.30 N, 72^{o}28’39.00 W), Democrat Point Jetty (40^{o}37’13.10 N, 73^{o}18’23.30 W) and three in protected areas on Long Island Sound: Stony Brook Harbor (40^{o}55’16.88 N, 73^{o}08’58.04 W), Flax Pond Inlet (40^{o}58’01.28 N, 73^{o}08’17.00 W) and Cedar Beach Jetty (40^{o}57’54.00 N, 73^{o}02’35.17

W). During the period of reproductive activity, near-shore wave height in the Atlantic sites varied from 1.0 to more than 4.0 meters, whereas in the Long Island Sound sites, it varied from 0.0 to 2.0 meters (NOAA National Weather Service Marine Forecasts; http://www.weather.gov/om/marine/home.htm). At each site, I haphazardly chose a focal barnacle in the mid-intertidal and measured the distance from its aperture to the aperture of its nearest neighbor. This distance represents the minimum distance that a penis would have had to reach to successfully fertilize the brood of the focal barnacle. Focal barnacles that had the scar of a recently deceased individual closer than the nearest living neighbor were not used. Each measurement was placed into one of five bins (0.01 to 0.5 cm, 0.51 to 1.0 cm, 1.01 to 1.5 cm, 1.51 to 2.0 cm, 2.01 to 2.5 cm). Barnacles farther than 2.5 cm from their nearest neighbors were not included. I removed the focal barnacle from the substrate, inspected the egg mass and recorded whether it had been fertilized. At this stage in brooding, fertilized egg masses were compressed, solid pellets that were distinguished visually from egg masses that had not been fertilized (see *fig. 1*). At each site I repeated the process until I had scored fertilization for 100 individuals in each distance bin. I included only barnacles that were healthy adults, and there were no significant differences in body sizes or population densities between the different sites.

I compared the percentage of fertilized barnacles for each distance class for exposed and protected sites. All percentage data were arcsine-square root transformed (Sokal and Rohlf, 1995). Because normality of the sample distribution and equality of variance assumptions of were not always met, I used non-parametric Wilcoxon rank-sum tests (JMP 5.1, SAS Institute 2004) to determine whether fertilization percentage was different between exposure levels (Sokal and Rohlf, 1995). I used logistic regression to determine the relative importance of the interaction between exposure level and neighbor distance on probability of fertilization. I coded each observed fertilized barnacle as an event (= 1) and each un-fertilized barnacle as a non-event (= 0) (Cody and Smith, 1997). I used a Hosmer-Lemeshow Goodness-of-fit-test (Quinn and Keough, 2002) to compare how well logistic regression models fit the data. I compared a model that included wave exposure level (with site assignments as binary variables: exposed = -1 and protected = 1), neighbor distance and the interaction between the two with a model that only included exposure and neighbor distance.

Results

(A) Penis morphology

Penises collected from barnacles in un-crowded aggregations (mean = 222.14 annulations, ± 5.025, n = 29) were significantly longer (DF = 74, *t* = -4.20, P < 0.0001) than those from crowded aggregations (mean = 198.31 ± 2.899 annulations, n = 47). There was no significant difference in the slope of the penis length-body volume relationship between aggregation types (ANCOVA, DF = 1, *F* = 3.21, P = 0.0773; see *fig. 2*). There was no difference (DF = 74, *t* = 1.15, P = 0.2533) in penis length between the protected site, Shinnecock County Park (mean = 203.83 ± 4.290 annulations, n = 35) and the wave-exposed site, Shinnecock Inlet Jetty (mean = 210.46 ± 3.990 annulations, n = 41) and no difference in slope (DF = 1, *F* = 0.17, P = 0.6855). Penis basal diameter was greater, however, (DF = 64, *t* = 2.21, P = 0.0307) at the exposed site (mean diameter =

0.76 ± 0.0319 mm, n = 36) than at protected sites (mean diameter = 0.66 ± 0.0282 mm, n = 32; see *fig. 3*). There was no difference between the penis basal diameter-body volume relationship for the two wave exposure levels (DF = 1, $F = 0.08$, P = 0.7793). Finally, there was no difference (DF = 50, $t = 0.51$, P = 0.6118) between mean penis basal diameter between crowded (mean diameter = 0.68 ± 0.0278 mm, n = 25) and un-crowded barnacles (mean diameter = 0.66 ± 0.0326 mm, n = 27). As with all the other tests, there was no significant difference in the penis basal diameter-body volume relationship (DF = 1, $F = 0.08$, P = 0.7772).

(B) Penis function

In both the Long Island Sound and the Atlantic Ocean sites, as neighbor distance increased, the likelihood of fertilization decreased (*fig. 4*). From 0.01 to 0.5 cm, in the exposed site 93% (s^2=0.000422) of broods were fertilized compared to 96% (s^2=0.000867) in the protected site (Wilcoxon: DF = 1, $X^2 = 1.263$, P = 0.2612). From 0.51 to 1.0 cm, in the exposed site 76% ($s^2 = 0.005489$) were fertilized compared to 88% ($s^2 = 0.004267$) in the protected site (Wilcoxon: DF = 1, $X^2 = 2.333$, P = 0.1266). For the three greater distance classes there were significant differences in the percentage of broods with fertilized eggs between exposed and protected sites. From 1.01 to 1.5 cm, 52% (s^2=0.003622) were fertilized in the exposed sites compared to 84% ($s^2 = 0.01680$) in the protected site (Wilcoxon: DF = 1, $X^2 = 3.857$, P = 0.0495); from 1.51 to 2.0 cm, 29% (s^2=0.001867) were fertilized in the exposed site compared to 66% ($s^2 = 0.022689$) in the protected site (Wilcoxon: 1, $X^2 = 3.857$, P = 0.0495) and from 2.01 to 2.5 cm, 13%

($s^2 = 0.006067$) were fertilized in the exposed site compared to 44% ($s^2 = 0.0150$) in the protected site (Wilcoxon: DF = 1, $X^2 = 3.857$, P = 0.0495).

Logistic regression indicated that the interaction between wave exposure and neighbor distance was important for the best fit model for the data. Including the exposure level (coefficient = 0.3196, DF = 1, SE = 0.1235, Wald $X^2 = 6.6933$, P = .0097), neighbor distance (coefficient = -1.8811, DF = 1, SE = 0.0780, Wald $X^2 = 581.4507$, $P<0.0001$) and the exposure x neighbor distance (coefficient = 0.2666, DF = 1, SE = 0.0780, Wald $X^2 = 11.6821$, P = 0.0006 resulted in a good fit to the data (Hosmer-Lemeshow goodness-of-fit test: $X^2 = 6.5312$, DF = 8, P = 0.5880). A model excluding the interaction term, was significantly different than the data (Hosmer-Lemeshow goodness-of-fit test: $X^2 = 18.9497$, DF = 8, P = 0.0151).

Discussion

Barnacles in less dense aggregations had longer penises than those in crowded aggregations (*fig. 2*). Barnacles that are not crowded have greater distances to their nearest potential mates where longer penises would be advantageous, providing a greater ability to reach partners. Conversely, barnacles from crowded aggregations (especially hummocks) have numerous potential mates within a very short distance. If sperm limitation constrains the maximum number of broods that a functional male can fertilize, densely settled barnacles should have no need to reach mates outside of a close range. The difference in allometry between penis length and body volume for the crowded and un-crowded barnacles was not statistically significant (p = 0.0773), although greater sample size in future studies may reveal that barnacles from sparse aggregations grow relatively longer penises than crowded barnacles of the same body volume.

Wave exposure likely affects penis basal diameter. While the penises of barnacles collected from greater wave exposure were not different in length than those from the protected waters, they had a significantly greater diameter at the base. This may reflect greater muscle mass developed to maintain function in a more turbulent environment, or may be a result of strengthening the penis against the risk of injury caused by the strong accelerations produced by wave impacts (Denny, 1987). There were no significant effects of aggregation density on penis basal diameter. Responses of penis morphology in *Semibalanus balanoides* to population density and wave action may involve adaptive plastic responses to increase fertilization success and ameliorate costs and risks associated with its presence.

The mating ability of barnacles varies across habitat types. Specifically, the ability of the penis to reach neighbors is reduced in wave exposed sites. Barnacles from areas protected from waves had a greater percentage of fertilized broods than those from exposed sites in all neighbor distance bins. The significant results for the three most distant bins suggest that barnacles within protected sites have a greater ability to reach distant mates than those at exposed sites. The comparison of logistic regression models indicates that the interaction between wave exposure and neighbor distance is an important factor in barnacle reproductive behavior. Specifically, the reduction in ability of barnacles to reach neighbors at increasing distance is magnified in sites exposed to ocean waves.

There are three components that may explain these findings; direct wave impact, behavioral response to wave impacts and biomechanical limitation. First, water motion in exposed sites may directly hinder the ability of a barnacle to reach a mate with its penis. A mating barnacle must search for receptive partners, sweeping the penis among its neighbors, then insert the penis and inject seminal fluid. This process takes several minutes (Murata, Imafuku and Abe, 2001), during which waves and water motion may physically interfere with penis activity. Second, barnacles have been observed to cease activities during periods of extreme wave action (Luke Miller, personal communication). In sites where strong forces imposed by waves are more frequent, the relative paucity of time available for mating may reduce the number of partners that a barnacle can locate and fertilize. If there is a critical threshold of water motion at which barnacles cease activity it is likely surpassed more often on coasts exposed to ocean waves. Finally, the thicker penises found in exposed sites may not have the ability to stretch as far as those from protected sites and may be simply unable to reach mates at greater distances. A combination of these factors could be responsible for the observed patterns: barnacles in wave exposed sites have less time when it is calm enough to attempt to search for a mating partner, and their thicker penises, which may be more resistant to damage, are unable to stretch as far as their thinner, calm-water counterparts. The data from this study suggests that the barnacle's ability to reach mates may vary from site to site due to both physical and biological factors.

Sex allocation theory predicts that for hermaphrodites, relative allocation to male function should increase with mating group size and local mate competition (Charnov, 1980; Charnov, 1982). Application of the data in this project to that theory generates several predictions. Since barnacles generally have greater ability to reach distant mates in protected situations, all other factors being equal, barnacles in areas protected from waves should have larger mating groups than those in exposed areas. The resulting functional mating groups will have higher levels of competition among functional males, leading to relatively greater investments into the male role. I would then predict that in areas exposed to waves, barnacles will invest relatively more energy into female function and produce larger broods of eggs. Given the higher number of males competing to mate with a single individual in areas protected from waves, individual broods are more likely to have a larger number of siring males and therefore greater genetic diversity among the offspring.

The barnacle penis is degenerate for most of the year, growing rapidly to a large size immediately prior to the mating season (Barnes, 1992). The pattern of penis growth and loss, along with the data from this project, suggest that the expression of the full-size penis is costly to a barnacle. Construction and maintenance of the penis, as well as opportunity costs (such as a reduction in feeding ability or space to brood offspring) must trade-off against fitness gained via male function. Increased penis length will add to the number of potential mates for a barnacle, but the rising costs associated with penis growth and maintenance will likely reach a point where continued investment in male function results in no net fitness gain. Such decelerating fitness gains are expected for simultaneous hermaphrodites; increasing investment in male function should eventually result in diminished returns (Charnov, 1979; McCartney, 1997). Overall, the observed variation in the condition and morphology of the penis may serve to ameliorate costs imposed by the environment, but add to the costs of construction and maintenance.

These trade-offs must be considered in studies comparing relative allocation to male function.

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Figures

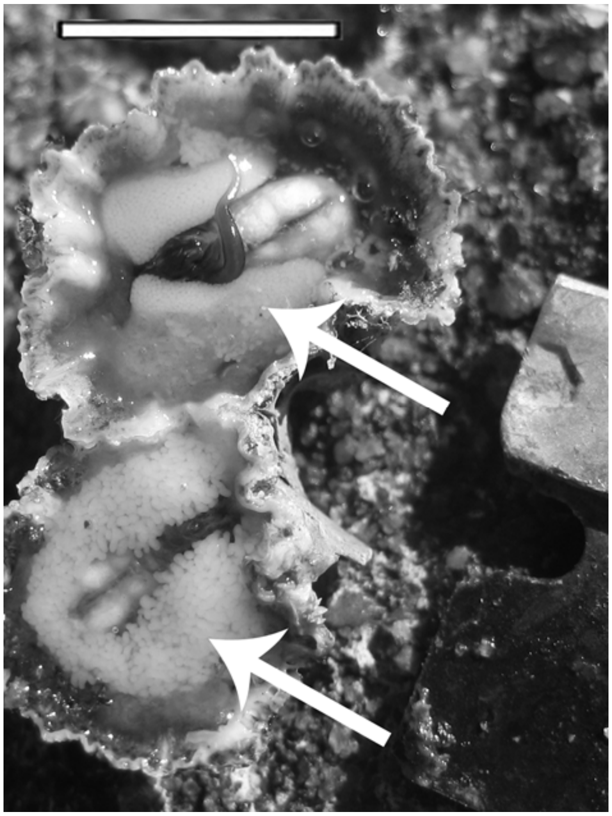

Fig. 1. A barnacle with fertilized eggs (above) and barnacle with unfertilized eggs (below). The fertilized egg masses are visible as compressed, solid pellets on either side of the body, with the penis and sperm mass are visible between them. The unfertilized egg mass is large and more diffuse, visible as globules. The scale bar is 1 cm.

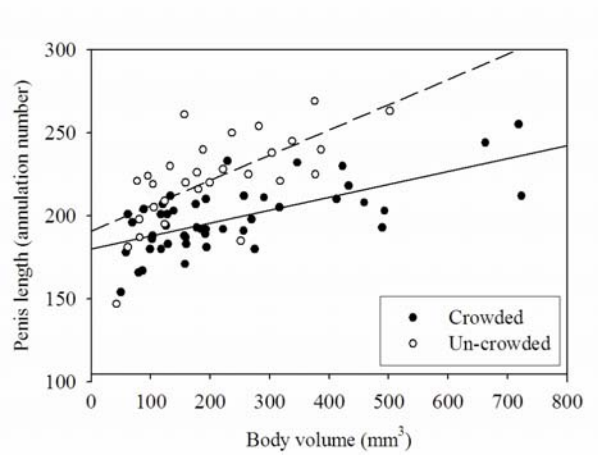

Fig. 2. Untransformed penis length (measured by number of annulations) plotted against body volume (mm^3) for barnacles from crowded (closed circles) and un-crowded (open circles) aggregations. The dashed line represents the OLS regression line for un-crowded aggregations; the solid line represents the OLS regression line for crowded aggregations.

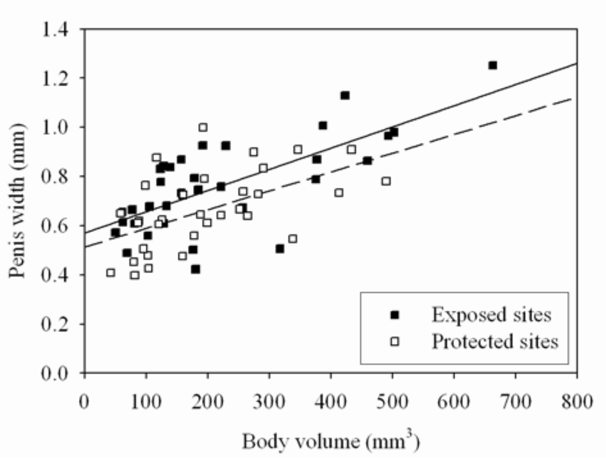

Fig. 3. Untransformed penis basal diameter (mm) plotted against body volume (mm^3) for barnacles from exposed (closed squares) and protected sites (open squares). The dashed line represents the OLS regression line for the protected site; the solid line represents OLS regression line for the exposed site.

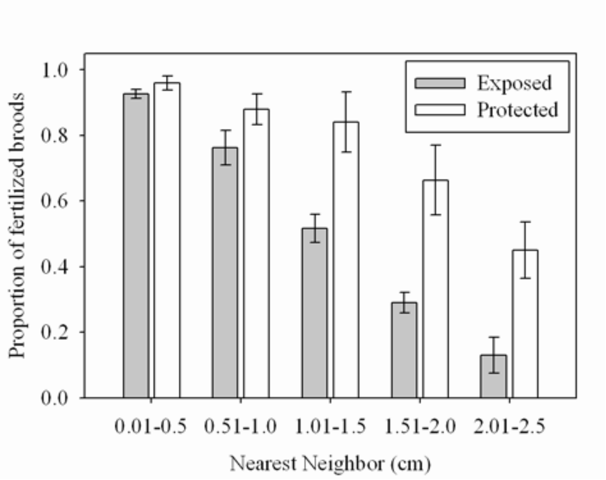

Fig. 4. Fraction of fertilized broods plotted against bins of nearest neighbor distance, comparing populations from sites exposed to waves (shaded) and protected sites (unshaded). Error bars show standard error.

References

Barnes, H., Barnes, M., Klepal, W., 1977. Studies on the reproduction of cirripedes. I. Introduction: Copulation, release of the oocytes and formation of the egg lamellae. Journal of Experimental Marine Biology and Ecology 27, 195-218.

Barnes, M., 1992. The Reproductive Periods and Condition of the Penis in Several Species of Common Cirripedes. Oceanography and Marine Biology 30, 483-525.

Bertness, M., Gaines, S., Yeh, S., 1998. Making mountains out of barnacles: the dynamics of acorn barnacle hummocking. Ecology 79, 1382-1394.

Brauer, V., Scharer, L., Michiels, N., 2007. Phenotypically flexible sex allocation in a simultaneous hermaphrodite. Evolution 61, 216-222.

Charnov, E., 1979. Simultaneous hermaphroditism and sexual selection. Proceedings of the National Academy of Sciences 76, 2480-2484.

Charnov, E., 1980. Sex Allocation and local mate competition in barnacles. Marine Biology Letters 1, 269-272.

Charnov, E., 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton, NJ,

Cody, R., Smith, J., 1997. *Applied Statistics and the SAS Programming Language, 4th edition*. Prentice Hall, Upper Saddle River, NJ.

Crisp, D.J., Patel, B.S., 1958. Relation between brooding and ecdysis in cirripedes. Nature 181, 1078-1079.

Denny, M.W., 1987. Life in the maelstrom: The biomechanics of wave-swept rocky shores. Trends in Ecology and Evolution 2, 61-66.

Fischer, E., 1980. The relationship between mating system and simultaneous hermaphroditism in the coral reef fish, *Hypoplectrus nigricans* (Serranidae). Animal Behavior 28, 620-633.

Fisher, R., 1930. *The Genetical Theory of Natural Selection*, (Variorum edition 1999, Bennett JH, Ed). Clarendon Press, Oxford.

Hamilton, W., 1967. Extraordinary sex ratios. Science 156, 477-488.

Klepal, W., Barnes, H., Munn, E.A., 1972. The morphology and histology of the cirripede penis. Journal of Experimental Marine Biology and Ecology 10, 243-265.

Lorenzi, M., Sella, G., Schleicherova, D., Ramella, L., 2005. Outcrossing hermaphroditic polychaete worms adjust their sex allocation to social conditions. Journal of Evolutionary Biology 18, 1341-1347.

Marchinko, K.B., 2003. Dramatic phenotypic plasticity in barnacle legs (*Balanus glandula* Darwin): Magnitude, age dependence and speed of response. Evolution 57, 1281-1290.

Marchinko, K.B., Palmer, A., 2003. Feeding in flow extremes: dependence of cirrus form on wave-exposure in four barnacle species. Zoology 106, 127-141.

McCartney, M., 1997. Sex allocation and male fitness gain in a colonial, hermaphroditic marine invertebrate. Evolution 51, 127-140.

Murata, A., Imafuku, M., Abe, N., 2001. Copulation by the barnacle *Tetraclita japonica* under natural conditions. Journal of Zoology 253, 275-280.

Quinn, G., Keough, M., 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge, 537 pp.

Raimondi, P.T., Martin, J.E., 1991. Evidence that mating group-size affects allocation of reproductive resources in a simultaneous hermaphrodite. American Naturalist 138, 1206-1217.

Rasband, W.S., 1997-2006. Image J. U.S. National Institute of Health, Bethesda, MA.

Scharer, L., Wedekind, C., 2001. Social situation, sperm competition and sex allocation in a simultaneous hermaphrodite parasite, the cestode *Schostocephalus solidus*. Journal of Evolutionary Biology 14, 942-953.

Scharer, L., Ladurner, P., 2003. Phenotypically plastic adjustment of sex allocation in a simultaneous hermaphrodite. Proceedings of the Royal Society, B: Biological Sciences 270, 935-941.

Sokal, R., Rohlf, F., 1995. *Biometry,* 3rd edition. W.H. Freeman and Company, New York, NY, 887 pp.

Tan, G., Govedich, F., Burd, M., 2004. Social group size, potential sperm competition and reproductive investment in a hermaphroditic leech, *Helobdella papillornata* (Euhirudinea: Glossiphoniidae). Journal of Evolutionary Biology 17, 574-580.

Trouve, S., Jourdane, J., Renaud, F., Durand, P., Morand, S., 1999. Adaptive sex allocation in a simultaneous hermaphrodite. Evolution 53, 1599-1604.

Wethey, D.S., 1984. Effects of crowding on fecundity in barnacles: *Semibalanus (Balanus) balanoides*, *Balanus glandula* and *Chthamalus dalli*. Canadian Journal of Zoology 62, 1788-1795.

Wu, R., Levings, C., Randall, D., 1977. Differences in energy partition between crowded and uncrowded barnacles (*Balanus balanoides* Darwin). Canadian Journal of Zoology 58, 559-566.

Chapter 3 - Adaptive plasticity of the penis in a simultaneous hermaphrodite

Abstract

Acorn barnacles are important model organisms for the study of sex allocation. They are sessile, non-selfing hermaphrodites that copulate with penises that have been suggested to be phenotypically plastic. On wave-exposed shores, *Semibalanus balanoides* develop penises with relatively greater diameter, while in wave-protected sites they are thinner. A reciprocal transplant experiment between wave-exposed and protected sites tested whether these exposure-specific morphologies have adaptive value. Mating success was compared over a range of distances to compare the ability of barnacles to reach mates. Barnacles that grew in the wave-protected site and mated in the wave-protected site fertilized more broods at increasing distances than those transplanted to the wave-exposed site. For barnacles that developed in the wave-exposed site, there was no difference in the ability to fertilize neighbors between sites of differing exposure. This study demonstrates the adaptive value of plasticity in penis morphology. The results suggest a trade-off between development of a penis adapted to wave exposure and the ability to fertilize distant mates. Barnacles in different physical environments are limited by different factors, which may limit numbers of potential mates, constrain optimal sex allocation strategies and alter reproductive behavior.

Introduction

Acorn barnacles live in the rocky intertidal zone over gradients of wave energy, from consistently calm locations to some of the most energetic environments found in nature (Denny 1987). They have developed several phenotypically plastic morphologies and behaviors in order to deal with the potential dangers in such habitats. When large waves crash, barnacles retreat into their shells to avoid injury (Miller 2007). Because avoiding waves is not always possible, barnacles have developed several morphological solutions to the risks imposed by wave impacts. Barnacles build shells that are more resistant to being crushed by debris in wave exposed areas (Pentcheff 1991), the feeding cirri of barnacles change size and shape dramatically in response to wave exposure (Arsenault et al. 2001; Marchinko 2003), as do their penises (Hoch 2008; Neufeld and Palmer 2008). In the case of the penis, the functional advantage of one exposure-specific morphology over another has yet to be explained.

Acorn barnacles are notable among sessile organisms in that they are among the few that reproduce by copulation (or more accurately, pseudo-copulation), which they accomplish with penises that can stretch up to several times the body length (Barnes 1992; Darwin 1854; Klepal et al. 1972). This limits their pool of potential mates to only those barnacles located within a penis-length (most intertidal acorn barnacles are not self-fertile). These properties make barnacles an excellent system for studying reproduction and sex allocation in simultaneous hermaphrodites. Barnacles are predicted to allocate resources to either sex role based on local mate competition (Charnov 1980). Indeed, barnacles have been shown to increase allocation to female function in relatively small mating groups (Raimondi and Martin 1991).

A barnacle acting as a functional male will typically search for a receptive functional female by sweeping the penis amongst its neighbors (Barnes et al. 1977). Once located, the penis is inserted into the functional female's mantle cavity and seminal fluid is deposited (Klepal 1990). This process may take several minutes (Murata et al. 2001), during which they are vulnerable to damage or disruption from breaking waves and predators. Barnacles brood their fertilized eggs and larvae for several weeks before releasing nauplius larvae into the water.

Most intertidal barnacles have a brief reproductive season and only fully develop a penis during this period (Barnes 1992). Long Island, NY populations of *Semibalanus balanoides* (Linnaeus), the focal species of this study, reproduce in late October and early November (Hoch 2008). For most of the year the penis is greatly reduced in size and not functional. However, over the course of about two months before reproductive activity, the penis grows rapidly to its maximum size over the course of several molts (Barnes 1992; Hoch unpublished data). Densely settled barnacles (those contacting other barnacles) develop shorter penises than sparsely settled barnacles of the same size (Hoch 2008). *S. balanoides* from areas exposed to waves tend to have thicker penises (relative to length) than their counterparts from protected embayments (Hoch 2008). *Balanus glandula* exhibit a similar pattern, which has been suggested to be the result of phenotypic plasticity (Neufeld and Palmer 2008). However, whether these morphological differences have impacts on the barnacle's ability to fertilize neighbors remains untested. Given the differential mating success observed for *S. balanoides* between sites of varying wave exposure (Hoch 2008), an adaptive explanation for the observed morphologies seems likely.

I tested the functional significance of these observed morphological differences between wave exposure with a reciprocal transplant experiment to compare penis function (ability to fertilize neighbors of varying distances) of barnacles in different physical environments. Barnacles that developed in either wave-exposed or wave-protected environments were reciprocally transplanted immediately prior to mating. Overall mating success and success at different inter-individual distances were assessed. This experiment was designed to test whether plasticity in penis form has adaptive importance for functioning in a given environment or if some other factor explains the observed patterns, such as a by-product effect of other morphological changes that barnacles undergo across habitats of varying wave intensity, like the length of the cirri (Marchinko 2003).

Materials and Methods

Experimental design

In June 2006, I set up experimental mating populations at two sites: Shinnecock Inlet jetty, on the wave-exposed Atlantic shore ($40^o50'23.19$ N, $72^o28'24.80$ W) and Stony Brook Harbor, a protected harbor off Long Island Sound ($40^o55'16.88$ N, $73^o08'58.04$ W). I collected live barnacles from mussel shells (*Mytilus edulis*) at Flax Pond inlet jetty on Long Island Sound ($40^o58'01.28$ N, $73^o08'17.00$ W), a site of intermediate wave exposure. Wave exposure was estimated by measuring the relative dissolution rate of 3 cm-diameter plaster spheres (Thompson and Glenn 1994) during the mating season the previous year (October, 2005). Briefly, spheres in Stony Brook Harbor, the protected site, lost 58.42% (N= 5, 95% CI= ±15.21%) of their mass over the course of two high tides, at Flax Pond Inlet, the intermediate site and location of collection, spheres lost 83.34% (N= 6, 95% CI= ±6.32%) of their mass and at Shinnecock Inlet jetty, the exposed site, spheres lost 91.89% of their mass (N= 6, 95% CI= ±11.93%). These qualitative exposure classifications were supported by near-shore wave heights reported over the duration of the experiment (NOAA National Weather Service Marine Forecasts, http://www.weather.gov/om/marine/zone/east/okxmz.htm; zones ANZ335 and ANZ350, June-December 2006). I chose mussel shells that were embedded amongst dense populations of mussels and barnacles in the mid intertidal zone. Barnacles at this location recruit yearly and have low winter survivorship, so most belong to a single age-class and are similar in size. To further reduce variability based on body size I used only medium-sized barnacles (4 to 9 mm aperture diameter). I removed individual barnacles from mussel shells with a rotary saw (Dremel moto-tool, model 275 with diamond wheel), taking care not to damage the barnacle. I created experimental arrays (treated as blocks in the statistical analysis) by fastening five 5-cm square limestone tiles to 45.75 cm metal straps. Each tile had four barnacles cemented (with JB-Kwik; JB-weld Company) in a square pattern with inter-individual distances (between apertures) of 0.5 cm, 1.0 cm, 1.5 cm, 2.0 cm and 2.5 cm. These distances represent the minimum distance that a penis would have to stretch to reach each barnacle; the diagonal distances in each case are greater (for example, barnacles from the 0.5 cm tile had diagonal neighbor distances of 0.71 cm, those from the 1.0 cm tile had diagonal distances of 1.41 cm, and so on). The order of distance treatments along the array was randomized. Previous studies with this species have shown that successful mating is extremely unlikely between individuals more than 5 cm apart (Hoch 2008), so all potential mates were restricted to a single tile (*figure 1*).

At each experimental site (exposed, Shinnecock Jetty and protected, Stony Brook Harbor) I bolted 35 metal straps (45.75 cm) to rocks and other permanent structures in the mid-intertidal zone. I chose areas in the middle of large barnacle populations to ensure high rates of survival through the summer and fall and to keep relative tidal height similar. I used nylon cable ties to attach each array (block of tiles) to the bolted straps. From 15 June until 15 September I visited each site weekly and replaced barnacles that had died with similarly-sized barnacles from the source population. Approximately 1/3 of newly affixed barnacles died, but with repeated replacements done before the start of the reproductive season, the number of living barnacles in the experiment remained high and stable. In June, penises were not developed and were only present as tiny rudiments. Dead barnacles were not replaced after mid-September, when penises began developing rapidly. This ensured that all penis development occurred completely within their original experimental site.

During the month of October, I monitored mating activity in natural populations at both sites twice each week. On October 25, after observing the first mated barnacle in a natural population I immediately re-collected all of the surviving arrays from both sites (28 from each site- seven arrays at each site were destroyed by such things as impacts of wave-borne debris, vandalism, entanglement with fishing gear etc.). At the next possible low tide (October 26), I replaced half of the arrays to their original site (14 to the exposed site, 14 to the protected site) and reciprocally transplanted the remainder to the opposite site. No mating activity was observed during the hours that the arrays were in the lab. This ensured that penis development occurred before the transplant and that mating occurred after the transplant. I had four treatments, each with 14 blocks of five neighbor distances: 1) barnacles that developed in the protected site and mated in the protected site, hereafter "p-p," 2) barnacles that developed in the protected site and mated in the exposed site, hereafter "p-e," 3) barnacles that developed in the exposed site and mated in the protected site, hereafter "e-p" and 4) barnacles that developed in the exposed site and mated in the exposed site, hereafter "e-e." By the end of November mating activity had ceased in natural populations. All surviving barnacles were collected and frozen until they could be examined.

Fertilized eggs masses (*figure 2*) are easily distinguished because they are compressed into two solid pellets at the bottom of the mantle cavity (Barnes et al. 1977; Hoch 2008). Tiles with only one or two surviving barnacles were excluded from analyses. For all others, I measured the aperture radius, the base radius and the height of the shell to approximate the volume of each barnacle (which was calculated as the volume of a truncated cone). I dissected the barnacles under a dissecting microscope and removed the penis. Penises were mounted on glass slides and photographed through a compound microscope (40x magnification) with an attached digital camera. The images were analyzed using the software *ImageJ* (Rasband 1997-2007). I measured the length of each penis and the diameter of each at its base. All measurements were carried out blindly with respect to sample origin and treatment.

Statistical analysis

I calculated the proportion of barnacles with fertilized broods on each tile. Because I was using proportion data, I used an arcsine-square root transformation to remove the dependency of the variance on the values (Sokal and Rohlf 1995). Arrays of tiles were treated as blocks, but I discarded tiles that had only one or two surviving barnacles and blocks that had fewer than three usable tiles. This left between 10 and 14 usable blocks in each treatment (see *figure 3* for sample sizes). I used a general linear model including site of penis development, mating site and the blocked factor, neighbor distance using the following model:

$$y_{ijkl} = \mu + \alpha_i + \gamma_j + \beta_k + \delta_{kl} + \alpha\gamma_{ik} + \alpha\beta_{lk} + \gamma\beta_{jk} + \alpha\gamma\beta_{ijk} + \alpha\delta\beta_{ijkl} + \gamma\delta\beta_{jkl} + \alpha\gamma\delta\beta_{ijkl}$$

where α is the effect of site of penis development, γ is the effect of site of mating, δ is the effect of distance treatment and β is the effect of the block (Cody and Smith 2006; Quinn and Keough 2002). I tested for differences in mating success between the four treatments (e-e, e-p, p-e and p-p) as a result of site of penis development, site of mating and the site of penis development by site of mating interaction using $MS_{\alpha\gamma\beta}$ as the error (Cody and Smith 2006). I tested for differences in ability to fertilize neighbors and a result of neighbor distance, site of origin, site of mating and all possible interactions using $MS_{\alpha\gamma\delta\beta}$ as the error term (Cody and Smith 2006). I used a Student-Newman-Keuls (SNK) Multiple Range Test (Cody and Smith 2006, SAS 9.1.3, SAS Institute 2004) to place the average fertilization success of the four transplant treatments into groups statistically different from one another.

I tested for site-specific phenotypic responses (following Hoch 2008) by comparing the diameter at the base of penises of barnacles that had developed in the protected site (the presumptive protected morphology) with those that developed in the exposed site (the presumptive exposed morphology). Because penis development occurred entirely before the transplant, the mating location was not included as a factor. I only used intact, un-damaged penises from blocks used in analysis of fertilization success. I used a general linear model (SAS 9.13, SAS Institute 2004), including the factors shell volume (to account for variation as a result of differences in body size) and site of development to account for variation in penis diameter. All data were log-transformed. I followed the same procedure to test for differences in length between penises that developed in the different sites. I noted many penises that bore evidence of injury, either broken off at the base or damaged along their length. Because mating activity had occurred entirely after the transplant, damage most likely occurred in the mating location. I tested for differences in penis-breakage rate between the treatments using a general linear model similar to the model used for the fertilization data, including site of penis development, site of mating, block and the proportion of broken penises as the response variable (arcsine-square root transformed, as above). I used a Student-Newman-Keuls (SNK) Multiple Range Test (Cody and Smith 2006, SAS 9.1.3, SAS Institute 2004) to place the average proportion of broken penises in the four transplant treatments into groups statistically different from one another.

Results

Barnacles that developed in the protected site and mated in the protected site (p-p) had relatively high fertilization success at all between-mate distances (*figure 3*). Barnacles that developed in the exposed site and mated in the exposed site (e-e) had relatively high fertilization success at short between-mate distances, but showed a steady decline as distance increased. Barnacles that developed in the exposed site but mated in the protected site (e-p) showed a very similar pattern to those from the e-e treatment: high relative success at short distances and steadily decreasing success with increasing distance. Barnacles that developed in the protected site and mated in the exposed site (p-e) showed intermediate success at short mate-distances with a steady decline in success as distance increased. The general linear model (*table 1*) indicated that significant effects on differences in mating success between blocks were mating site ($F_1 = 16.47$, $p = 0.0002$) and the origin by mating site interaction ($F_1 = 9.83$, $p = 0.0029$). Within blocks, mate distance ($F_4 = 10.30$, $p < 0.0001$) was the only significant effect. The SNK multiple range test showed that across all distances, 'p-p' (untransformed mean = 65.11% fertilized) was significantly different from all other treatments and formed its own group (*A*); 'e-e' (31.1%) and 'e-p' (38.3%) were not significantly different from each other and formed a group (*B*) and 'e-e' (31.1%) and 'p-e' (15.87%) were not significantly different from each other (group *C*).

The average basal diameter of penises (*figure 4*) developed in the exposed site (mean = 0.830 mm, 95% CI = ±0.0381, n = 89) was significantly greater ($F_1 = 5.07$, $p = 0.0255$; *table 2*) than the average of those developed in the protected site (mean = 0.779, 95% CI = ±0.0294, n = 106). Shell volume ($F_1 = 255.71$, $p < 0.0001$) and the interaction between shell volume and site of origin ($F_1 = 6.82$, $p = 0.0097$) also significantly affected penis diameter. The only significant effect on penis length was shell volume ($F_1 = 34.62$, $p < 0.0001$). There were large numbers of damaged penises in all treatments. Of the barnacles that developed in the exposed site and mated in the exposed site (e-e), 45.33% had broken penises (95% CI = ±8.52%, n= 66). 64.71% of penises in the 'e-p' treatment were broken 95% CI = ±8.71%, n= 64), 34.70% were broken in the 'p-e' treatment (95% CI = ±7.56%, n= 67) and 55.68% were broken in the 'p-p' treatment (95% CI = ±8.78%, n= 69). The general linear model identified only site of mating as a significant source of variation (F_1= 14.65, p= 0.0003; *table 3*). The SNK multiple range test showed that 'e-e' and 'p-e' were not significantly different from each other (group A), 'e-e' and 'p-p' were not significantly different from each other (group B) and 'p-p' and 'p-e' were not significantly different from each other (group C) (*figure 5*).

Discussion

The morphology of the penis in barnacles is phenotypically plastic, and this phenotypic plasticity produces a morphology that out-performs the alternate form in a given environment. Barnacles from sites protected from waves develop penises with relatively thinner diameter that are better able to reach distant mates in wave-protected sites than the thicker penises transplanted from exposed sites (significant, SNK test). However, these thinner penises have reduced performance in wave-exposed sites (significant, SNK test). Penises that develop in wave-exposed sites have approximately equal mating ability in wave-exposed sites and protected sites (NS, SNK test), but lower performance in the protected sites than barnacles that are raised and mate in the protected site (significant, SNK test). These comparisons suggest a tradeoff between these two morphologies; penises that develop in the wave-exposed sites are less able to reach distant mates, but maintain mating ability over short distances in wavy conditions. Barnacles from protected sites develop penises capable of reaching mates at great distances, but which do not perform as well in wavy conditions. Mating in the protected site appears to be limited by intrinsic biomechanical limitations on the ability of the penis to stretch, while in the wave-exposed site limitations appear to be imposed by extrinsic physical factors, like wave action.

The barnacles in this experiment have indeed developed the same site-specific morphologies observed in other studies (Hoch 2008; Neufeld and Palmer 2008). Penises that developed in wave-exposed sites had greater diameter than those from protected sites ($p = 0.0255$). A comparison of the two un-transplanted control groups shows that generally, barnacles in protected sites (p-p) are better able to reach mates at a greater distance than those in sites exposed to waves (e-e). This study supports previous results based on observations of barnacles in the field (Hoch 2008) and suggests that the experimental design of this study did not affect the natural mating activities of these barnacles. Between transplant treatments, the proportion of barnacles that were successfully fertilized was significantly affected by the mating site ($p = 0.0002$) and the interaction between mating site and penis development site ($p = 0.0029$). That is, given mating in a specific site, the site where penis development occurred is an important factor the barnacle’s ability to reach mates. Within blocks, only neighbor distance significantly affected the ability of barnacles to mate ($p < 0.0001$); barnacles are more likely to fertilize the broods of closer neighbors.

Plastic morphologies and behaviors increased mating success in the environments where they were expressed. Barnacles that developed the protected-site penis morphology had greater success, at greater mate distances in the protected site (the p-p treatment) than when transplanted to the exposed site (p-e). Within the exposed site, barnacles transplanted from the protected site had slightly poorer (15.2% lower, NS SNK test) performance than the non-transplanted exposed-morphology barnacles (e-e). There are several non-exclusive explanations for this: 1) the protected-site penis morphology may be sub-optimal in an exposed environment and barnacles are physically constrained from reaching distant mates or 2) barnacles transplanted to the exposed site may behave differently in the exposed site and attempt to mate less frequently. Barnacles actively feeding in rough water have been observed to retract into their shells when waves crash (Miller 2007). Barnacles from protected sites may have a lower threshold for wave action and retract into their shells more frequently in the rougher exposed environment, potentially interrupting the mating act or leaving less time available for searching for receptive mates. Barnacles from the ‘p-e’ treatment had by far the lowest proportion of damaged penises (34.7%), suggesting overall reduced activity (and reduced exposure to risk). The thinner penises of these barnacles may also be more easily disturbed during mating activities than the thicker penises of barnacles reared in the exposed site. Beam theory predicts thicker penises should better resist bending due to drag and should thus be more resistant to wave forces (Neufeld and Palmer 2008; Vogel 2003).

Barnacles that develop the exposed-site penis morphology have similar performance whether transplanted to a protected site (e-p) or returned to the exposed site (e-e) (not significantly different, SNK). The ability of barnacles transplanted to the protected site (e-p) to reach mates is poorer than un-transplanted controls from the protected site (p-p) (significantly different, SNK), demonstrating that even with the absence of wave action, they are unable to increase their ability to reach distant mates. There are two possible explanations for this; 1) penises that develop the thicker morphology seen in the exposed sites may not be able to stretch to as great a length as those from protected sites or 2) the transplanted barnacles may behave differently in the site and attempt to mate less frequently. Penis biting predators (Stubbings 1975) may be more common during high tide in the protected site and the exposed-origin barnacles may have increased susceptibility or a lower threshold for avoidance behaviors than those from the protected site. I observed a significantly greater proportion of penis injury in the protected site ($p = 0.0003$), especially in barnacles transplanted from the exposed site (64.7% damaged), indicating that risk of damage may play an important role in limiting mating opportunities. Reduced flexibility and stretch may be necessary to maintain function in the exposed sites, as forces caused by waves may bend, stretch or deform active penises. Material properties and physical constraints of the penis that allow continued function in the exposed site may hinder the ability of barnacle to reach distant mates in the absence of wave action. The penis of the barnacle is extended by hydrostatic pressure (Klepal 1990) and a penis with greater diameter, like those with the exposed morphology, should require a greater volume of fluid to extend it to the same length as a thinner penis. Because penises are approximately conical, all else being equal, given an increase in diameter, the volume required to stretch to a length will increase by the square of that increase. In this study, penises from exposed sites were on average 5.6% greater in diameter than those from protected sites. In order to stretch to a given distance, penises from the wave-exposed site would need 11.5% greater fluid volume than those from the protected site. The penis is so greatly enlarged during the mating season, that this may be a significant portion of the body volume. If the thicker penises observed in exposed sites are tougher or less flexible, they may also require greater internal pressure to extend. Such limitation may constrain the length to which a penis can stretch. Behavioral alterations and physical constraints may act synergistically to reduce the overall distance over which a penis developed in a wave-exposed site can reach.

Phenotypic plasticity of the penis may have important consequences for other aspects of reproduction for these hermaphrodites. First, barnacles at different wave exposures seem to be allocating different amounts of energy and resources to penis development and function (length is unaffected by exposure, but diameter increases, resulting in an increase in total penis size). This investment will directly affect an individual barnacle's mating ability, and may tradeoff with other aspects of its life processes. For example, the penis is located between the feeding cirri. In this study the penises were on average 10.98 times longer than the first cirrus, and it is likely that such a bulky addition to the active portion of the barnacle's body would reduce efficiency. The relative allocation to the sex roles for simultaneously hermaphroditic barnacles is predicted to be driven by mating group size and local mate competition. Specifically, as the number of individuals competing for mates increases, the relative allocation to the male role is predicted to increase towards an asymptote at 50% (Charnov 1980; Charnov 1982). In wave exposed sites, barnacles are not able to reach distant mates as easily as those in protected sites (owing to reduced ability, reduced search time or differing behavior). Individual barnacles will thus have a smaller available mating group, fewer potential mates and will experience less intense mate competition. In such an environment, less allocation to male function is predicted, following Charnov's model. Moreover, barnacles in these exposed sites are presumably investing more into their stouter penises (owing to increased volume). This increased investment into male-specific fixed structures may trade off with sperm production and further reduce mate competition. In the protected site the opposite case exists: barnacles are demonstrating a significant level of control over the size of their mating group. Without variation in the ability to reach distant mates, barnacles would be limited to mating opportunities within a fixed distance. When freed of constraints placed by wave action, barnacles improve their ability to fertilize the broods of increasingly distant neighbors and are actively increasing the intensity of mate competition. This is predicted to translate directly into greater investment into male function. All else being equal, barnacles in exposed sites are predicted to allocate relatively more to the female role.

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Tables

Table 1: Statistical results from the general linear model on fertilization success from the reciprocal transplant experiment. Analyses were performed on arcsine-square root transformed values.

Between site comparisons of fertilization success, using $MS_{\alpha\gamma\beta} = 0.3076$ as the error term

Source	*DF*	*Type III SS*	*MS*	*F Value*	*Pr > F*
Development site	1	0.0882	0.0882	0.29	0.5948
Mating site	1	5.0678	5.0678	16.47	0.0002
Devel*Mate	1	3.0241	3.0241	9.83	0.0029

Within block comparison of fertilization success, using $MS_{\alpha\gamma\delta\beta} = 0.2471$ as the error term

Source	*DF*	*Type III SS*	*MS*	*F Value*	*Pr > F*
Neighbor distance	4	10.1784	2.5446	10.30	<0.0001
Devel*dist	4	1.8645	0.4661	1.89	0.1153
Mate*dist	4	1.5107	0.3776	1.53	0.1963
Devel*mate*dist	4	2.2531	0.5632	2.28	0.0630

Table 2: Statistical results from general linear model of penis basal diameter for barnacles from different sites of origin. Analysis was performed on log-transformed data.

Comparison of penis width from different development sites, using MS_{error} = 0.0151

Source	*DF*	*Type III SS*	*MS*	*F Value*	*Pr > F*
Body volume	1	5.18316325	5.18316325	255.71	<0.0001
Development site	1	0.10278557	0.10278557	5.07	0.0255
Volume*site	1	0.13823674	0.13823674	6.82	0.0097

Table 3: Statistical results from general linear model of proportion of broken penises for barnacles from different treatments. Analyses were performed on arcsine-square root transformed values.

Comparison of penis width from different development sites, using $MS_{\alpha\gamma\beta}$ = 0.4184 as the error term

Source	*DF*	*Type III SS*	*MS*	*F Value*	*Pr > F*
Development site	1	0.94527998	0.94527998	2.26	0.1389
Mating site	1	6.12974989	6.12974989	14.65	0.0003
Devel*Mate	1	0.00007882	0.00007882	0.00	0.9891

Figures

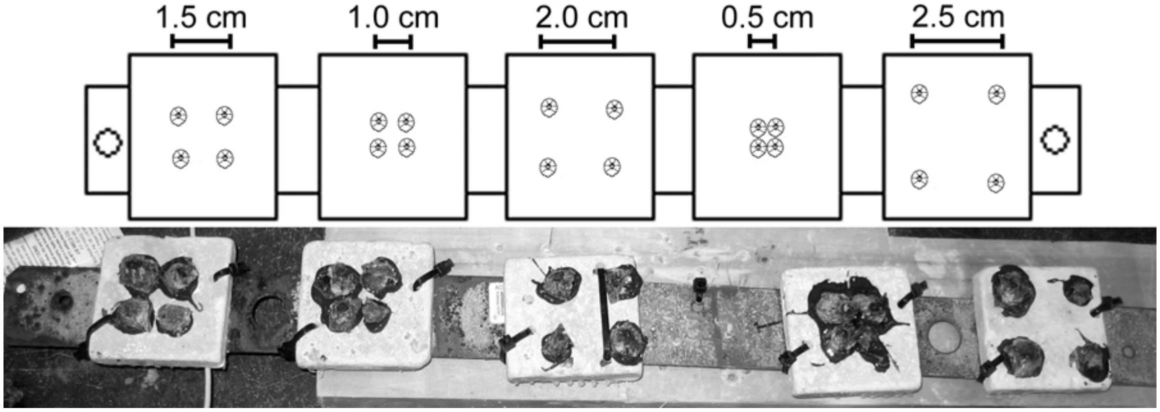

Fig. 1: Schematic of an experimental array showing spacing of individual barnacles and a photograph showing an actual array used in the experiment. Arrays of five tiles were built with four barnacles per tile, with 0.5, 1.0, 1.5, 2.0 or 2.5 cm between individuals. Each barnacle only had potential mates on its own tile. The order of tiles was randomized on each array.

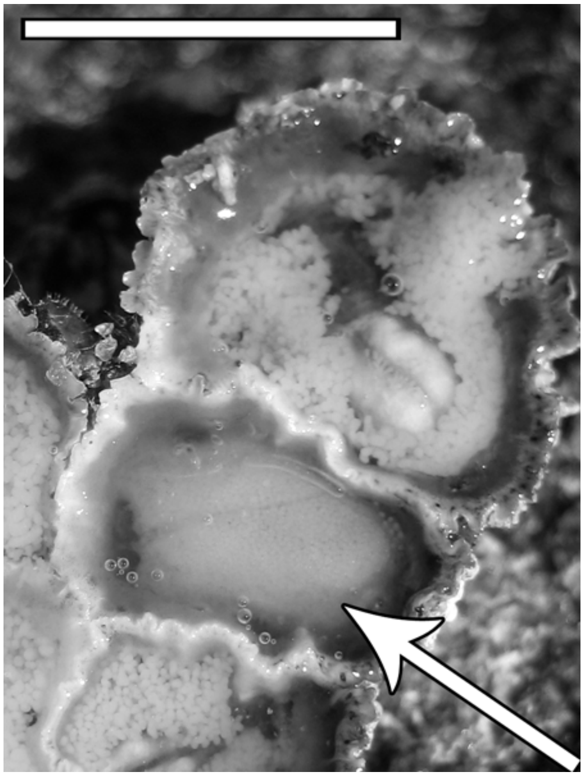

Fig. 2: Several barnacles removed from a rock, with the eggs and testes visible. The middle barnacle (indicated with arrow) has been mated and the fertilized eggs are compressed into two solid pellets. The barnacle above has not been fertilized. The scale bar is 1 cm.

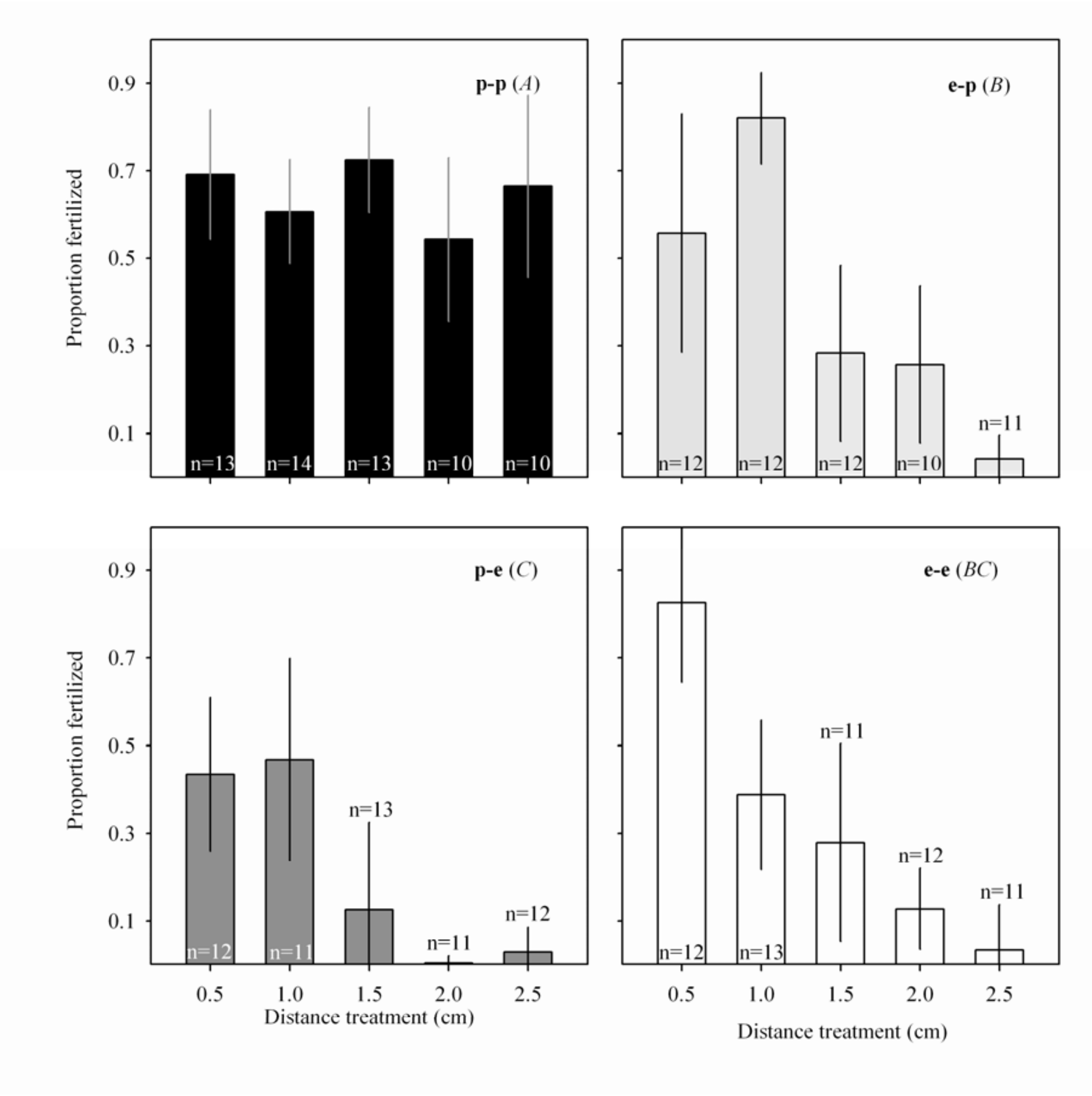

Fig. 3: Fertilization percentages (untransformed) for barnacles from the four treatments of the experiment. The within block neighbor distance treatments are listed on the *x-axes* and proportion fertilized on the *y-axes*. The identity of the treatment is listed in the upper right corner of each graph, with the SNK grouping in parentheses. "p-p" refers to barnacles whose penises developed in the protected site and mated in the protected site, "p-e" refers to barnacles whose penises developed in the protected site and mated in the

exposed site, "e-e" refers to barnacles whose penises developed in the exposed site and mated in the exposed site, and "e-p" refers to barnacles whose penises developed in the exposed site and mated in the protected site. The number of surviving tiles from each neighbor distance grouping (n) is presented on each bar. Error bars show 95% confidence intervals.

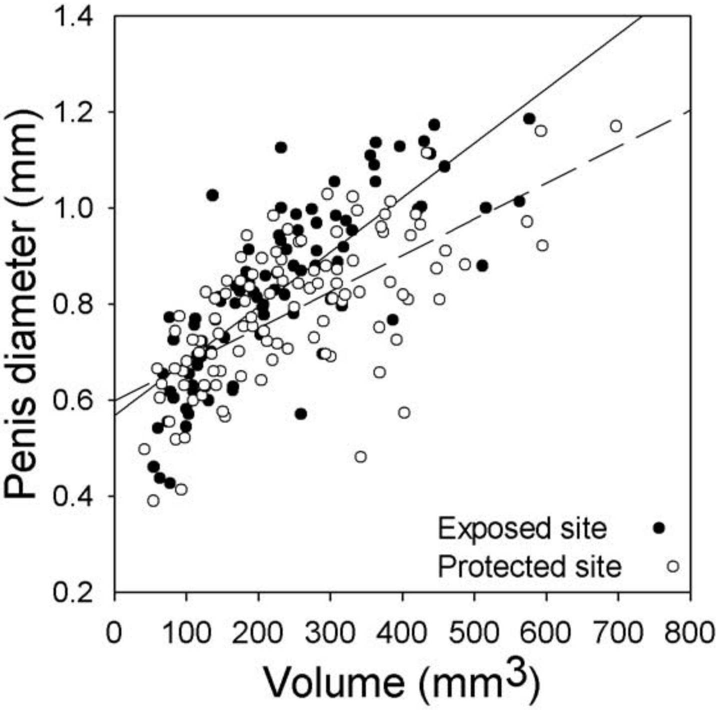

Fig. 4: Untransformed penis basal diameter of barnacles whose penis developed in the exposed site (dark circles) and protected site (open circles) plotted against shell volume. The graph only includes data from penises that were not damaged from barnacles that were included in the fertilization analysis. The solid line represents the best-fit trend-line from the exposed site; the dashed line represents the best-fit trend-line from the protected site.

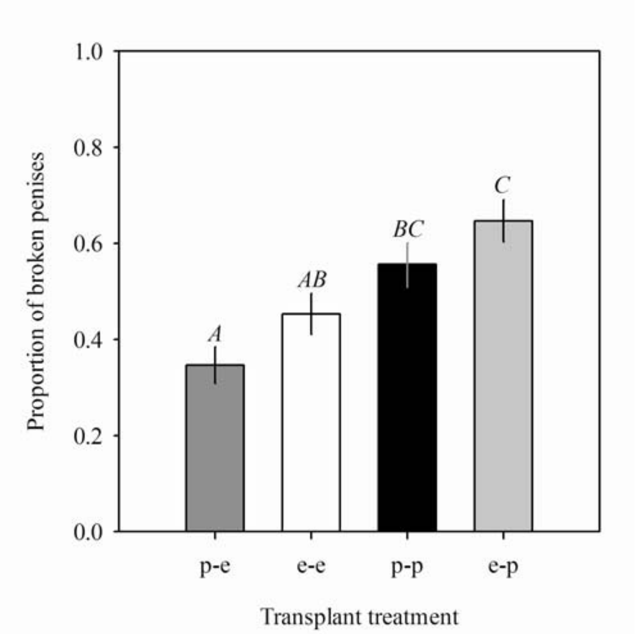

Fig. 5: Untransformed proportions of damaged or broken penises in the four transplant treatments. Letters represent treatments non-significantly different from each other as revealed by the SNK test. Error bars show 95% confidence intervals.

References

Arsenault, D. J., K. B. Marchinko, and A. R. Palmer. 2001. Precise tuning of barnacle leg length to coastal wave action. Proceedings of the Royal Society B: Biological Sciences 268:2149-2154.

Barnes, H., M. Barnes, and W. Klepal. 1977. Studies on the reproduction of cirripedes. I. Introduction: copulation, release of the oocytes and formation of the egg lamellae. Journal of Experimental Marine Biology and Ecology 27:195-218.

Barnes, M. 1992. The reproductive periods and condition of the penis in several species of common cirripedes. Oceanography and Marine Biology 30:483-525.

Charnov, E. 1980. Sex allocation and local mate competition in barnacles. Marine Biology Letters 1:269-272.

Charnov, E. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton, NJ.

Cody, R. P., and J. K. Smith. 2006. *Applied Statistics and the SAS Programming Language*. Pearson Prentice Hall, Upper Saddle River, NJ.

Darwin, C. 1854. *The Balanidae (or Sessile Cirripedes); the Verrucidae, etc.: A Monograph of the Sub-class Cirripedia*. The Ray Society, London, UK.

Denny, M. W. 1987. Life in the maelstrom: The biomechanics of wave-swept rocky shores. Trends in Ecology and Evolution 2:61-66.

Hoch, J. M. 2008. Variation in penis morphology and mating ability in the acorn barnacle, *Semibalanus balanoides*. Journal of Experimental Marine Biology and Ecology 359:126-130.

Klepal, W. 1990. The fundamentals of insemination in cirripedes. Oceanography and Marine Biology 28:353-379.

Klepal, W., H. Barnes, and E. A. Munn. 1972. The morphology and histology of the cirripede penis. Journal of Experimental Marine Biology and Ecology 10:243-265.

Marchinko, K. B. 2003. Dramatic phenotypic plasticity in barnacle legs (*Balanus glandula* Darwin): Magnitude, Age dependence and speed of response. Evolution 57:1281-1290.

Miller, L. P. 2007. Feeding in extreme flows: behavior compensates for mechanical constraints in barnacle cirri. Marine Ecology-Progress Series 349:227-234.

Murata, A., M. Imafuku, and N. Abe. 2001. Copulation by the barnacle *Tetraclita japonica* under natural conditions. Journal of Zoology 253:275-280.

Neufeld, C., and A. Palmer. 2008. Precisely proportioned: intertidal barnacles alter penis form to suit coastal wave action. Proceedings of the Royal Society B: Biological Sciences 275:1081-1087.

Pentcheff, N. 1991. Resistance to crushing from wave-borne debris in the barnacle *Balanus glandula*. Journal of Crustacean Biology 110:399-408.

Quinn, G. P., and M. J. Keough. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.

Raimondi, P. T., and J. E. Martin. 1991. Evidence that mating group-size affects allocation of reproductive resources in a simultaneous hermaphrodite. American Naturalist 138:1206-1217.

Rasband, W. S. 1997-2007. ImageJ. US National Institute of Health, http://rsb.info.nih.gov/ij/, Bethesda MD.

Sokal, R. R., and F. J. Rohlf. 1995. *Biometry, third edition*. W.H. Freeman and Company, New York.

Stubbings, H. 1975. *Balanus balanoides.* Liverpool University Press, Liverpool.

Thompson, T. L., and E. P. Glenn. 1994. Plaster standards to measure water motion. Limnology and Oceanography 39:1768-1779.

Vogel, S. 2003. *Comparative biomechanics: life's physical world*. Princeton University Press, Princeton, NJ.

Chapter 4 - The relationship of crowding and wave exposure to morphological differences in the penises and cirri of the acorn barnacle, *Semibalanus balanoides*

Abstract

Recent work has demonstrated that the penis of the acorn barnacle, *Semibalanus balanoides,* varies with wave exposure and with characteristics of the mating neighborhood. Barnacles in wave-exposed sites grow penises with thicker diameters than those in protected sites and barnacles in dense aggregations have functionally shorter penises than those from sparse groups (based on the number of annulated folds in the cuticle). I used field experiments to determine if differences in penis length were a response to crowding, neighbor distance or potential mate number. Crowding was the only factor shown to have an important effect on penis length. I also compared morphology of the penis and the feeding cirri across a gradient of wave exposure. As has been found in other species, cirri form was related to wave exposure.

Introduction

Acorn barnacles are obligate-outcrossing, simultaneous hermaphrodites that reproduce by copulating (or more accurately, pseudo-copulating) with their neighbors. Owing to their sessile lifestyle, they accomplish this with a long, flexible penis (Darwin

1854), which they also may use as a sensory organ during the search for mates (Munn et al. 1974). For most of the year, the penis is degenerate and non-functional; penises are rapidly developed over a short time before the reproductive season (Klepal et al. 1975, reviewed in Barnes 1992). The penis has an annulated exoskeleton (with accordion-like folds), allowing it to stretch to many times its relaxed length (Klepal et al. 1972). Mating occurs when an individual becomes receptive as a functional female (Barnes et al. 1977). Neighbors are most likely attracted to chemical signals from functional females and search using the sensory penis (Munn et al. 1974). The penis is inserted into the mantle cavity of the functional female, where sperm is deposited and fertilization occurs (Barnes et al. 1977, Klepal et al. 1977, Klepal 1990). Copulation may last for up to several minutes (Murata et al. 2001). After fertilization, embryos are brooded in the mantle cavity until they are released as nauplius larvae. The North Atlantic acorn barnacle, *Semibalanus balanoides*, mates in the fall and broods it larvae until their release in mid-winter, which settle as cyprids in the spring (Crisp and Patel 1958, Stubbings 1975, Hoch 2008).

Recent work on mating behaviors of acorn barnacles has yielded some interesting information on functional morphology of the barnacle penis. *S. balanoides* growing in densely crowded groups have penises with significantly fewer annulations and are inferred to have reduced capacity to stretch than those from sparsely crowded groups (Hoch 2008). *S. balanoides* from wave-exposed sites also grow penises with a greater basal diameter than those from sites protected from waves (Hoch 2008). This pattern is also present in the Pacific acorn barnacle, *Balanus glandula*, which grows a shorter, thicker, more massive penis in wave-exposed conditions (Neufeld and Palmer 2008).

Experimental work has shown that this is the result of phenotypic plasticity in response to wave action and that the observed morphologies are adaptively matched to the environments in which they are found (Neufeld and Palmer 2008, Hoch 2009). These patterns of variation in penis morphology are similar to patterns of phenotypic plasticity observed in the feeding cirri of several species of barnacles (Arsenault et al. 2001, Marchinko 2003).

I used field experiments to test several hypotheses concerning the functional morphology of penises and cirri for the North Atlantic acorn barnacle, *S. balanoides*. First, I sought to determine whether population factors, such as aggregation density and neighborhood size contribute to plasticity in penis length. Previous work showed a difference in annulation number between densely crowded and sparse aggregations (Hoch 2008). However, the barnacles from that study could have been responding to a number of cues, including physical contact between neighbors, the number of neighbors within the immediate vicinity or the distance to near mates. I set up factorial experiments in several sites, creating artificial mating groups with varying numbers of mates available and differing levels of crowding to test if either factor was significantly related to penis length. At the same time, I measured the distances between potential mates to determine if that affected penis length. The sites that I used also varied in wave exposure. I tested whether wave exposure affected penis diameter, penis mass and cirri length. The relationship between cirrus size and penis size was also examined. These may be correlated or they may tradeoff with the two structures, as both are located at the distal segment of the barnacle's body and are subject to similar risks when extended into the water column.

Materials and Methods

The common intertidal barnacle on Long Island, NY, USA, *Semibalanus balanoides* was the focus of this research. In the late summer of 2005 I created experimental mating aggregations in the intertidal zone at six sites on Long Island: Stony Brook Harbor (40°55'17.20"N, 73° 8'59.06"W), Port Jefferson Harbor (40°56'46.96"N, 73° 4'19.29"W), Cedar Beach Dock (40°57'54.59"N, 73° 2'33.56”W), Flax Pond Inlet Jetty (40°58'1.59"N, 73° 8'16.01"W), Shinnecock Inlet Jetty (40°50'18.73"N, 72° 28'29.44"W), Robert Moses Beach Jetty (40°37'13.11"N, 73° 18'23.23"W). Barnacles do not begin development of their penis and other reproductive tissue until mid-September, so there was ample time for condition-specific phenotypes to develop (Barnes 1992, Hoch 2008). Patches were manipulated in the middle third of the vertical range of barnacles in the intertidal zone to hold tidal height and emersion time constant among sites. At each site I haphazardly set up plots divided into four quarters. I selectively removed individuals from these quarters, with two factors in mind: the number of individuals and the level of crowding. I made treatments of high numbers (greater than ten individuals) and low numbers (fewer than four individuals) and densely crowded (the tests of individual barnacles contact one another) or sparse groups (no physical contact between neighbors). Barnacles in the densely crowded treatments made physical contact with the tests of neighbors, that is, the test of each barnacle grew together with the test of more than one neighbor. This resulted in four treatments (figure 1) in each plot: high numbers of individuals, densely crowded (the HD treatment), high numbers of individuals, sparsely crowded (the HS treatment), low numbers of individuals, densely crowded (the LD treatment) and low numbers of individuals, sparsely crowded (the LS treatment). The barnacles were then left alone and allowed to develop until their reproductive season. The sites were located over a gradient of wave exposures. Shinnecock Inlet Jetty and Robert Moses Beach Jetty are both on the exposed shore of the Atlantic Ocean and are almost constantly exposed to waves. Port Jefferson Harbor and Stony Brook Harbor and in protected embayments of Long Island Sound and are very infrequently exposed to large or constant waves. Cedar Beach Dock and Flax Pond Inlet Jetty are both exposed to the waves of Long Island Sound and appeared to be intermediate wave exposure. I quantified relative wave exposure by comparing the loss of mass of plaster spheres. Mass loss is positively correlated with water motion (Thompson and Glenn 1994), but can also be affected by temperature, sediment scour and water chemistry. In the week before I began collecting barnacles (when penises are fully developed and weather conditions are similar to that of the mating period), I bolted six 3-cm plaster spheres to the rocks among the barnacle treatments at all of the sites except Cedar Beach Dock, where storms had buried the plots with sand and pebbles, scouring all the barnacles. This site was abandoned. Plaster spheres were left in the intertidal zone for six high tides (approximately three days), after which they were dried and weighed to determine the percent of plaster lost from each.

On Long Island, *S. balanoides* begin to mate in mid-November (Stubbings 1975, Barnes 1992). Between 2 November and 8 November 2005 I collected barnacles from the four treatments in each plot and preserved them in 70% ethanol. I measured the distance from each barnacle's aperture to that of its nearest neighbor as a measure of the smallest possible distance over which it could mate. I estimated the size of its mating group by counting the number of neighbors within 2.5 cm of the focal individual (which is about the limit of penis reach for this species; Hoch 2008). Overall, I collected 350 barnacles from the five sites (Flax Pond: 15 from HD, 33 from HS, 13 from LD, 28 from LS; Moses: 13 from HD, 16 from HS, 15 from LD, 18 from LS; Port Jefferson Harbor: 14 from HD, 16 from HS, 16 from LD, 14 from LS; Stony Brook Harbor: 15 from HD, 22 from HS, 16 from LD, 21 from LS; Shinnecock: 16 from HD, 17 from HS, 15 from LD, 17 from LS). Not all of these were used in each analysis (see results for samples sizes in each test).

In the laboratory I measured the height and the radii if the aperture and base of each barnacle's test. Test volume was estimated by considering it as a truncated cone (following Hoch 2008). I dissected the penis and one of the central cirri (cirrus number six, following Marchinko 2003) from each barnacle, wet-mounted them on glass slides and photographed them through a compound microscope at 40x magnification (figure 2). Penises were dried at 50° C for 24 hours and mass was determined with a fine-scale microbalance (accurate to 0.001 mg). Photographs were analyzed with ImageJ (Rasband 1997-2007). I measured the length along the center axis of each penis, the diameter of each penis at its base (point of attachment to the body) and the length of each cirrus. For the images that were of high enough quality (N = 291; 62.5% of those photographed), the number of annulations were counted as an alternate measure of length. Preservation, dissection and handling commonly damage or stretch the penis; the number of annulations represents a convenient estimation of penis length and ability to extend that is not affected by preparation (Barnes 1992, Hoch 2008).

Statistical Analysis

I used a general linear model and Tukey HSD test to compare the percent mass lost from each plaster sphere in each site (using the arcsin-square root transformation, as suggested for percentage data by Sokal and Rohlf 1995). All morphological data (including test volume, penis length, annulation numbers, penis diameter and cirrus length) were log-transformed prior to analysis in SAS (SAS 9.2; SAS Institute 2003-2008). I used two-way ANCOVA with test volume as covariate, to test whether penis length varied in response to the main effects, number of individuals in the mating group and level of crowding. Both the absolute penis length (in mm) and number of annulations were tested in this way. A Tukey-Kramer post-hoc test was used to determine which treatments were significantly different from each other. I used regression analysis to determine the relationship between penis length (measured both in mm and as number of annulations) and neighbor distance and another to determine the relationship between the residual variation of penis length (with the effect of body size removed) when plotted against neighbor distance. I used ANCOVA with test volume as covariate to examine the effects of wave exposure on penis diameter and penis mass. I used Tukey-Kramer post-hoc tests to determine which levels of wave exposure had penises of significantly different diameters and masses. I used model II (RMA) regression to assess the relationship between penis mass and penis volume (calculated by estimating the penis as a cone (volume = $(\pi r^2 h) / 3$). I could not use ANCOVA with body volume as a covariate to assess the effects of water motion on cirral length because the slopes were not homogenous. However, slopes were homogenous when separated to individual sites, so I compared cirri length between sites. I used a Tukey-Kramer post-hoc test to determine which sites had average cirri lengths that were different from each other. Finally, I tested for a tradeoff between cirrus length and penis volume by plotting the residuals of cirrus (on test volume) against the residuals of penis volume (also on test volume) and testing the relationship using model II (RMA regression).

Results

The differences in plaster dissolution rates confirmed that the sites differed in water motion (figure 3). The Tukey HSD test indicated that Robert Moses Beach jetty mean = 99.6%, 95% C I= +/- 3.3%, N = 6) and Shinnecock Inlet jetty (mean = 95.4%, 95% CI = +/- 14.1%, N = 6) did not differ significantly in water motion ($p < 0.05$), and that Shinnecock Inlet Jetty and Flax Pond Inlet Jetty (mean = 83.7%, 95% CI = +/- 6.9%, N = 6) did not differ significantly in water motion ($p < 0.05$). Stony Brook Harbor (mean = 58.6%, 95% CI = +/- 14.4%, N = 5) and Port Jefferson Harbor (mean = 25.2%, 95% CI = +/- 3.7%, N = 6) each had progressively less loss of plaster mass and were significantly different from all other sites. For some analyses, I grouped Robert Moses Beach jetty and Shinnecock Inlet jetty (the two sites on the ocean) as “exposed,” Flax Pond Inlet jetty (on Long Island Sound) as “intermediate” and Stony Brook Harbor and Port Jefferson Harbor (the two sites within harbors) as “protected.”

Penis length measured in millimeters did not vary with numbers of neighbors (two-way ANCOVA, $p = 0.5668$), crowding ($p = 0.8357$) or the interaction between number and crowding ($p = 0.6518$). However, when annulation number (figure 4) was used as the measure of length, there was a significant effect of crowding ($p < 0.0001$).

The number of neighbors ($p = 0.4551$) and the interaction ($p = 0.9667$) were still not significant. The Tukey-Kramer post-hoc test showed that the annulation number of the HS treatment (back-transformed least square mean = 222.92, 95% CI = +/- 6.51, N = 52) was significantly greater than that of the HD treatment (back-transformed l. s. mean = 204.69, 95% CI = +/- 6.58, N = 43; $p = 0.0009$) and the LD treatment (back-transformed l. s. mean = 206.89, 95% CI = +/- 6.23, N = 49; $p = 0.0031$) (which were not significantly different from each other; $p = 0.9641$). The annulation number of LS treatment (back-transformed l. s. mean = 225.60, 95% CI = +/- 6.24, N = 58) was also significantly greater than the HD ($p < 0.0001$) and LD treatments ($p = 0.0003$) and was not significantly different than the HS treatments ($p = 0.9363$). The slope of regression of penis length in millimeters on neighbor distance was not significantly different from zero (slope = 0.00776, $p = 0.6682$, $r^2 = 0.0007$). The slope of the regression of annulation number of neighbor distance was positive and significantly different from zero (slope = 0.03384, $p < 0.0001$, $r^2 = 0.0930$). There was no relationship between penis length (mm or annulations) and wave exposure.

Penis diameter (figure 5) was significantly affected by wave exposure (ANCOVA, $p < 0.0001$). Barnacles from wave-exposed sites (back-transformed l. s. mean = 0.725 mm, 95% CI = +/- 0.021 mm, N = 113) had significantly greater (Tukey-Kramer, $p < 0.0001$) penis diameter than those from protected sites (back-transformed l. s. mean = 0.652 mm, 95% CI = +/- 0.019 mm, N = 106). Interestingly, penises from the intermediate site (back-transformed l. s. mean = 0.714 mm, 95% CI = +/- 0.026 mm, N = 67) were not significantly different than those from the most wave-exposed sites ($p = 0.7831$), but were different than those from the protected site ($p = 0.0005$).

Penis mass (figure 6) was also significantly affected by water motion (ANCOVA, $p < 0.0001$). Penis mass was greatest at the intermediate site (back-transformed l. s. mean = 0.0957 mg, 95% CI = +/- 0.0138 mg, N = 71) and significantly greater (Tukey-Kramer, $p = 0.0199$) than those from the protected site (back-transformed l. s. mean = 0.0745 mg, 95% CI = +/- 0.0084 mg, N = 115). Penises from the exposed site (back-transformed l. s. mean = 0.0863 mg, 95% CI = +/- 0.0097 mg, N = 118) did not differ from those from either the intermediate ($p = 0.5045$) or protected site ($p = 0.1712$).

The model II (RMA) regression of penis mass against penis volume showed a significant positive relationship (slope = 1.091, 95% CI = +/- 0.095, $r^2 = 0.4816$). The regression of the residuals showed a weak positive relationship between the cirri length and penis volume (slope = 2.826, 95% CI = +/- 3365, $r^2 = 0.02255$).

The slopes of cirral length against body volume (figure 7) for the three levels of exposure were significantly different from each other ($p < 0.0001$), so ANCOVA could not be performed. However, when considered by site rather than exposure level, the slopes were homogenous ($p = 0.4183$). ANCOVA showed that both test volume ($p < 0.0001$) and site ($p < 0.0001$) significantly contributed to variation in cirral length. The Tukey-Kramer post-hoc tests showed that the longest cirri were found in Port Jefferson Harbor (back-transformed l. s. mean = 2.97 mm, 95% CI = +/- 0.144 mm, N = 39), which were significantly larger than cirri from all other sites (P.J.H.-Flax $p = 0.0349$, P.J.H.-Moses $p < 0.0001$, P.J.H.-S.B.H. $p < 0.0001$, P.J.H.-Shinn. $P = 0.0002$). The cirri of barnacles from Flax Pond (back-transformed l. s. mean = 2.72 mm, 95% CI = +/- 0.095 mm, N = 69) and Shinnecock Inlet (back-transformed l. s. mean = 2.59 mm, 95% CI = +/- 0.095 mm, N = 62) were not significantly different from each other ($p = 0.3946$), but they were different than all other sites (Flax-Moses $p < 0.0001$, Flax-S.B.H. $p < 0.0001$, Shinn.-Moses $p < 0.0001$, Shinn.-S.B.H. $p < 0.0001$). Finally, the shortest cirri were found in Robert Moses Beach jetty (back-transformed l. s. mean = 2.21 mm, 95% CI = +/- 0.094 mm, N = 49) and Stony Brook Harbor (back-transformed l. s. mean = 2.25 mm, 95% CI = +/- 0.096 mm, N = 51); they were not significantly different from each other ($p = 0.9757$), but were significantly different than all other sites.

Discussion

The number of annulations in the penis were phenotypically plastic and responded primarily to the density of crowding. Barnacles in densely crowded groups grew penises with fewer annulations than those from sparse groups. Although not shorter in their relaxed state, penises from animals grown in crowded condition probably have a reduced ability to stretch owing to fewer folded annulations in the cuticle. Penis length and annulation number did not change in response to the number of neighbors and showed a weak, although significant response to the distance of the nearest neighbor. The density of crowding must be a reliable cue for estimating mate distance, as barnacles whose tests touch those of their neighbors always had a shorter distance over which to reach mates. Increasing the number of annulations of the penis most likely allows mating barnacles to stretch to more distant neighbors, allowing greater opportunity for mating. Densely crowded barnacles may have diminishing marginal returns as mate number increases, so increasing the ability to reach would not increase fitness. Potential costs of a longer penis, such as increased risk of damage or greater metabolic effort, may also restrain densely crowded barnacles from growing penises with more annulations. At the extreme levels of dense settlement, barnacles form "hummocks;" tests of neighbors grow together and grown into tall columns rather than short cones, eventually getting wider at the aperture, forming trumpet-like shapes (Barnes and Powell 1950, Crisp and Barnes 1954). These hummocked barnacles have different proportions of soft-tissue to test and different growth rates, owing to neighbors sharing skeletal elements and growth in test height outstripping somatic growth (Wu et al. 1977, Wethey 1984, Bertness et al. 1998). For this reason, I intentionally avoided barnacles showing the hummock growth form. Hummocked barnacles would have an even greater number of neighbors within a very short distance, so I predict even fewer penis annulations would be found in barnacles from such aggregations. Penis length (whether measured in millimeters or by annulation number) did not change with wave exposure, matching the results of previous studies (Hoch 2008, Hoch 2009), although it is know from those studies that functional ability to reach mates does change with wave exposure.

The results of this experiment also confirm the results of prior studies showing a difference in penis basal diameter among sites varying for water motion (Hoch 2008, Neufeld and Palmer 2008). In areas protected from wave activity barnacles produce thinner penises that are able to reach more mates in wave protected sites (Hoch 2009). For another barnacle, *Balanus glandula,* penises were shown to be more massive in wave-exposed sites than in protected sites (Neufeld and Palmer 2008). For *S. balanoides*, animals exposed to greater water motion did not produce penises with increased mass than those from protected sites. However, barnacles from intermediate sites did produce penises with greater mass than the protected site. Other factors, such as wave period may vary among sites and be important for development of penis mass. Cirri length did not vary with wave exposure as would have been initially expected based on work from other species (Arsenault et al. 2001, Marchinko 2003). Barnacles from sites of greater relative exposure produced shorter cirri on average. The exception was the barnacles from Stony Brook Harbor, which had cirri as short as those from the site of greatest exposure, Robert Moses Beach jetty. A possible explanation for this is differences in barnacle feeding behavior related to differences in flow regime, but unrelated to wave exposure. Barnacles in Stony Brook Harbor are somewhat protected from Long Island Sound by seawalls, floating docks and islands and experiences little wave action except during storms. However, because of its location on a channel between the main body of Stony Brook Harbor and Smithtown Bay, it experiences very strong tidal flow on both the falling and rising tides. Differences in feeding behavior of barnacles under different flow conditions are well documented. In slow currents, barnacles create their own flow through cirral beating and pumping; in stable, faster currents they switch to cirral extension (Crisp and Southward 1961). During the most extreme wave events barnacles retreat into their tests and do not attempt to feed at all (Miller 2007). Indeed these different feeding behaviors have been associated with differences in size of the cirral net (Crisp and MacLean 1990). The clear inference is that barnacles at the Stony Brook Harbor site obtain the majority of their food through passive capture with extended cirri during the periods of strong tidal exchange. Relatively shorter cirri will reduce the metabolic demand of extension in this steady flow, capture more than enough particles to sustain growth and reproduction and reduce the risks and metabolic costs of increased, unnecessary drag.

I also tested the hypothesis that there is a relationship between penis and cirri morphology. The penis is attached at the distal end of the barnacle's body directly in between the central feeding cirri. The rapid growth and loss of the penis suggests that there was a strong cost to penis presence (Barnes 1992, Hoch 2008). There may be a developmental tradeoff between the two, a tradeoff as a result of metabolic limitation or increased costs owing to greater drag. Conversely, hindrance of feeding behavior owing to the presence of the large penis' presence may necessitate larger cirri to maintain rates of food capture. However, neither regressions of cirri size against penis volume, nor regressions of the residuals of the two (accounting for body size) suggest either a positive or negative relationship between penis and cirral form. There is evidence that during reproductive periods, barnacles forfeit much growth and maintenance that they enjoy for the majority of the year; after copulating, they generally do not molt until their broods are released (Crisp and Patel 1958), despite molting about every two weeks for the rest of the year (Barnes and Barnes 1982). Barnacles may sustain themselves during their reproductive period with lowered activity, reduced energy demand and with stored reserves.

Barnacles are important model organisms for studies of sex allocation in hermaphrodites (Charnov 1980, Charnov 1982, Raimondi and Martin 1991). This research highlights phenotypically plastic allocation to a fixed male-specific cost, the penis, which is necessary for reproduction (Heath 1977, Charnov 1982). Penises from this study were on average 9.8% of the mass of the total male system (sum of sperm, testes, penis etc.), a non-trivial amount. Further, penises define the limits of the mating group of barnacles. That is, all neighbors within reach of a focal barnacle's penis and all of those that can reach it form its mating group. Relative allocation to male function in simultaneously hermaphroditic acorn barnacles is predicted to be driven by sperm competition with other members of the mating group. As mating group size increases, barnacles are predicted to allocate a greater proportion of resources to male function, towards an asymptote at 50% (Charnov 1980, Charnov 1982). This study did not identify a tradeoff between penis and cirri form, even though it initially seemed likely. It is likely that despite the lack of a tradeoff, the presence of the penis does hinder other aspects of the barnacles' functions. Opportunity costs associated with building the penis, such as reduced scope for growth, reduced feeding efficiency and cessation of feeding and molting are very likely to be imposed by the presence of the penis. With more research on metabolic and physical constraints imposed by the penis, these costs of reproduction could be fruitfully incorporated into future models of the costs of sex and costs paid for male specific function in hermaphrodites.

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Table 1: Statistical tables

Results of two-way ANCOVA

Variation in penis length (mm) with factors of number of neighbors and level of crowding

Source	DF	SS	MS	F Value	Pr > F
Volume	1	4.15794825	4.15794825	49.00	<0.0001
Number	1	0.02790882	0.02790882	0.33	0.5668
Crowding	1	0.00365517	0.00365517	0.04	0.8357
Number*Crowding	1	0.01731873	0.01731873	0.20	0.6518

Results of two-way ANCOVA

Variation in penis length (annulations) with factors of number of neighbors and level of crowding

Source	DF	SS	MS	F Value	Pr > F
Volume	1	0.28658413	0.28658413	25.14	<0.0001
Number	1	0.00638377	0.00638377	0.56	0.4551
Crowding	1	0.36847740	0.36847740	32.33	<0.0001
Number*Crowding	1	0.00001996	0.00001996	0.00	0.9667

Results of ANCOVA

Variation in penis diameter (mm) with factor of wave exposure

Source	DF	SS	MS	F Value	Pr > F
Exposure	2	0.68361211	0.34180605	14.53	<0.0001
Volume	1	4.06616237	4.06616237	172.89	<0.0001

Results of ANCOVA

Variation in penis mass (mg) with factor of wave exposure

Source	DF	SS	MS	F Value	Pr > F
Exposure	2	2.92454822	1.46227411	3.90	0.0214
Volume	1	32.42858831	32.42858831	86.41	<0.0001

Results of ANCOVA

Variation in cirri length (mm) with factor of site

Source	DF	SS	MS	F Value	Pr > F
Site	4	2.84414714	0.71103679	33.11	<0.0001
Volume	1	2.26490634	2.26490634	105.48	<0.0001

Results of GLM

Variation in percent mass of plaster loss at each site

Source	DF	SS	MS	F Value	Pr > F
Site	4	3.62279896	0.90569974	51.48	<0.0001

Figures

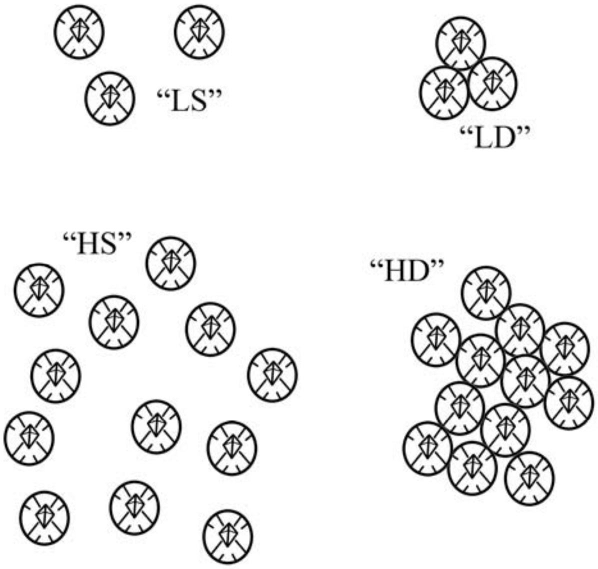

Fig. 1: Schematic of the four treatments from the experiment: Low numbers of individuals, sparsely aggregated (LS), low numbers of individuals, densely aggregated (LD), high numbers, sparsely aggregated (HS) and high numbers, densely aggregated (HD). Each plot contained one of each treatment, haphazardly positioned.

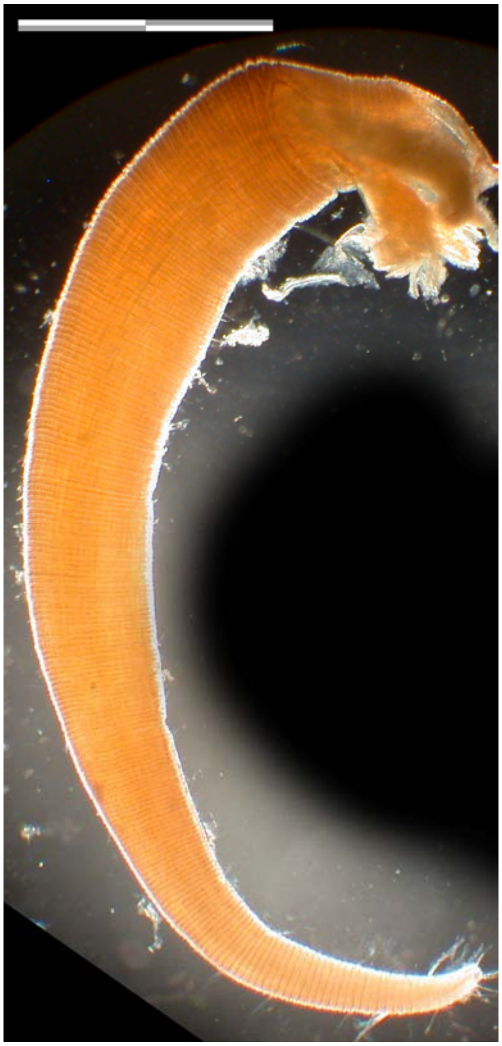

Fig. 2: A penis dissected from *Semibalanus balanoides*. Note the annulations (ring-like folds) down the length of the penis. The scale bar is one millimeter.

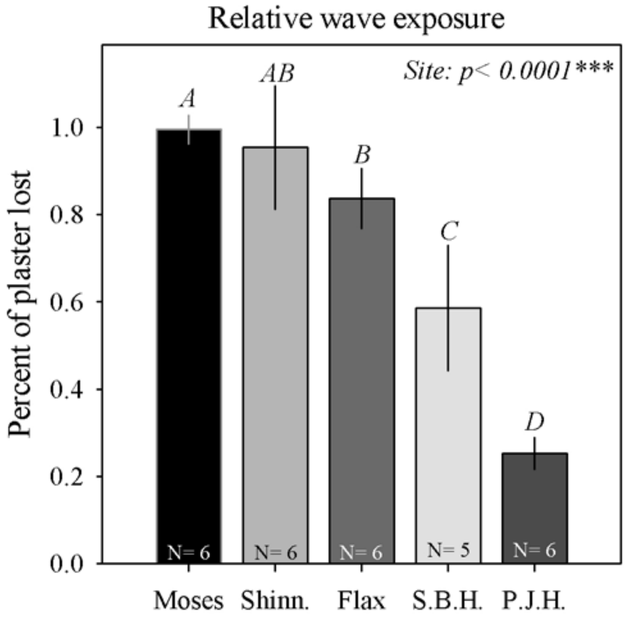

Fig. 3: Average percent plaster mass lost in each site as a proxy for relative water motion in each site. Error bars show 95% confidence intervals. Bars labeled with the same letter are not significantly different from each other. Sample size is listed in the bar for each site. Raw data is shown, although transformed data was used for statistical analysis.

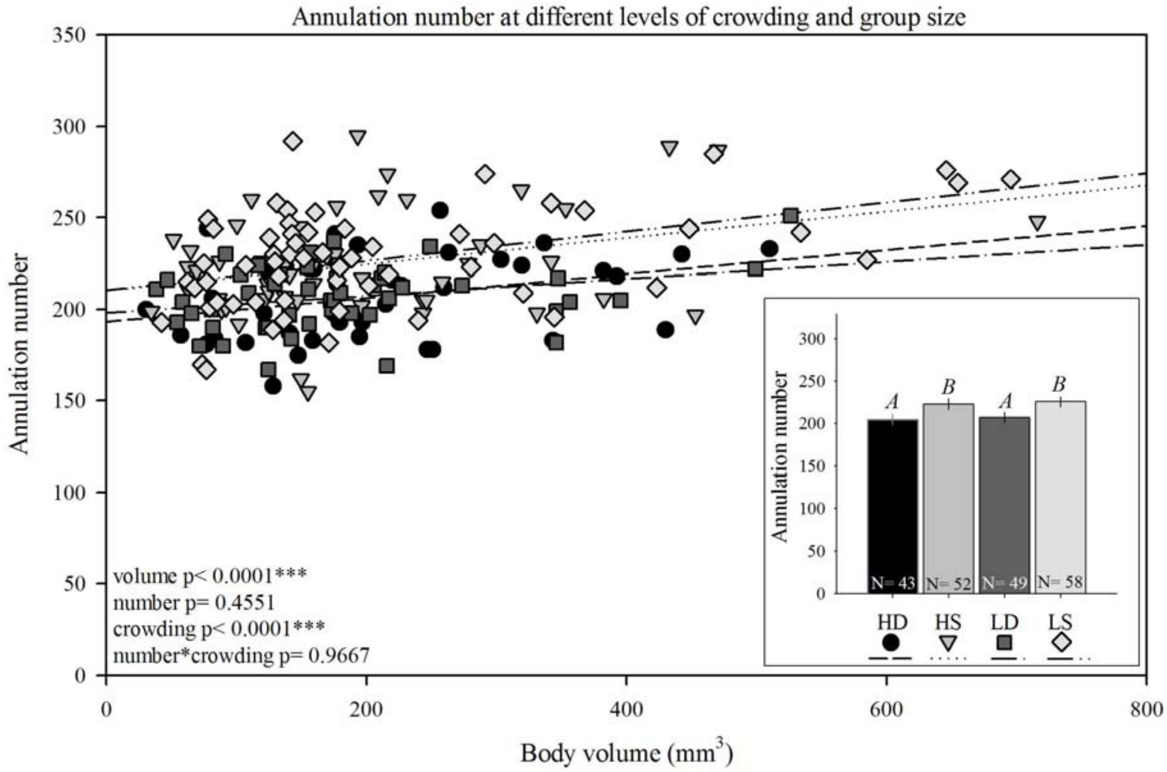

Fig. 4: The number of annulations on the penis plotted against test volume for the four experimental treatments. The lines represent the best fit line for the high number, densely crowded treatment (HD- dashed), the low number, sparsely crowded treatment (LD- dash and one dot), the high number, sparsely crowded treatment (HS- dotted) and the low number, sparsely crowded treatment (LS- dash with two dots). Raw data are shown, although transformed data were used for statistical analysis. Inset: Back-transformed least square means of the number of annulations on the penis plotted for the four experimental treatments. Error bars show 95% confidence intervals. Different letters above each bar indicate that values are significantly different (Tukey-Kramer $p < 0.05$). Sample size is listed in the bar for each site.

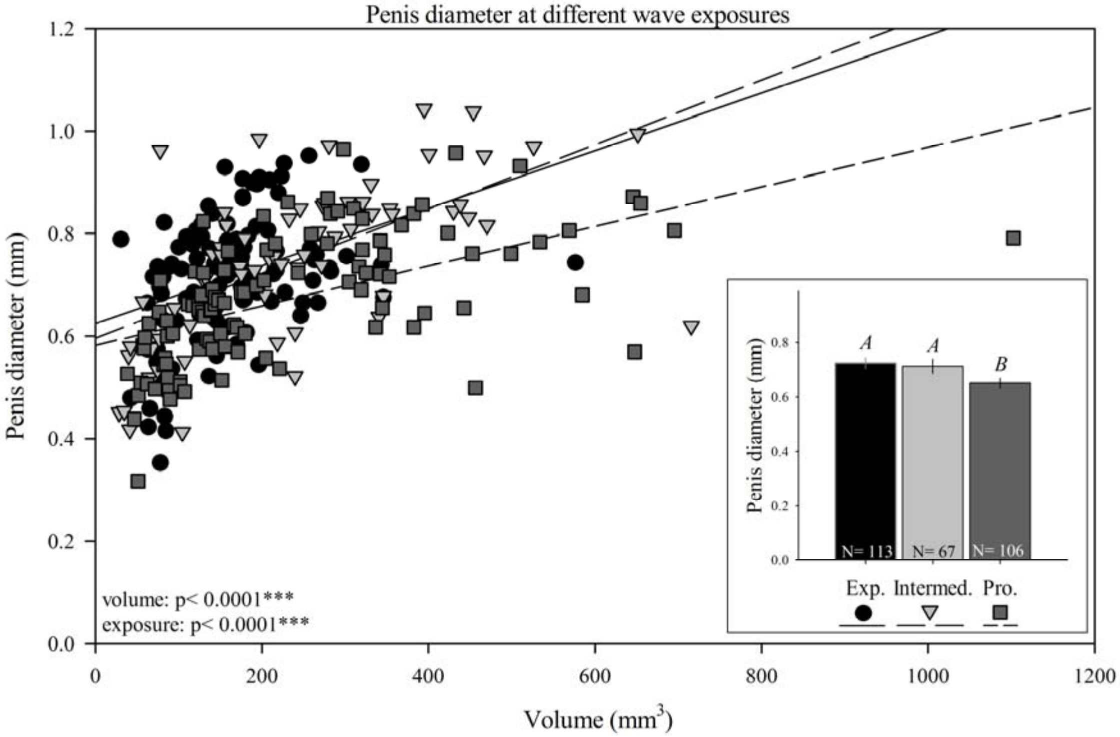

Fig. 5: Penis diameter at its base plotted against test volume. The lines represent the best fit line for the exposed sites (Exp.- solid), the intermediate sites (Intermed.- long dashes) and the the protected sites (Pro.- short dashes). Raw data is shown, although transformed data were used for statistical analysis. Inset: Back-transformed least square means of penis diameter from sites differing for wave exposure. Error bars show 95% confidence intervals. Different letters above each bar indicate that values are significantly different (Tukey-Kramer $p < 0.05$). Sample size is listed in the bar for each site.

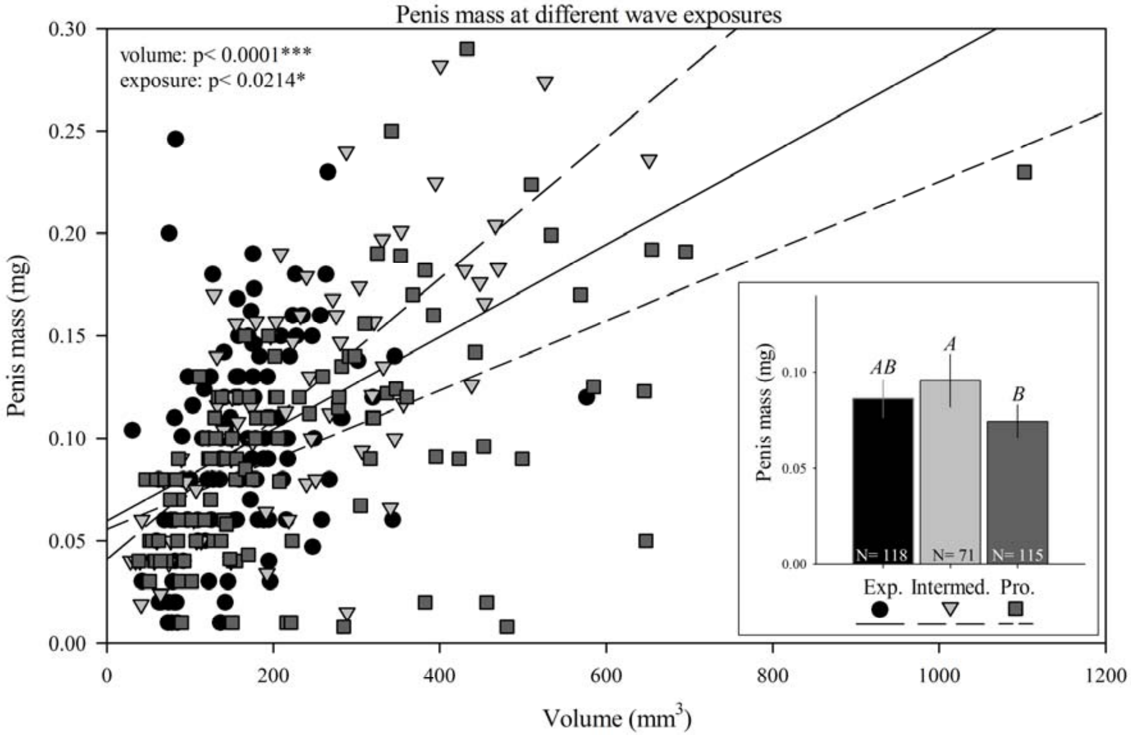

Fig. 6: Penis mass (mg) plotted against test volume. The lines represent the best fit line for the exposed sites (Exp.- solid), the intermediate sites (Intermed.- long dashes) and the protected sites (Pro.- short dashes). Raw data are shown, although transformed data were used for statistical analysis. Inset: Back-transformed least square means of penis mass from sites differing for wave exposure. Error bars show 95% confidence intervals. Different letters above each bar indicate that values are significantly different (Tukey-Kramer $p < 0.05$). Sample size is listed in the bar for each site.

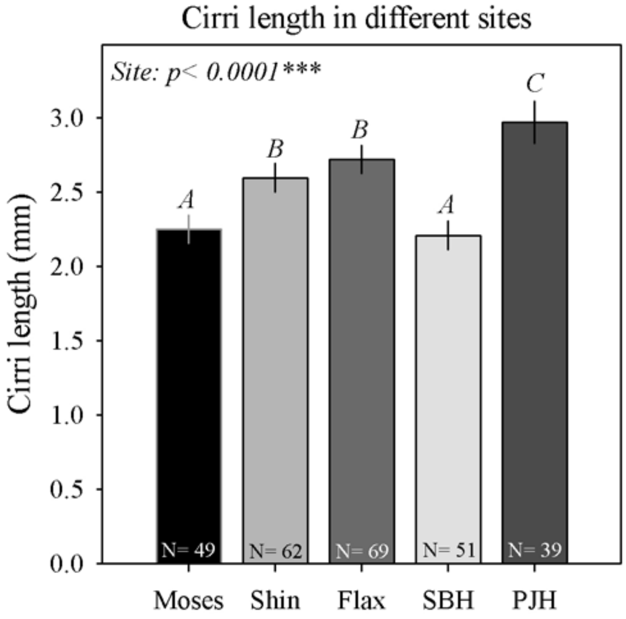

Fig. 7: Least square means of cirral length for each site. Error bars show 95% confidence intervals. Letters above each bar indicate that its values are significantly different from bars labeled with different letters. Sample size is listed in the bar for each site. Raw data are shown, although transformed data was used for statistical analysis.

References

Arsenault, D. J., K. B. Marchinko, and A. R. Palmer. 2001. Precise tuning of barnacle leg length to coastal wave action. Proceedings of the Royal Society B: Biological Sciences **268**:2149-2154.

Barnes, H. and M. Barnes. 1982. Effect of turbulence on the feeding and molting of the cirripede *Balanus balanoides* (L) given and algal diet. Journal of Experimental Marine Biology and Ecology **65**:163-172.

Barnes, H., M. Barnes, and W. Klepal. 1977. Studies on the reproduction of cirripedes. I. Introduction: copulation, release of the oocytes and formation of the egg lamellae. Journal of Experimental Marine Biology and Ecology **27**:195-218.

Barnes, H. and H. T. Powell. 1950. The development, general morphology, and subsequent elimination of barnacle populations after heavy initial settlement Journal of Animal Ecology **19**:175-179.

Barnes, M. 1992. The reproductive periods and condition of the penis in several species of common cirripedes. Oceanography and Marine Biology **30**:483-525.

Bertness, M., S. Gaines, and S. Yeh. 1998. Making mountains out of barnacles: the dynamics of acorn barnacle hummocking. Ecology **79**:1382-1394.

Charnov, E. 1980. Sex allocation and local mate competition in barnacles. Marine Biology Letters **1**:269-272.

Charnov, E. L. 1982. *The Theory of Sex Allocation.* Princeton University Press, Princeton NJ.

Crisp, D. J. and H. Barnes. 1954. The orientation and distribution of barnacles at settlement with particular reference to surface contour. Journal of Animal Ecology **23**:142-162.

Crisp, D. J. and MacLean. 1990. The relationship between the dimensions of the cirral net, the beat frequency and the size and age of the animal in *Balanus balanoides* and *Eliminius modestus*. Journal of the Marine Biological association of the UK **70**:505-514.

Crisp, D. J. and B. S. Patel. 1958. Relation between brooding and ecdysis in cirripedes. Nature **181**:1078-1079.

Crisp, D. J. and A. J. Southward. 1961. Different types of cirral activity of barnacles. Philosophical Transactions of the Royal Society of London B **243**:271-307.

Darwin, C. 1854. *The Balanidae (or Sessile Cirripedes); the Verrucidae, etc.: A Monograph of the Sub-class Cirripedia.* The Ray Society, London, UK.

Heath, D. J. 1977. Simultaneous hermaphroditism: cost and benefit. Journal of Theoretical Biology **81**:151-155.

Hoch, J. 2008. Variation in penis morphology and mating ability in the barnacle, *Semibalanus balanoides*. Journal of Experimental Marine Biology and Ecology **359**:126-130.

Hoch, J. M. 2009. Adaptive plasticity of the penis in a simultaneous hermaphrodite. Evolution **63**:1946-1953.

Klepal, W. 1990. The fundamentals of insemination in cirripedes. Oceanography and Marine Biology **28**:353-379.

Klepal, W., H. Barnes, and M. Barnes. 1975. Variability in the 'loss' of the penis in *Balanus balanoides* (L.) under natural and experimental conditions and histology of degeneration. Pages 275-286 *in* Proceedings of the 9th European Marine Biological Symposium. Aberdeen University Press.

Klepal, W., H. Barnes, and M. Barnes. 1977. Studies on reproduction in cirripedes. VI. Passage of spermatozoa into the oviducal sac and closure of pores. Journal of Experimental Marine Biology and Ecology **27**:289-304.

Klepal, W., H. Barnes, and E. A. Munn. 1972. The morphology and histology of the cirripede penis. Journal of Experimental Marine Biology and Ecology **10**:243-265.

Marchinko, K. B. 2003. Dramatic phenotypic plasticity in barnacle legs (*Balanus glandula* Darwin): Magnitude, Age dependence and speed of response. Evolution **57**:1281-1290.

Miller, L. P. 2007. Feeding in extreme flows: behavior compensates for mechanical constraints in barnacle cirri. Marine Ecology-Progress Series **349**:227-234.

Munn, E. A., W. Klepal, and H. Barnes. 1974. The fine structure and possible function of the sensory setae of the penis of *Balanus balanoides* (L.). Journal of Experimental Marine Biology and Ecology **14**:89-98.

Murata, A., M. Imafuku, and N. Abe. 2001. Copulation by the barnacle *Tetraclita japonica* under natural conditions. Journal of Zoology **253**:275-280.

Neufeld, C. and A. Palmer. 2008. Precisely proportioned: intertidal barnacles alter penis form to suit coastal wave action. Proceedings of the Royal Society B: Biological Sciences **275**:1081-1087.

Raimondi, P. T. and J. E. Martin. 1991. Evidence that mating group-size affects allocation of reproductive resources in a simultaneous hermaphrodite. American Naturalist **138**:1206-1217.

Rasband, W. S. 1997-2007. ImageJ. US National Institute of Health, http://rsb.info.nih.gov/ij/, Bethesda MD.

Sokal, R. R. and F. J. Rohlf. 1995. *Biometry, third edition*. W. H. Freeman and Company, New York.

Stubbings, H. 1975. *Balanus balanoides.* Liverpool University Press, Liverpool.

Thompson, T. L. and E. P. Glenn. 1994. Plaster standards to measure water motion. Limnology and Oceanography **39**:1768-1779.

Wethey, D. S. 1984. Effects of crowding on fecundity in barnacles: *Semibalanus (Balanus) balanoides*, *Balanus glandula* and *Chthamalus dalli*. Canadian Journal of Zoology **62**:1788-1795.

Wu, R., C. Levings, and D. Randall. 1977. Differences in energy partition between crowded and uncrowded barnacles (*Balanus balanoides* Darwin). Canadian Journal of Zoology **58**:559-566.

Chapter 5 - Variation in penis morphology and function in response to differing physical and biotic conditions for the acorn barnacle, *Balanus glandula*

Abstract

Acorn barnacles are simultaneous hermaphrodites that copulate with a long penis. Morphological variation in the penis of the barnacle, *Balanus glandula*, was evaluated in an experiment testing the effects nearest neighbor distance, the level of crowding in a mating group and the number of potential mates. I measured the proportion of individuals fertilized over inter-neighbor distances and compared these proportions among the sites that vary in wave exposure. There were no strong effects of population parameters on penis length, but penis diameter and penis mass were both significantly affected by wave exposure. The proportion of individuals with fertilized broods decreased as mate distance increased. This effect was most pronounced in the sites with greatest wave exposure and less apparent in the protected sites, demonstrating that wave action is correlated with reduced ability of barnacles fertilize mates at relatively greater distances. Variation in penis morphology and mating ability should lead to differences in mate competition and sex allocation between sites differing for wave exposure.

Introduction

Most acorn barnacles are simultaneous hermaphrodites (Darwin 1854) and copulate with a long, flexible penis that is capable of stretching up to several times the barnacle's body length (Klepal et al. 1972). Functional males (hermaphroditic individuals acting as males), search for receptive functional females by sweeping the penis from neighbor to neighbor (Murata et al. 2001). They eventually insert the penis and deposit sperm into the functional female's mantle cavity (Klepal 1990). Upon fertilization the eggs are compressed into pellet-like masses and form lamellae in the mantle cavity (Barnes et al. 1977, Klepal et al. 1977). The Pacific acorn barnacle, *Balanus glandula* (Darwin 1854), has its major reproductive period in the winter, although low levels of reproduction may occur in other parts of the year (Strathmann 1987). For most of the year, reproductive tissues are not present in large quantities and the penis is greatly reduced in size. The penis rapidly re-grows each year, prior to the major peak of reproductive activity (Barnes 1992).

Balanus glandula has phenotypically plastic penis morphology over gradients of wave exposure, with thicker, more massive penises developing in areas of greater wave action (Neufeld and Palmer 2008). This pattern is similar to that found in *Semibalanus balanoides* in the Atlantic (Hoch 2008). Thin penises the developed in wave-protected sites have greatly reduced ability to reach distant mates when transplanted to wave-exposed sites. Barnacles from wave-exposed sites with thick penises do not show increased ability to fertilize distant neighbors when the threat of waves is removed, likely owing to a reduced ability to stretch (Hoch 2009). The penis of *S. balanoides* also varies with population conditions. Barnacles in densely crowded mating groups have functionally shorter penises than those from sparse aggregations, based on the number of annulations in the penis's exoskeleton (Hoch 2008). This morphological change is a response to density of crowding and not a response to neighbor distance alone or the number of nearby potential mates (*Chapter 3*). Phenotypic plasticity has also been observed in the feeding cirri of several species of barnacles in response to water motion, including *B. glandula*, *Chthamalus dalli*, *Pollicipes polymerus*, *Semibalanus cariosus* (Arsenault et al. 2001, Marchinko 2003, Marchinko and Palmer 2003) and *S. balanoides* (*Chapter 3*).

I tested several hypotheses concerning the relationship between penis morphology and differing levels of crowding and wave exposure for the barnacle *Balanus glandula*. First, I tested the hypotheses that penis length in *B. glandula* varies with level of crowding, number of neighbors and distance of near neighbors. At the same time I investigated the effects of wave exposure on penis length, penis diameter and penis mass. Owing to their close physical proximity and the possibility of developmental interaction or functional tradeoffs, I also investigated the possibility of correlations between the structure of the penis and cirri. Finally, to place morphological variation in penis structure into a functional context, I investigated penis performance, by testing the hypothesis that successful fertilization in barnacles will decline progressively neighbor distance and at greater magnitudes of wave exposure.

Methods

In late July and early August 2005, I set up experimental aggregations of the acorn barnacle, *Balanus glandula*, at six sites on San Juan Island, Washington, USA: Friday Harbor Laboratory dock (48°32'44.27"N, 123°0'45.14"W), Collins Cove (48°33'1.47"N, 123°0'19.91"W), Cattle Point (48°27'11.10"N, 122°57'44.57"W), Eagle Cove (48°27'39.15"N, 123°1'53.75"W), False Bay (48°29'15.55"N, 123°4'24.27"W) and Westcott Bay (48°35'33.43"N, 123°9'3.49"W). I began work much earlier than the major reproductive peak reported for this species of barnacle (January and February, Strathmann 1987). This allowed ample time for barnacles to respond to experimental conditions. At each site I haphazardly chose half-meter-square plots (with at least one meter between plots) within the middle third of the vertical range of barnacles in the intertidal (approximately 1 to 1.5 m above mean lower low water). I divided each plot into four quarters and adjusted the number of barnacles and density of crowding by removing adult barnacles. This resulted in four treatments per plot: high numbers of individuals, densely crowded (the HD treatment), high numbers of individuals, sparsely crowded (the HS treatment), low numbers of individuals, densely crowded (the LD treatment) and low numbers of individuals, sparsely crowded (the LS treatment). Low numbers treatments had between two and four individuals; high number treatments had greater than ten. Densely crowded treatments had barnacles with tests physically touching the tests of their neighbors (and less than 1 cm to between the apertures of nearest neighbors); sparsely crowded treatments had barnacles that were not in physical contact with their neighbors (and greater than 1 cm between the apertures of nearest neighbors; see *figure 1*).

The sites were characterized for relative wave exposure by comparing the dissolution rate of 3-cm diameter plaster spheres (Thompson and Glenn 1994). Dissolution rate of plaster is strongly correlated to water velocity. I mounted spheres among experimental plots simultaneously at each site for 24 hour periods (from low tide to the low tide of the following day). I measured the percent of plaster that was lost as to estimate rates of dissolution. Plaster dissolution rates may also be strongly affected by temperature, sediment content of the water and other aspects of environmental chemistry, but visual observations of wave action over the course of the experiment support the notion that these sites have strong differences in water motion. Cirri have been shown to be strongly correlated with wave exposure (Arsenault et al. 2001, Marchinko 2003, Marchinko and Palmer 2003), so to further confirm relative exposure level, I compared cirral length between sites.

I monitored barnacles outside of the experimental plots at each of these sites starting in December 2005 and by January 2006 gametes and reproductive structures were fully developed: reproductive activity was imminent. Between 1 January and 30 January 2006 I collected individuals from each site noting their density/number treatment, the distance to the aperture of its nearest neighbor (the minimum distance that a penis would have to reach for mating to occur) and the number of individuals within a 3-cm radius (the approximate boundary of the barnacle's mating group). I collected multiple barnacles from each treatment in each plot (Cattle Point: 152 individuals from 25 plots, Collins Cove: 132 individuals from 24 plots, FHL dock: 163 individuals from 24 plots, Eagle Cove: 165 individuals from 23 plots, False Bay: 133 individuals from 24 plots, Westcott Bay: 159 individuals from 30 plots; see figures for exact sample sizes for each analysis). Barnacles were preserved in 70% ethanol until dissection and analysis. I also collected a small sample of barnacles from a site on the outer Pacific coast, Ocean Shores, WA (46°55'35.71"N, -124°10'34.4467"W), which has a much greater magnitude of wave exposure than the experimental plots on San Juan Island. This sample size was small in numberand is only included in later results for qualitative comparisons.

In the laboratory, the height of each test and the diameters of the base and aperture were measured with digital calipers with accuracy to 0.01 mm. I estimated the volume of each test by treating it as a truncated cone. I removed the body from each test, removed the penis and one of the central feeding cirri (the sixth cirrus) using fine watchmaker's forceps and wet-mounted each onto glass slides and photographed them through a compound microscope with a digital camera at 40x magnification (see *figure 2*). The penises were dried at 50° for 24 hours and the mass of each was determined with a digital microbalance accurate to 0.001 mg. I measured the length and the diameter at the base of each penis and the length of each cirrus from photographs with the software imageJ (Rasband 1997-2007).

In February 2007 I returned to each site to measure fertilization success in natural populations in close proximity to my experimental plots. Fertilized barnacles are easily detected, as a fertilization sac compresses the egg mass into a sold pellet that is easily distinguished from an egg-mass that has not been fertilized (Barnes et al. 1977). At each site I haphazardly located individual barnacles in the mid-intertidal with nearest neighbors between 0.0 and 0.5 cm between apertures. This distance is the minimum distance that a penis would have had to reach across to fertilize that focal barnacle. I removed the focal barnacle and inspected it for fertilization. Within one meter of the first focal barnacle, I measured fertilization for nine more individuals with neighbor distances between 00 and 0.5 cm. I collected ten barnacles from neighbor distance bins of 0.51-1.0 cm, 1.01-1.5 cm, 1.51-2.0 cm. 2.01-2.5 cm and 2.51-3.0 cm. I repeated this entire process at ten plots in each site (each at least 3 meters from the nearest plot), for a total of 100 barnacles from each neighbor distance bin at each site. I sampled similarly at two sites on the open Pacific Ocean on the outer coast of Washington: Ocean Shores, WA and Westport, WA (46°54'34.92"N, -124°8'40.12"W).

Statistical analysis

All morphological data were log-transformed prior to analysis in SAS (SAS 9.2; SAS Institute 2003-2008). I performed two-way ANCOVA with test volume as covariate, to compare how penis length varied in response to number of individuals in the mating group, level of crowding and the interaction between them. I used a Tukey-Kramer post-hoc test to determine specific differences between high or low mate numbers, high or low density (crowding) and the interaction of number and density (differences between the HD, HS, LD and LS treatments). I used regression analysis to determine the relationship between penis length and neighbor distance. I also used regression analysis to detect the relationship between the residual variation of penis length (with the effect of body size removed) and neighbor distance. I used a general linear model and a SNK multiple range test (Cody and Smith 2006) to compare the percent mass lost from each plaster sphere in each site (using the arcsin-square root transformation, as suggested for percentage data by Sokal and Rohlf 1995). I divided these results into three general exposure categories, exposed, intermediate and protected, which were used as exposure categories in later analysis (see results). I used ANCOVA with test volume as covariate to compare both penis diameter and penis mass at the different exposure levels (exposed, intermediate and protected). I used Tukey-Kramer post-hoc tests to determine which levels of wave exposure had penises of significantly different diameters and masses. I used model II (RMA) regression to determine the relationship between penis volume (penis volume calculated as a cone, using radius at the base and length) and penis mass (Bohonak and van der Linde 2004). Finally, I tested for a tradeoff between cirral length and penis volume by using model II (RMA) regression to identify the relationship between cirral length and penis volume and the relationship between their residuals (removing the effect of body volume) (Bohonak and van der Linde 2004). I also compared the length of cirri of barnacles among the sites with differing degrees of wave exposure to determine if the differences in magnitude of exposure were great enough to elicit previously observed phenotypically plastic responses (e.g., Marchinko 2003). I repeated all exposure-related data analysis and included data from the small sample of barnacles from the Ocean Shores Jetty, in Ocean Shores, WA. These sample sizes were much smaller than those of the other groups, so these are presented for illustrative purposes only.

The effects of site, neighbor distance and site-by-neighbor distance interaction on fertilization success were compared with a factorial general linear model. I compared the effects of site and site-by-neighbor distance interaction for all possible pairs of sites. The arc-sine square root transformation was used on proportion data and the log transformation was used on distance data (Sokal and Rohlf 1995). Differences as a result of site can be thought of as differences in magnitude of overall mating activity in each site (that is, overall mating success for the site); differences as a result of the site by distance interaction can be thought of as differences among sites in ability of barnacles to reach to mates at greater distances with their penises (the magnitude in the decline of ability to fertilize neighbors at greater distances).

Results

Penis length varied significantly with body volume ($p < 0.0001$) and with the interaction between number of neighbors and density of crowding ($p = 0.0051$), but not with number of neighbors ($p = 0.8216$) or density of crowding alone ($p = 0.5763$). Singificant differences in least-square mean penis length among the four treatments (HD, HS, LD, LS) were not detected (Tukey Kramer $p > 0.05$, figure 3). The regression of penis length against nearest neighbor distance had a significantly positive slope (slope = 0.0458, $R^2 = 0.0266$, $p < 0.0001$) and the regression of residual variation in length (after removing the effect of body size) did as well (slope = 0.0283, $R^2 = 0.0123$, $p < 0.0049$).

Of the six sites (figure 4), Eagle Cove had significantly greater relative plaster loss (Mean = 100%, N = 2, 95% CI = 0.0000, SNK $p < 0.05$) than all other sites. It was interpreted as having the greatest relative water motion. Cattle Point had significantly greater plaster loss (Mean = 95.65%, N = 4, 95% CI = 1.22%, SNK $p < 0.05$) than every site except Eagle Cove. False Bay (Mean = 92.38%, N = 4, 95% CI = 1.89%) and Collins Cove (Mean = 92.38%, N = 3, 95% CI = 1.63%) did not have significantly different (SNK $p > 0.05$) relative plaster loss but both were significantly (SNK $p < 0.05$) greater than the FHL dock (Mean = 81.32%, N = 4, 95% CI = 2.13%) and Westcott Bay

(Mean = 56.15%, N = 4, 95% CI = 3.09%). The FHL dock and Westcott Bay also had significantly different rates than each other (SNK $p < 0.05$). For later analysis, Eagle Cove and Cattle Point were classified as "exposed," False Bay and Collins Cove were classified as "intermediate," and Westcott Bay and FHL Dock were classified as "protected." Barnacles from exposed sites (mean = 4.29 mm, 95% CI = 0.1355 mm, N = 247) had significantly shorter cirri than those from protected sites (mean = 4.99 mm, 95% CI = 0.1770 mm, N = 207, $p < 0.0001$), which in turn has significantly shorter cirri than intermediate sites (mean = 5.36 mm, 95% CI = 0.1883 mm, N = 201, $p = 0.0147$). When ocean sites were included they had shorter cirri than all other exposure levels (mean = 2.87 mm, 95% CI = 0.267 mm, N = 28, $p < 0.0001$ for each comparison).

Body volume ($p < 0.0001$) and magnitude of water motion ($p < 0.0001$) both significantly affected penis basal diameter (figure 5). Tukey-Kramer post hoc tests showed that barnacles from exposed sites (mean = 0.779 mm, 95% CI = 0.0149, N = 256) had significantly greater basal diameters than those from both intermediate (mean = 0.728 mm, 95% CI = 0.0156, N = 203, $p < 0.0001$) and protected sites (mean = 0.698 mm, 95% CI = 0.0145, N = 228, $p < 0.0001$). Barnacles from sites of intermediate water motion had significantly ($p = 0.0175$) greater penis diameters than those from the protected sites. When the barnacles from outer coast, open ocean site were included, there was no difference in basal diameter as compared to those from the exposed sites on San Juan Island (mean = 0.804 mm, 95% CI = 0.0467, N = 28, $p = 0.6607$). Body volume ($p < 0.0001$) and level of exposure ($p < 0.0001$) both significantly affected penis mass (figure 6). Barnacles from the exposed sites (mean = 0.1414 mg, 95% CI = 0.0104, N = 277) had significantly more massive penises than those from the intermediate (mean

= 0.1055 mg, 95% CI = 0.0082, N = 253, $p < 0.0001$) and protected sites (mean = 0.1102 mg, 95% CI = 0.0081, N = 301, $p < 0.0001$), which were not different from each other ($p = 0.7069$). When ocean sites were included in this analysis, their penis masses (mean = 0.1170 mg, 95% CI = 0.0230, N = 28) fell in between those of the exposed and the intermediate/protected sites, and were not significantly different from any of them (exposed $p = 0.4694$, intermediate $p = 0.7771$, protected $p = 0.9311$). Penis volume increases with length and with the square of the radius (when estimated as a cone). Model II (RMA) regression showed that penis mass increases with penis volume (slope = 1.285, 95% CI of slope = 0.075, $r^2 = 0.4432$). The length of the sixth cirrus was positively related to penis volume; the regression of cirri length against penis volume was positive (slope = 0.5537, 95% CI = +/- 0.0384, $r^2 = 0.2346$) as was the regression of cirri residuals against penis residuals positive (removing the effect of body size; slope = 0.6637, 95% CI = +/- 0.0517, $r^2 = 0.0.03602$).

Neighbor distance, site and the interaction between distance and site significantly affected the proportion of fertilized barnacles ($p < 0.0001$ for each). In most sites, barnacles had relatively high proportions of fertilized broods at close neighbor distances and decreasing fertilization success as distances increased (figure 7). I used the effect of site, that is, differences in average proportion fertilized from all distance bins to estimate relative magnitude of mating activity. Barnacles in Eagle Cove had the greatest proportion of fertilized broods, (un-transformed mean = 60%, 95% CI = 3.89%, N = 100), not significantly different than those from Cattle Point (51%, 95% CI = 4.25%, N = 100). Collins Cove had the lowest proportion of individuals carrying fertilized broods of all the sites (21%, 95% CI = 2.81%, N = 100). The relationship of distance to fertilization proportion was a significant factor for all sites, but this relationship also varied between sites (site*distance $p < 0.0001$). (See table 1 for p-values of pairwise comparisons and figure 7 for groups that did not have different effects of site or the site by neighbor distance interaction).

Discussion

Barnacle penis length varied with density of crowding and with number of neighbors in a complex fashion. The basal diameter of penises did vary with wave exposure, as did the mass. I did not detect a relationship between penis condition and cirral length. Although there was a significant effect of neighbor distance on penis length, it explained very little of the overall variance in penis length. For another species of acorn barnacle, *Semibalanus balanoides*, measured length of the penis is not appreciably affected by any of the above factors; however the number of annulations in the penis's cuticle is strongly affected by settlement density (*Chapter 3*), however annulation number was not evaluated in this study.

For this study, I used the gradient of wave exposure available on San Juan Island. Cattle Point and Eagle Cove (the exposed sites) face the large confluence of the Strait of Juan de Fuca and Haro Strait. The waves here have much greater fetch than those of the other sites. The intermediate site Collins Cove faces the much smaller San Juan Channel and the intermediate False Bay plots were on sheltered interior walls within False Bay. Barnacles at Friday Harbor Labs Dock were within the protected bend of Friday Harbor and further protected by a large floating dock; Westcott Bay is extremely protected and receives no wave action from Puget Sound. This gradient did not provide as extreme values of wave exposure, turbulence and force as is available along the outer Pacific coast, but did cover enough variation to allow general conclusions and predictions. The use of dissolution rates of plaster spheres as a measure of water motion is useful for relative comparisons of water motion, but suffers from some weaknesses. For example, differing levels of suspended particles in the water may amplify dissolution rate. For this reason I also compared length of cirri of barnacles, which are known to be strongly related to wave-exposure (Arsenault et al. 2001, Marchinko and Palmer 2003) and used those results to confirm my qualitative estimates of relative wave exposure. Cirri were significantly shorter in the sites classified as ‘exposed’, but longest in the ‘intermediate’ sites. This may be because feeding behaviors, such as pumping, beating and extension are correlated with subtle differences in water motion (Crisp and Southward 1961) and these behaviors are often associated with cirral morphology (Crisp and MacLean 1990). Despite the potential weaknesses of these quantitative classifications of wave exposure, my qualitative observations over the course of the mating season of these barnacles suggest that they are essentially accurate.

Barnacles from exposed sites grew penises that were on average (least square means) thicker at the base than those that grew in intermediate sites, which in turn, were thicker than those in protected sites. For the barnacles from the outer Pacific coast, penises were not significantly different in diameter than those from the exposed sites on San Juan Island. Penis mass was also greatest in exposed sites, although the intermediate and protected sites were not significantly different from each other. The penises from the outer coast were also less massive than those from San Juan Island. The intermediate values of diameter for barnacles from the sites of intermediate exposure, combined with the low values of mass suggest that increased diameter alone provides some advantages when attempting to mate in moving water. Bending beam theory suggests that thicker cylinders do provide greater resistance to deformation in flow and barnacles with increased penis thickness may benefit from that property (Vogel 2003, Neufeld and Palmer 2008). The increase in mass between penises from intermediate sites and exposed sites suggests that above a threshold of water motion, mass may provide additional resistance to interruption, perhaps owing to increases in muscle structure.

Overall penis volume (which is proportional to length and the square of the radius) is positively related to penis mass, providing some evidence that as diameter increases, muscle mass increases as well, and not just surface area of the penis. There was no evidence for a tradeoff between penis and cirri structure. Penis volume and cirri length instead showed a slightly positive relationship as did the residuals of penis volume and cirrus length. Whether penises impede cirral activity, hinder food capture efficiency or impart other opportunity costs for feeding and growth are important questions for future study. Acorn barnacles go through a period of anecdysis (non-molting) after reproduction and this may be a result in part of reduced growth and repair capacity owing to reduced available energy during the reproductive period (Crisp and Patel 1958).

Acorn barnacles clearly have reduced ability to reach mates at progressively greater distances when exposed to waves. The reduction in ability to reach neighbors in the largest distance classes is especially apparent in barnacles from the two Pacific coast sites, Ocean Shores and Westport. Despite high levels of mating activity, as shown by the relatively high fertilization percentages at close distances, they much less often reach neighbors at greater distances. It is not clear if this lowered ability to reach neighbors in wave exposed sites is a result of direct interference by waves or reduced flexibility of the thicker penises in those sites. Results from the Atlantic acorn barnacle, *Semibalanus balanoides*, suggest that it is a combination of these two factors (Hoch 2009). In protected sites, there is a less severe decline in fertilization rates as distance increases. This is particularly obvious in the Collins Cove and FHL Dock sites, which have much lower rates of decline across all distances, despite a generally low level of activity. The low overall proportion of fertilizations in some of the sites may indicate asynchrony in mating or other factors preventing mating among the sites.

This work shows that the mating group of barnacles is subject to more variation than just that which is imposed by the distribution of individuals within a population. Barnacles with distant neighbors may compensate for their lowered likelihood of fertilizing neighbors by growing slightly longer penises. Barnacles in sites exposed to waves appear to invest more into penis structure to maintain function, although overall ability to reach neighbors at greater distance is lost. My results show that for *Balanus glandula*, static estimates of mating group size may not be sufficient to fully capture variation over physical gradients. Further, limits of the mating group may vary with average neighbor distance of the groups in question, owing to plasticity in penis morphology.

Mating group size is an important factor in studying reproduction of hermaphrodites (Charnov 1982). The relative allocation to either sex role is predicted to skew towards female-bias at low group sizes and to move towards an asymptote at fiftypercent as mate numbers approach infinity (Charnov 1980). This hypothesis is based on Bateman's principle and the idea that with greater competition between males, fitness is increased by investing more into becoming a strong competitor for the eggs of neighbors (Bateman 1948, Arnold 1994). Raimondi and Martin (1991) found evidence supporting this model in the barnacle *Catomerus polumerus*. They found greater relative allocation to the female role in small mating groups. They estimated mating group size by predicting the ability of penises to reach mates using laboratory observations. The results from this experiment show that, all else being equal, mating groups in wave exposed sites will contain fewer individuals. These smaller groups would then be predicted to allocate relatively more resources to female function. Individuals functioning as females in wave exposed sites are predicted to carry eggs fertilized by fewer sires per brood. All of these predictions are based on the existence of a trade-off between male and female function, which has not been convincingly demonstrated in *B. glandula* (Hoch, *dissertation chapter 5*).

There is much in this field that would provide fruitful areas for future research. Molecular markers could be used to measure the number of sires in broods of individual females and compare this value across gradients of wave exposure. Further investigation into the development of the penis is warranted, especially with respect to identifying what advantages are provided by increases in diameter. The large size of the penis and its rapid development may constrain construction of other body parts. I suggest that growth or maintenance of the cirri may be reduced by growth of the penis, despite the lack of a tradeoff seen in this work. Comparing cirri sizes from the reproductive period with periods when reproduction is not occurring may inform on this question. The overall patterns of phenotypic plasticity in penis structure and function have been demonstrated for two species of barnacle, *B. glandula* and *S. balanoides* (Hoch 2008, Neufeld and Palmer 2008, Hoch 2009, this study). Work in other species of barnacles experiencing different physical and biotic gradients would be useful to understand the constraints of barnacle mating behavior.

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Table 1:

This table shows significance of pairwise comparisons of the effect of site and the site by distance interaction for fertilization success. Significant differences are presented in italics.

Site	**effect**	**Cattle**	**Collins**	**Eagle**	**False**	**Dock**	**Ocean**	**Westcott**
Collins	site	*< 0.0001*						
	site*dist	0.1066						
Eagle	site	0.088	*< 0.0001*					
	site*dist	0.7046	0.2146					
False	site	0.7889	*< 0.0001*	*0.0392*				
	site*dist	0.2721	*0.0067*	0.1242				
Dock	site	*< 0.0001*	*0.0186*	*< 0.0001*	*< 0.0001*			
	site*dist	0.8254	0.1646	0.8811	0.1851			
Ocean	site	0.0501	< 0.0001	0.0002	0.0747	< 0.0001		
	site*dist	*< 0.0001*	*< 0.0001*	*< 0.0001*	*< 0.0001*	*< 0.0001*		
Westcott	site	0.1321	*< 0.0001*	*0.0006*	0.1955	*< 0.0001*	0.5207	
	site*dist	0.3032	0.4807	0.5128	*0.0212*	0.4333	*< 0.0001*	
Westport	site	0.7198	*< 0.0001*	*0.0374*	0.9157	*< 0.0001*	0.1076	0.2618
	site*dist	*< 0.0001*	*< 0.0001*	*0.0001*	*< 0.0001*	*< 0.0001*	*0.0036*	*< 0.0001*

Figures

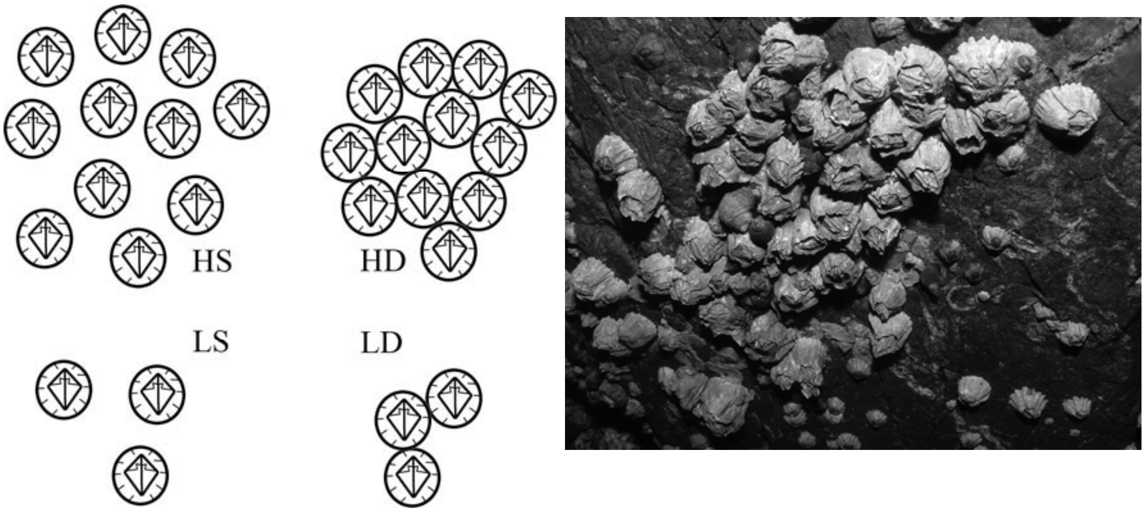

Fig. 1: *Left*: a schematic diagram of an experimental plot, showing the high number, sparse treatment (HS), high number, dense treatment (HD), low number, sparse treatment (LS) and low number dense treatment (LD). The tests of neighbors in sparse treatments did not touch one another (with greater than one cm between the apertures of nearest neighbors); in dense treatments tests were in contact (with less than one cm between apertures). Low number treatments had four or fewer barnacles; high number treatments had greater than ten. The position of treatments within a plot was randomized. There was a great enough distance between the treatments (> 5 cm) so that potential mates were only within a single treatment. *Right*: An experimental treatment of the acorn barnacle, *Balanus glandula*. This treatment is a high number (greater than ten), densely crowded (tests of neighbors touching) treatment (HD). Some sparsely aggregated barnacles (tests of neighbors not in contact) are visible in the lower right of the image, as well as some individuals of the genus *Chthamalus*.

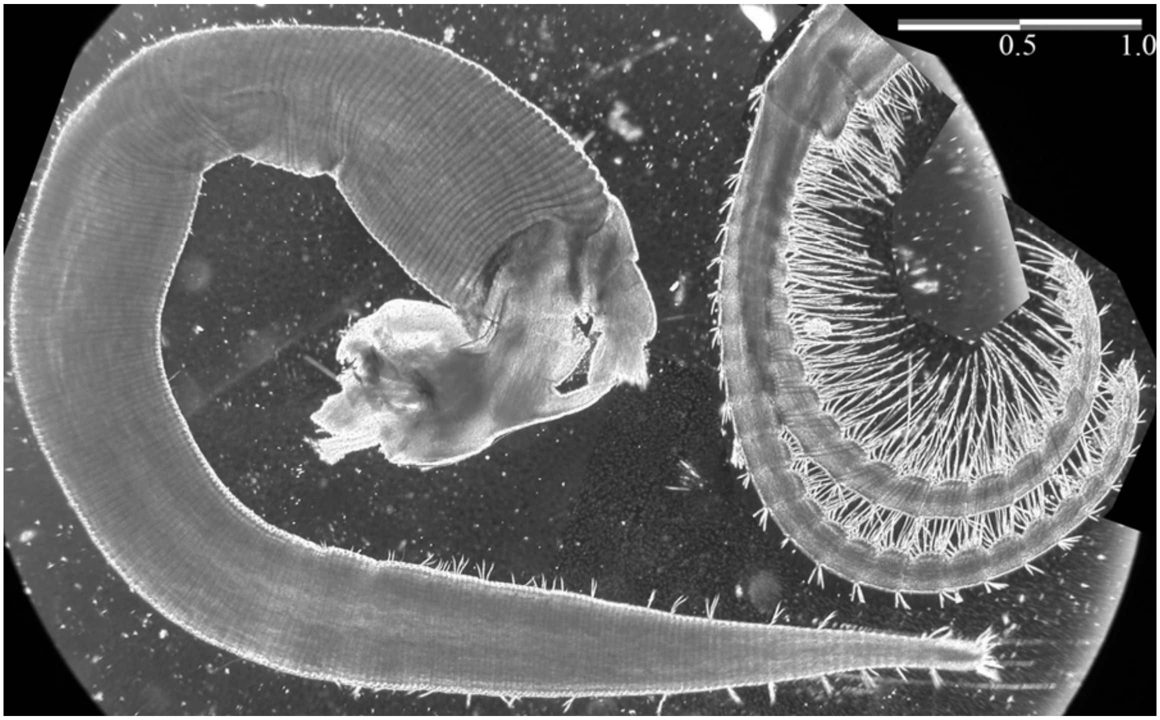

Fig. 2: A penis from the acorn barnacle, *Balanus glandula* and one of its cirri. Note the annulations (ring-like folds in the exoskeleton) along the entire length of the penis. The scale bar is one millimeter.

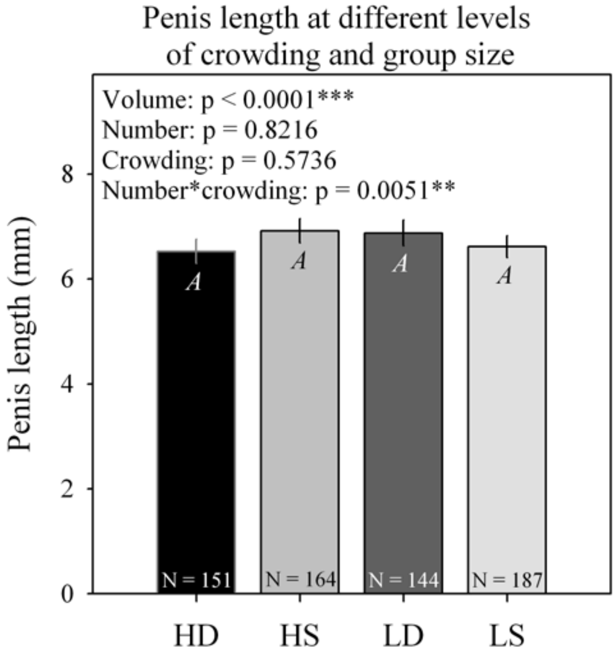

Fig. 3: Mean penis length (in millimeters) for penises from the four experimental treatments, high numbers-densely crowded (HD), high numbers-sparsely crowded (HS), low numbers-densely crowded (LD) and low numbers-sparsely crowded (LS). The plot shows least square means, generated by factorial ANCOVA and post hoc tests that have been back transformed. Bars labeled with the same letter are not significantly different from each other. Sample size is presented at the bottom of each bar. Error bars show 95% confidence intervals.

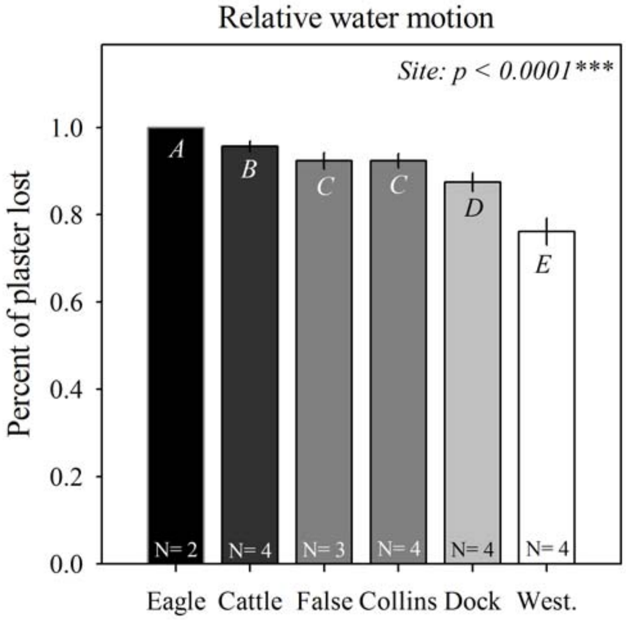

Fig. 4: Average percent plaster mass lost in each site as a proxy for relative water motion in each site. Error bars show 95% confidence intervals. Bars labeled with the same letter are not significantly different from each other. Sample size is listed in the bar for each site. Raw data is shown, although transformed data was used for statistical analysis. Site abbreviations are as follows: Eagle- Eagle Cove, Cattle- Cattle Point, False- False Bay, Collins- Collins Cove, Dock- Friday Harbor Laboratory dock, West.- Westcott Bay.

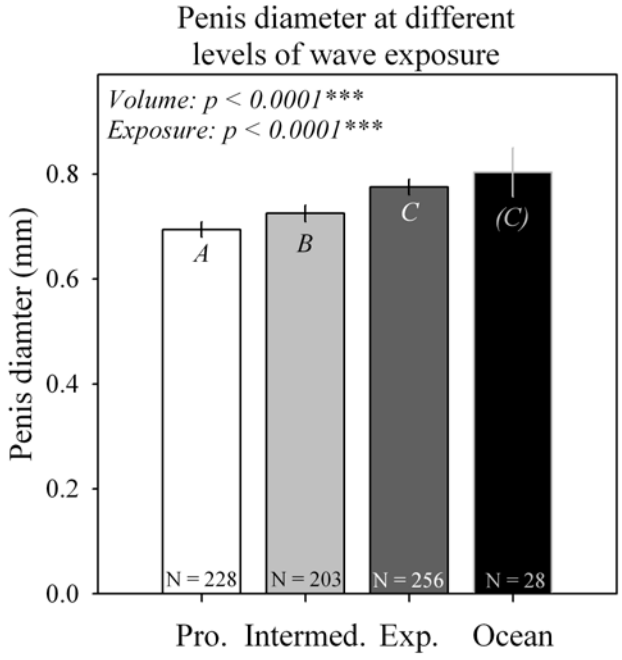

Fig. 5: Least square mean penis basal diameter (in millimeters) for penises from the three exposure levels on San Juan Island. Diameters from a sub-sample of penises collected from Ocean Shores, Washington, on the open pacific coast are included only to illustrate penis diameter at a greater extreme of wave exposure. Bars labeled with the same letter are not significantly different from each other. The Ocean bar is labeled for significant differences that would have been present had it been included in the full analysis. Sample size is presented at the bottom of each bar. Error bars show 95% confidence intervals.

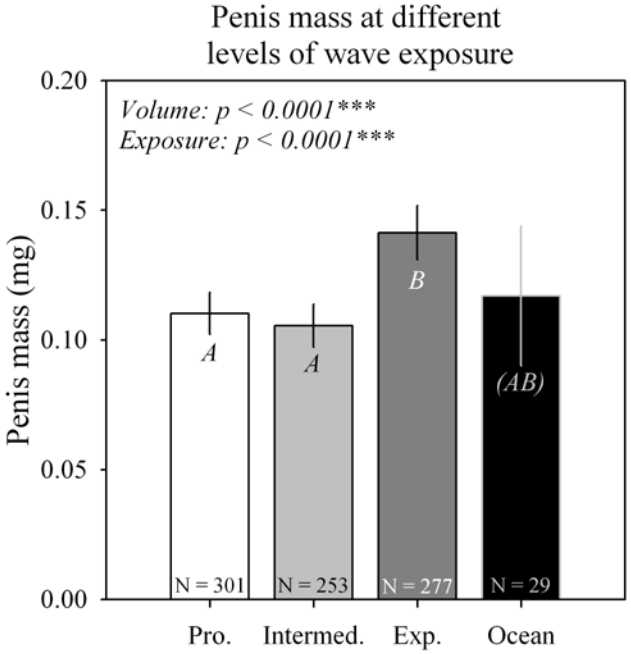

Fig. 6: Least square mean penis mass (in milligrams) for penises from the three exposure levels on San Juan Island. Masses from a sub-sample of penises collected from Ocean Shores, Washington, on the open pacific coast are included only to illustrate penis mass at a greater extreme of wave exposure. Bars labeled with the same letter are not significantly different from each other. The Ocean bar is labeled for significant differences that would have been present had it been included in the full analysis. Sample size is presented at the bottom of each bar. Error bars show 95% confidence intervals.

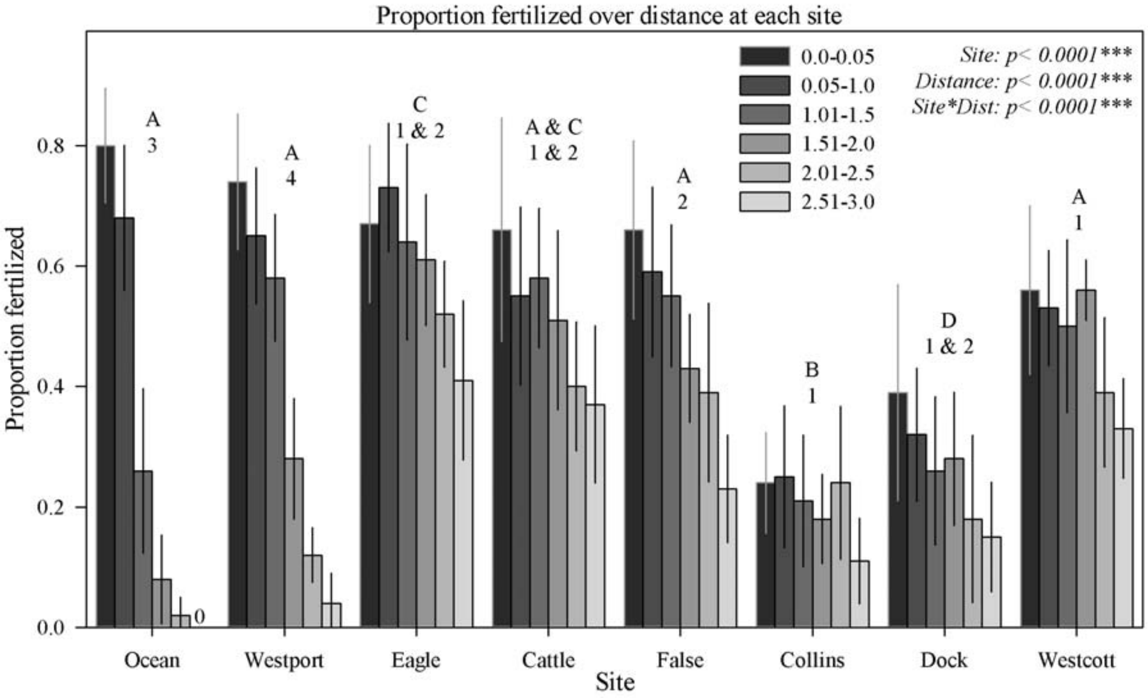

Fig. 7: Average proportion of barnacles carrying fertilized broods in each distance bin, at each site. Sites are presented in descending order of approximate wave exposure. Error bars show 95% confidence intervals. Groups of bars labeled with the same letter do not have significantly different effects as a result of site. Groups of bars labeled with the same numbers do not have significantly different effects of the site by distance interaction. Sites with greater wave exposure tend to have reduced proportions fertilized at greater neighbor distances, but mating activity is not synchronous between sites. For example, there seems to be far lower mating activity at Collins Cove than at Westport, but barnacles have better ability to reach neighbors at greater distances. Raw data is shown, although transformed data was used for statistical analysis. Site abbreviations are as follows: Ocean- Ocean Shores, WA, Westport- Westport, WA, Eagle- Eagle Cove, Cattle- Cattle Point, False- False Bay, Collins- Collins Cove, Dock- Friday Harbor Laboratory dock, Westott- Westcott Bay.

References

Arnold, S. 1994. Bateman's principle and the measurement of sexual selection in plants and animals. American Naturalist **144**:s126-s149.

Arsenault, D., K. B. Marchinko, and A. R. Palmer. 2001. Precise tuning of barnacle leg length to coastal wave action. Proceedings of the Royal Society B: Biological Sciences **268**:2149-2154.

Barnes, H., M. Barnes, and W. Klepal. 1977. Studies on the reproduction of cirripedes. I. Introduction: Copulation, release of the oocytes and formation of the egg lamellae. Journal of Experimental Marine Biology and Ecology **27**:195-218.

Barnes, M. 1992. The reproductive periods and condition of the penis in several species of common cirripedes. Oceanography and Marine Biology **30**:483-525.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity **2**:349-368.

Bohonak, A. J. and K. van der Linde. 2004. RMA: Software for Reduced Major Axis Regression, Java version. http://www.kimvdlinde.com/professional/rma.html. .

Charnov, E. 1980. Sex Allocation and local mate competition in barnacles. Marine Biology Letters **1**:269-272.

Charnov, E. L. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton NJ.

Cody, R. P. and J. K. Smith. 2006. *Applied Statistics and the SAS Programming Language. 5th edition*. Pearson Prentice Hall, Upper Saddle River, NJ.

Crisp, D. J. and MacLean. 1990. The relationship between the dimensions of the cirral net, the beat frequency and the size and age of the animal in *Balanus balanoides* and *Eliminius modestus*. Journal of the Marine Biological association of the UK **70**:505-514.

Crisp, D. J. and B. S. Patel. 1958. Relation between brooding and ecdysis in cirripedes. Nature **181**:1078-1079.

Crisp, D. J. and A. J. Southward. 1961. Different types of cirral activity of barnacles. Philosophical Transactions of the Royal Society of London B **243**:271-307.

Darwin, C. 1854. *A monograph on the sub-class Cirripedia, with figures of all the species. The Balanidæ, (or sessile cirripedes); the Verrucidæ, etc. etc. etc*. The Ray Society, London.

Hoch, J. 2008. Variation in penis morphology and mating ability in the barnacle, *Semibalanus balanoides*. Journal of Experimental Marine Biology and Ecology **359**:126-130.

Hoch, J. 2009. Adaptive plasticity of the penis in a simultaneous hermaphrodite. Evolution **63**: 1946-1953.

Klepal, W. 1990. The fundamentals of insemination in cirripedes. Oceanography and Marine Biology **28**:353-379.

Klepal, W., H. Barnes, and M. Barnes. 1977. Studies on reproduction in cirripedes. VI. Passage of spermatozoa into the oviducal sac and closure of pores. Journal of Experimental Marine Biology and Ecology **27**:289-304.

Klepal, W., H. Barnes, and E. A. Munn. 1972. The morphology and histology of the cirripede penis. Journal of Experimental Marine Biology and Ecology **10**:243-265.

Marchinko, K. B. 2003. Dramatic phenotypic plasticity in barnacle legs (*Balanus glandula* Darwin): Magnitude, Age dependence and speed of response. Evolution **57**:1281-1290.

Marchinko, K. B. and A. R. Palmer. 2003. Feeding in flow extremes: dependence of cirrus form on wave-exposure in four barnacle species. Zoology **106**:127-141.

Murata, A., M. Imafuku, and N. Abe. 2001. Copulation by the barnacle *Tetraclita japonica* under natural conditions. Journal of Zoology **253**:275-280.

Neufeld, C. and A. R. Palmer. 2008. Precisely proportioned: intertidal barnacles alter penis form to suit coastal wave action. Proceedings of the Royal Society B: Biological Sciences **275**:1081-1087.

Raimondi, P. T. and J. E. Martin. 1991. Evidence that mating group-size affects allocation of reproductive resources in a simultaneous hermaphrodite. American Naturalist **138**:1206-1217.

Rasband, W. S. 1997-2007. ImageJ. US National Institute of Health, http://rsb.info.nih.gov/ij/, Bethesda MD.

Sokal, R. R. and F. J. Rohlf. 1995. *Biometry, third edition*. W. H. Freeman and Company, New York.

Strathmann, M. 1987. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. University of Washington Press, Seattle.

Thompson, T. L. and E. P. Glenn. 1994. Plaster Standards to measure water motion. Limnology and Oceanography **39**:1768-1779.

Vogel, S. 2003. *Comparitve Biomechanics: Life's Physical World*. Princeton University Press, Princeton, NJ.

Chapter 6 - Sex allocation in two species of simultaneously hermaphroditic acorn barnacles

Abstract

Sex allocation theory for simultaneous hermaphrodites predicts increases in allocation to male-specific function as competition for mates increases. Theoretical models developed for acorn barnacles competing for mates make specific predictions as to the relative allocation to the sex roles. I used experimental manipulations to investigate how mate competition affected both relative and absolute allocation to the sex roles for two species of acorn barnacle: *Semibalanus balanoides* and *Balanus glandula*. Both species showed increased allocation to male function (estimated as total mass of sex specific tissues) with increased population density. Allocation to the female role seemed to be limited by the ability to produce and brood eggs. Both male and female function were positively related to body size. There was no tradeoff between male and female function, a key assumption of prior models. These results are largely in agreement with predictions made by size-dependent sex allocation models developed for other hermaphroditic animals.

Introduction

Fitness advantages as a result of skewed sex ratios were originally recognized by W.D. Hamilton (1967). Since then, a major goal of sex allocation theory has been to understand how sex ratios can maximize fitness (Charnov 1982). For organisms with separate sexes, sex ratio is the proportion of individuals of either sex in the population. For simultaneous hermaphrodites this is the relative investment into male and female function in a single individual. Given competition between functional males, fitness is predicted to be optimized when the proportion of reproductive resources devoted to male function (q) are allocated following Charnov (1980):

$$q = (k - 1) / (2k - 1)$$

Where $k + 1$ is the size of the mating group and k is the number of individuals competing with a focal individual for mates. The ratio allocated to male function (q) ranges from zero in the smallest mating groups (actually the smallest number of sperm that could possibly fertilize all of a single mate's eggs) to an asymptote of 50% as k approaches infinity. As k increases, q must increase for functional males to remain competitive. This model, known as the local mate competition model (LMC), assumes a tradeoff between male and female function and that allocation to the sex roles are limited by resources. Bateman's principle suggests that fecundity for females tends to be limited by resources available for egg production and that male fecundity is limited by access to females (Bateman 1948, Arnold 1994, Morgan 1994). In simultaneous hermaphrodites this applies to male and female functional roles. Charnov's LMC model is in accord with Bateman's principle in terms of male function: competitive advantage accrues to individuals with greater investments into sperm. However, according to Bateman's principle, fitness gained as a female is predicted to yield diminishing returns, owing to limitations of energy and resources available for egg production. Charnov's LMC model assumes energy limitation and a tradeoff with male function, but does not consider other potentially limiting resources that may not trade off with male function. Space available for brooding eggs may be such a limitation. Barnacles brood their eggs within their mantle cavity from their initial production until they are released as nauplius larvae (Barnes and Barnes 1968). Indeed, brood space limitation is often associated with the evolution of hermaphroditism (Heath 1979, Strathmann 1987).

Charnov's model of local mate competition was originally developed for acorn barnacles (Charnov 1980, see also Fischer 1980). Acorn barnacles are ideal animals for studies of sex allocation and reproductive success. They are typically obligate out-crossing, simultaneous hermaphrodites that copulate using a long penis, limiting potential mates to nearby neighbors and allowing assessment of mate identity and competition (Klepal 1990, Anderson 1994, Hoch 2008). Competitive interactions between functional males are likely mediated by sperm competition after multiple matings by a single functional female (Murata et al. 2001, David Rand personal communication). For this reason, "local sperm competition" (as described by Scharer 2009) may be a better term to describe the competitive interactions influencing sex allocation in these hermaphrodites. Barnacle anatomy is well suited for comparisons of allocation to either sex role (*figure 1*). Egg lamellae are packed discreetly within the mantle cavity and are easily removed. Testes and male-specific tissue can be extracted from the rest of the body. Embryos are brooded for several weeks, allowing one to collect offspring and measure mating success. Barnacles settle gregariously (Crisp and Barnes 1954), and mating group sizes range from very small and sparse to large and dense (Barnes and Powell 1950, Bertness et al. 1998). This natural variation is useful for comparisons of reproduction among mating groups of different size. Experimental manipulations of population size and density are straightforward and transplants between environments are possible (Connell 1961).

Raimondi and Martin (1991) found support for the general predictions of Charnov's LMC model, and reported that the barnacle *Catomerus polymerus* allocated relatively greater amounts of resources to female function in mating groups of smaller size. Much work has been completed on various aspects of the model in other taxa, including flatworms (Scharer et al. 2001, Scharer and Ladurner 2003, Vizoso and Sharer 2007), cestodes (Scharer and Wedekind 2001), trematodes (Trouve et al. 1999), leeches (Tan et al. 2004), bryozoans (McCartney 1997, Yund 1998) and fish (Peterson and Fischer 1996), recently reviewed by Scharer (2009). However, given the primacy of barnacles in the original modeling of hermaphrodite sex allocation, surprisingly little work has been done using the barnacle system.

I investigated the dynamics of sex allocation in two species of barnacle: *Semibalanus balanoides* on Long Island, NY, USA and *Balanus glandula* on San Juan Island, WA, USA. The penises of both *S. balanoides* and *B. glandula* have been shown to display morphological plasticity in response to local mate density (Hoch 2008, *chapters 3 and 4*) and wave exposure (Neufeld and Palmer 2008, Hoch In press). Barnacles in sparse aggregations (those whose tests are not in contact with the tests of their neighbors) have significantly longer penises than those from dense aggregations (those where tests are in contact with their neighbors). Barnacles are capable of recognizing and responding to settlement and aggregation density (*Chapters 3 and 4*). It has been suggested that barnacles may use the sensory ability of the penis to detect numbers of potential mates (Munn et al. 1974, Raimondi and Martin 1991). This is unlikely for the species used in this study. Both species have a relatively shorter reproductive period than *C. polymerus* and do not possess a fully developed penis outside of this period (Barnes 1992). *S. balanoides* has a single bout of reproductive activity for about three weeks in late October and early November (Hoch 2008). *B. glandula* has its major reproductive period in January and February, although it occasionally has minor periods of reproductive activity in the later spring and early summer (Strathmann 1987). Because the buildup of gametes prior to the reproductive period is concurrent with the development of the penis, it is unlikely that the sensory setae of the penis are useful for detecting mate number by which to make allocation decisions. Barnacles may have other ways of detecting their neighborhood size. For example, barnacles may detect waterborne cues of neighborhood size or they may be able to perceive contact with the tests of their neighbors.

I investigated the dynamics of sex allocation in two species of barnacle: *Semibalanus balanoides* on Long Island, NY, USA and *Balanus glandula* on San Juan Island, WA, USA. The goal of this study was to determine if patterns of sex allocation observed in acorn barnacles are better explained by Charnov's LMC model, alternate interpretations of Bateman's principle or by other organizing principles. I used full factorial experiments to test the hypotheses that allocation to male function, allocation to female function and the relative proportion of male function were related to the number of individuals or the density of crowding in a mating aggregation. I also tested whether body size was a major contributor to differences in sex allocation patterns, as has been suggested for other simultaneously hermaphroditic taxa, such as the sea hare, *Aplysia californica* (Angeloni et al. 2003).

Materials and Methods

In July of 2005 I manipulated naturally settled aggregations of the intertidal acorn barnacle, *Balanus glandula* on San Juan Island, Washington. I used six sites: Friday Harbor Labs dock (48°32'44.27"N, 123°0'45.14"W), Collins Cove (48°33'1.47"N, 123°0'19.91"W), Cattle Point (48°27'11.10"N, 122°57'44.57"W), Eagle Cove (48°27'39.15"N, 123°1'53.75"W), False Bay (48°29'15.55"N, 123°4'24.27"W) and Westcott Bay (48°35'33.43"N, 123°9'3.49"W). I began the work much earlier than major reproductive season of *B. glandula* (in the winter) so that experimental barnacles would have ample time to adjust to experimental conditions (Strathmann 1987).

In the first two weeks of September 2005 I manipulated natural aggregations of the intertidal acorn barnacle, *Semibalanus balanoides*, on Long Island, New York. I used six sites: Stony Brook Harbor (40°55'17.20"N, 73°8'59.06"W), Port Jefferson Harbor (40°56'46.96"N, 73°4'19.29"W), Cedar Beach Dock (40°57'54.59"N, 73°2'33.56"W), Flax Pond Inlet Jetty (40°58'1.59"N, 73°8'16.01"W), Shinnecock Inlet Jetty (40°50'18.73"N, 72°28'29.44"W), Robert Moses Beach Jetty (40°37'13.11"N, 73°18'23.23"W). In New York, *S. balanoides* have an acute, temporally restricted mating season in mid-November (Barnes 1992). Previous work has shown that they rapidly build reproductive structures and reserves during the months of September and October (Hoch, unpublished data).

In both regions treatments were established with sufficient time for the animals to respond to the experimental conditions. At each site I located large populations of barnacles in the mid-intertidal and haphazardly chose plots (~0.5 m^2) approximately in the middle third of the vertical range of barnacles (to hold tidal height and time immersed by water constant across sites). I divided each plot into quarters and randomly assigned each of four treatments: high numbers of individuals-dense aggregations (HD), high numbers of individuals-sparse aggregations (HS), low numbers of individuals-dense aggregations (LD) and low numbers of individuals-sparse aggregations (LS) (*figure 2*). Treatments were created by selective removal of individual barnacles from the rocks so that each treated quarter formed a single mating group (approximately 20 cm^2 and at least 5 cm from other treatments, so that each barnacles in each treatment were unable to mate with conspecifics outside of the mating group). In "low number" treatments, I removed all but three or four individuals; in "high number" treatments I left greater than ten individuals (some aggregations had as many as 25 potential mates). These numbers provide the barnacles in each treatment with ample opportunity for mating, but are predicted by the LMC model to have relatively large differences in sex allocation ratio (for example, groups of three are predicted to allocate approximately 33.3% of their reproductive resources to male function, compared to 48% for a group of 15) . In "dense" treatments, the tests of barnacles made physical contact with those of their neighbors; in "sparse" aggregations there was no contact between the tests of neighbors.

I created 30 plots at each of the six sites on San Juan Island, Washington and 25 plots at each site on Long Island, NY.

I collected barnacles in the time preceding the major bout of mating activity for that species. I collected *S. balanoides* from the Long Island New York sites from 2 November to 8 November (before most mating had occurred; intense mating activity was first observed 15 November 2005). I collected one or two barnacles from each treatment in each plot (Flax Pond: 73 barnacles from 15 plots, Robert Moses: 57 barnacles from 16 plots, Port Jefferson Harbor: 57 barnacles from 17 plots, Shinnecock: 64 individuals from 15 plots, Stony Brook Harbor: 72 barnacles from 15 plots), for a total of 323 individuals. *S. balanoides* were removed from rocks with a razor blade, so as not to damage the basal membrane, damage the test or lose egg masses. Storms had moved great masses of sand and pebbles at the Cedar Beach site, so these barnacles were not included. I collected *B. glandula* from the Washington sites over the course of their somewhat longer mating season; this analysis only includes barnacles collected between 1 January and 30 January (also before most mating had occurred; mating activity peaked in early February 2006). I collected up to four barnacles from each treatment in each plot (Cattle Point: 69 barnacles from 14 plots, Collins Cove: 66 barnacles from 13 plots, FHL Dock: 87 barnacles from 13 plots, Eagle Cove: 68 barnacles from 13 plots, False Bay: 87 individuals from 13 plots, Westcott Bay: 88 barnacles from 12 plots) for a total of 465 individuals. I removed each individual of *B. glandula* with a chisel, as their calcareous basal plates anchor them much more strongly to rocks. Care was taken not to damage the test or lose eggs. Individuals with damage to the rostrum, carina or laterals were not included. Many of the barnacles at the Westcott Bay site were dead, so I only collected apparently healthy individuals. I recorded the numbers of neighbors within the limits of penis reach for each collected barnacle (the number of individuals in the mating group). Field observations and prior data suggested that this limit was at about 2.5 cm for *S. balanoides* (Hoch 2008), and about 3.0 cm for *B. glandula* (*Chapter 4*). All specimens were preserved in 70% ethanol until dissection and analysis.

I recorded the height of each test and radii of the aperture and base and estimated the volume by treating it as a truncated cone. To test how well measures of the test as a truncated cone predict internal volume of the test, I collected a random sampling of *S. balanoides* tests. I measured the dry mass each test and then filled it with paraffin wax. Holding the test with the base facing upwards, I used the first few drops of melted wax to seal the aperture and any other cracks in the test. I then filled the mantle cavity with wax until a meniscus bulged up above open base of the test. After the wax had solidified, I used a razor blade to remove excess wax and cut the bulged meniscus so that the surface of the wax was flush with the base of the test. I measured the mass of each and divided the mass of paraffin in each test by the density of paraffin (measured from large blocks) to estimate the internal volume of each test. I used linear regression (on log-transformed data) to determine how well body volume as a truncated cone predicted internal volume as measured by wax. The truncated cone estimate proved to be an accurate (table 1, $N = 196$, $slope = 0.9467$, $R^2 = 0.8839$, $p < 0.0001$) predictor of internal volume (*figure 3*). Test volume estimated as a truncated cone was used as a covariate in many of the analyses.

The egg masses, penis, testes, seminal vesicles and all other reproductive organs were separated from all other tissues, dried for 24 hrs at 50° C and weighed with a fine scale microbalance (accurate to 0.0001 mg). I used egg mass to estimate allocation to female function (F) and the sum of the mass of all parts of the male system (including testes, sperm, seminal vesicle and penis) to estimate allocation to male function (M). Although mass alone does not exactly represent energetic investment into the sex roles (especially if eggs are significantly more energetically costly than an equivalent mass of sperm), it represents a consistent relative measure of investment into tissues and materials of the reproductive system (methods of estimating sex allocation in simultaneous hermaphrodites reviewed in Scharer 2009).

Statistical analysis

All analyses were performed on both *Semibalanus balanoides* and *Balanus glandula* using SAS (SAS 9.2; SAS Institute 2003-2008) (except where otherwise noted). Morphological, distance and length data were log-transformed and proportion data were arc-sine square root transformed (Sokal and Rohlf 1995). I used linear regression to test the hypothesis that the number of neighbors (possible mates) was a significant predictor of the proportion of male allocation ([M / (M + F)]; *q* in Charnov's (1980) model). I also used linear regression to test whether number of neighbors was a significant predictor of absolute allocation to male and female function (that is, the mass of sex specific reproductive tissue, M and F, independently).

I used linear regression to determine the relationship between body volume and male mass and the relationship between body volume and female mass. I used a general linear model to test whether the slopes of the regression lines of male mass and female mass against body volume were different. I took the residuals of both male mass and female mass against body volume and examined the relationship between them using model II (RMA) regression (Bohonak and van der Linde 2004) to test for a tradeoff between male and female function.

I used general linear models to test the effects of estimated test volume, site, mate number and aggregation density on relative male allocation (*q*), absolute male allocation (M) and absolute female allocation (F). I averaged the body volume, relative male mass (*q*), absolute male mass (M) and absolute female mass (F) for individuals from the same treatment in a plot, to correct for lack of independence. The interaction effects of site and mate number, site and aggregation density and the three-way interaction were non-significant for each test (except for absolute male function [M] in *B. glandula*, see below), so I pooled each species' data for all sites. I tested the hypothesis that the relative male allocation, *q*, differed with mate number, aggregation density or the interaction between them (HD, HS, LD, LS) with two-way ANCOVA using test volume as a covariate. I used Tukey-Kramer post hoc tests to determine whether mate number or aggregation density significantly affected relative allocation (*q*). Similar analyses were used to test for absolute differences in male function (M) and female function (F) among treatments. Since there was a significant interaction between site and mate number for absolute male allocation (M) in *B. glandula*, I included site as a factor in the ANCOVA model.

Results

Semibalanus balanoides

The number of barnacles in the mating group was a significant predictor of relative allocation to male function (*q*) (table 1, N = 308, slope = -0.0554, $R^2 = 0.0237$, p = 0.0068) and absolute male mass (N = 312, slope = -0.0469. $R^2 = 0.0153$, p = 0.0290), but not absolute female mass (N = 312, $R^2 = 0.004$, p = 0.2632). Body volume was a significant predictor of both male (table 1, N = 323, slope = 0.8016, $R^2 = 0.4988$, $p < 0.0001$) and female mass (N = 323, slope = 1.0822, $R^2 = 0.6256$, $p < 0.0001$) and explained much more of the variance. The slope of female mass when plotted against body volume was significantly greater than the slope of male mass (N = 323, $p < 0.0001$). The model II (RMA) regression of the residuals of male mass plotted against the residuals of female mass revealed a positive relationship between relative male function and relative female function (slope = 0.9526, 95% CI = +/- 0.0893, $R^2 = 0.2741$).

Two-way ANCOVA showed that only body volume (table 2, $p < 0.0001$) was a significant contributor to variation in *q*. Back-transformed least square means of *q* for each treatment were: HD = 0.2925, N = 62; HS = 0.26801, N = 77; LD = 0.2864, N = 70; LS = 0.3086, N = 71. According to the Tukey-Kramer post hoc tests, no treatments had significantly different least square means. Two-way ANCOVA showed that body volume (table 2, $p < 0.0001$) and aggregation density (p = 0.0305) contributed significantly to differences in absolute male mass (M). Barnacles from dense treatments (back-transformed least square mean = 1.0958 mg, N = 134) had significantly greater male mass than those from sparse treatments (back-transformed least square mean = 0.9665 mg, N = 148). Least square means (back-transformed) of male mass for each treatment were: HD = 1.1336, N = 64; HS = 0.9420, N = 77; LD = 1.0592, N = 70; LS = 0.9917, N = 71. According to the Tukey-Kramer post hoc test, none of these were significantly different from each other. Two-way ANCOVA showed that body volume (table 2, $p < 0.0001$), mate number ($p = 0.0166$) and aggregation density ($p = 0.0308$) all contributed significantly to differences in absolute female mass. Barnacles from groups of high numbers had significantly greater female mass (back-transformed least square mean = 2.8951 mg, N= 138) than those form groups of low numbers (back-transformed least square mean = 2.5295 mg, N = 141). Barnacles from dense groups (back-transformed least square mean = 2.8749 mg, N = 131) also had significantly greater female mass than those from sparse groups (back-transformed least square mean = 2.5472 mg, N = 148). Least square means (back-transformed) of female mass for each treatment were: HD = 3.0603, N = 61; HS = 2.7388, N = 77; LD = 2.7008, N = 70; LS = 2.3690, N = 71. According to the Tukey-Kramer post hoc test, only HD and LS were significantly different from each other ($p = 0.0100$).

Balanus glandula

The number of barnacles in the mating group was not a significant predictor of relative allocation to male function (q) (table 1, N = 463, $R^2 = 0.0021$, $p = 0.3303$), absolute male function (N = 463, $R^2 = 0.0021$, $p = 0.3202$) or absolute female function (N = 463, $R^2 = 0.0000$, $p = 0.9006$). Body volume was a significant predictor of both male (table 1, N = 465, slope = 0.8016, $R^2 = 0.8874$, $p < 0.0001$) and female mass (N = 465, slope = 1.3971, $R^2 = 0.6648$, $p < 0.0001$). The slopes of male mass and female mass were significantly different from each other when plotted against body volume (N = 465, $p < 0.0001$). The model II (RMA) regression of the residuals of male mass plotted against the residuals of female mass revealed a positive relationship between male function and female function (slope = 0.7246, 95% CI = +/- 0.05782, $R^2 = 0.2237$).

Two-way ANCOVA showed that only body volume (table 2, $p < 0.0001$) contributed significantly to differences in *q*. Back-transformed least square means of *q* for each treatment were: HD = 0.2683, N = 65; HS = 0.2713, N = 69, LD = 0.2825, N = 67; LS = 0.2343, N = 69. According to the Tukey-Kramer post hoc test, none of these were significantly different from each other. Multi-way ANCOVA showed that body volume (table 2, $p < 0.0001$), site ($p < 0.0001$), density ($p = 0.0010$) and the interaction between site and number of mates ($p = 0.0258$) contributed significantly to differences in absolute male mass. Dense aggregations (back-transformed least square mean = 2.5737 mg, N = 132) had significantly greater male masses than sparse aggregations (back-transformed least square mean = 2.2205 mg, N = 138). Least square means (back-transformed) of male mass for each treatment were: HD = 2.6064, N = 65; HS = 2.3826, N = 69; LD = 2.5414, N = 67; LS = 2.0694, N = 69. According to the Tukey-Kramer post hoc test, LS was significantly different from HD ($p = 0.0018$), and LD ($p = 0.0063$). Two-way ANCOVA showed that body volume (table 2, $p < 0.0001$) was the only significant contributor to variation in female mass. Least square means (back-transformed) of female mass for each treatment were: HD = 7.2139, N = 65; HS = 6.8563, N = 69; LD = 6.7198, N = 67; LS = 7.2916, N = 69. According to the Tukey-Kramer post hoc test, none of these were significantly different from each other.

Discussion

Interpretation of results

For *Semibalanus balanoides*, the number of individuals within the mating group was a significant predictor of relative allocation to male function (*q*) and absolute allocation to male function (M). However, the number of individuals within a mating group explained a small amount of the variance in sex allocation of these barnacles (2% for q and 1.5% for M). Mate number was not a significant predictor of relative allocation to female function for *S. balanoides*. For *Balanus glandula*, the number of individuals within the mating group was not a significant predictor of relative allocation to male function (*q*), absolute allocation to male mass or absolute allocation to female mass. The results suggest that barnacles are not altering their relative sex allocation (*q*) or absolute sex-specific allocation (M, F) in direct response to the number of individuals in their mating group. Neither the number of individuals in the mating group nor aggregation density significantly affected relative sex allocation (*q*) in either species (*figure 7*).

The relative proportion of male function, *q*, was used as one of several response variables in this study because of its use as an indicator of sex allocation strategies in Charnov's (1980) LMC model. A central assumption of the use of this variable is that there is a direct trade-off between male and female function (Charnov 1980, Scharer 2009). No tradeoff was observed; rather, there was a positive relationship between male and female function (*figure 5*) and between the residuals of male function and the residuals of female function (*figure 6*). For this reason, in acorn barnacles, strategic sex allocation patterns may be better inferred from absolute allocation to the male and female roles independently. This observed lack of tradeoff may be the result of different limiting factors for each sex role or an overall lack of energy limitation (Scharer et al. 2005). Barnacles brood their eggs as lamellae in the mantle cavity (*figure 1*); this brood space may be more limiting than energy needed for egg production. Space limitation in the mantle cavity would be unrelated to male function, as the testes are confined to the thorax and prosoma (Darwin 1854) and the penis remains coiled around the thorax when not in use (personal observation). The positive correlation between male and female function is likely also related to the condition of the individual. Individuals in environments with more food or that have higher fitness owing to their genetic makeup may be able to allocate relatively more to reproductive function.

Taken separately, absolute allocation to male function (total male mass) in both species was significantly affected by aggregation density, but not by mate number (*figure 7*). Barnacles of both species increased absolute allocation to the male role when densely aggregated. For *S. balanoides*, these responses are similar to those seen in phenotypically plastic responses of penis morphology to aggregation density. Barnacles from densely settled groups have relatively shorter penises than those from sparse groups (Hoch 2008), but changes in neighbor distance alone are insufficient to produce such changes in morphology (chapter 3, Yuen and Hoch unpublished manuscript). This supports one of the predictions of the LMC model: barnacles increase allocation to male function with greater levels of mate competition. This potential competition is perceived through cues associated with aggregation density and test-to- test contact. Male allocation in *B. glandula* was also significantly affected by the interaction between mate number and site. This may be a result of differing levels of wave exposure in the different sites. Waves may affect the number of mates available to a barnacle owing to changes in behavior in the presence of waves or direct interference by turbulence and drag forces.

Female mass in *S. balanoides* was affected by both number of mates and by aggregation density. In *B. glandula*, neither mate number nor aggregation density significantly affected allocation to female function (*figure 7*). The different responses of female allocation to mate number and aggregation density in the two species may be a result of differences in life history between these species and different allocation strategies as a result of that. For example, *B. glandula* tend to survive for much longer than *S. balanoides* and the difference seen here may reflect strategies related to tradeoffs between survival and reproduction (Stubbings 1975, Strathmann 1987).

Body volume was a significant predictor of both male and female mass for *S. balanoides* and *B. glandula*. Female function increased much more with body volume (*figure 5*) than did male function for both species. The strong positive relationship between the residuals of male and female mass provide evidence of a positive relationship in investments to male and female function. Both species are investing relatively more into female function as body size increases, suggesting that as size increases, gaining fitness through the female role becomes more important. Finally, the steep slope and high percentage of variation in female mass explained by body volume (*figure 5*), suggests that constraints imposed by body size may be more important limitations on sex allocation than parameters related to the characteristics of the mating group.

Factors that one must take into consideration when comparing absolute allocation to either sex role are the distinction between static and dynamic investments into a sex role and the distinction between fixed and variable costs of investment into sex roles. Dynamic allocation includes such material as sperm and secretion products that are continually constructed and used over a reproductive period, as opposed to static costs in which an investment remains constant over time (Scharer et al. 2004, Vizoso and Sharer 2007). Estimates of sex-specific allocation based on "snapshots" of dynamic structures (such as sperm mass) are usually assumed to be strongly correlated to overall investment into that function. A major reason that the two species of barnacle from this project make excellent study organisms is that they both have a long period of gamete build-up before a relatively shorter period of mating. *S. balanoides*, in particular builds up egg and sperm for several months in the late summer and early fall before a much shorter period (< 3 weeks) of mating activity in mid-fall (Barnes 1992, Hoch 2008). Measures of sex-specific mass immediately prior to mating can be considered an almost complete picture of those investments. A fixed investment into a sex role is a cost that must be paid for reproduction to be possible, but that does not directly enhance fertility (Heath 1977, Charnov 1979, Scharer 2009). The penis of the barnacle is an example of this (Hoch 2008), whereas eggs and sperm would be considered variable investments, since changes in allocation to those will have direct effects on fertility. Again, these distinctions should not be important for the barnacles from this study, as penises have a clear, male-only function and for *S. balanoides* averaged 9% of total male investment (by mass) and for *B. glandula* 7%. They exist only as degenerate stubs outside of the mating season, and grow rapidly to their full size over the same period that variable investments are being made. Because of the brief and defined mating season and rapid development of the penis, concurrent with development of eggs and testes, investment into penises was not treated differently than investments into testes.

Implications for studies of sex allocation

The results from this project shed light on several aspects of sex allocation. Barnacles seem to use the density of crowding to gauge the extent of sperm competition that they will face. They respond to this by increasing allocation to male function, which meets the predictions of LMC theory (Hamilton 1967, Charnov 1982). Owing to the lack of a tradeoff between male and female function, the predictions of relative allocation to the female role are not met. Clutches of eggs in mated barnacles tend to be 100% fertilized. That is, there are no partially fertilized broods and there is no evidence of sperm limitation (personal observation). If any mating partners are present, these eggs would almost certainly be fertilized and it would therefore be adaptive to invest as much as possible to female function. Any individual investing less than the maximum amount possible in female function will be at a fitness disadvantage.

In this study, body size of the barnacle was by far the most important factor in determining sex allocation. Size dependent sex allocation is prevalent in other simultaneous hermaphrodites, including sea hares (Yusa 1996, 2008), snails (Chaine and Angeloni 2005, Norton et al. 2008), fish (Peterson and Fischer 1996) and plants (reviewed in Klinkhamer et al. 1997). Size and relative fitness gain is also important for sex changes in sequential hermaphrodites (Ghiselin 1969, Charnov 1982, Berglund 1986). For all of these, fitness gained through the female role increases faster than that of the male role as body size increases. Many models predicting changes in sex allocation with body size include factors such sperm storage (Charnov 1996, Angeloni et al. 2003), reciprocal mating (Greeff and Michiels 1999) and the size distribution of the population (Angeloni et al. 2003). Angeloni et al (2002) point out that these predictions are not dependent on the specific factors included in the models, but on the specific biology of the organisms and the shapes of the fitness gain curves. Overall, the results of this study generally support the predictions of these size-dependent models: greater relative allocation to female function with increasing size. Barnacles are likely to experience intense competition between males (Barnes et al. 1977, Klepal 1990, Murata et al. 2001) and broods are often fertilized by numerous functional males (David Rand, personal communication). This competition must result in a saturating male-fitness curve, leading to a smaller increase in allocation to male function (than female) with body size. Generally, these results also provide further support for the application of Bateman's principle to simultaneous hermaphrodites: female function is limited by the capacity to build and brood eggs and male function is limited by access to females.

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Table 1: Regressions

Semibalanus balanoides

***q* as a function of number of mates**

R^2= 0.0237

Source	DF	SS	MS	F Value	Pr > F
Model	1	41.85	41.85	7.43	0.0068
Error	306	1723.95	5.63		

Variable	DF	Parameter estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.315	0.197	6.68	<0.0001
Number	1	-0.055	0.020	-2.73	0.0068

Male mass as a function of number of mates

R^2= 0.0153

Source	DF	SS	MS	F Value	Pr > F
Model	1	30.97	30.97	4.81	0.0290
Error	310	1996.22	6.43		

Variable	DF	Parameter estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.687	0.208	3.31	0.0011
Number	1	-0.046	0.021	-2.19	0.0290

Female mass as a function of number of mates

R^2= 0.004

Source	DF	SS	MS	F Value	Pr > F
Model	1	7.67	7.67	1.26	0.2632
Error	310	1893.26	6.10		

Variable	DF	Parameter estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.442	0.203	7.09	<0.0001
Number	1	-0.023	0.020	-1.12	0.2632

Male mass as a function of body volume

R^2= 0.4988

Source	DF	SS	MS	F Value	Pr > F
Model	1	86.19	86.19	319.46	<0.0001
Error	321	86.60	0.27		

Variable	DF	Parameter estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-4.093	0.231	-17.70	<0.0001
Volume	1	0.801	0.044	17.87	<0.0001

Female Mass as a function of body volume

R^2= 0.6256

Source	DF	SS	MS	F Value	Pr > F
Model	1	152.96	152.96	529.66	<0.0001
Error	317	91.55	0.29		

Variable	DF	Parameter estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-4.597	0.242	-18.93	<0.0001
Volume	1	1.082	0.047	23.01	<0.0001

Calculated body volume predicts internal volume as measured by wax

R^2= 0.8839

Source	DF	SS	MS	F Value	Pr > F
Model	1	76.96	76.96	1477.41	<0.0001
Error	194	10.11	0.05		

Variable	DF	Parameter estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-0.415	0.128	-3.24	0.0014
Calculated vol.	1	0.946	0.025	38.44	<0.0001

Balanus glandula Regressions

q as a function of number of mates

R^2= 0.0021

Source	DF	SS	MS	F Value	Pr > F
Model	1	0.45	0.45	0.95	0.3303
Error	461	222.23	0.48		

Variable	DF	Parameter estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-0.589	0.049	-11.99	<0.0001
Number	1	-0.005	0.006	-0.97	0.3303

Male mass as a function of number of mates

R^2= 0.0021

Source	DF	SS	MS	F Value	Pr > F
Model	1	0.33	0.33	0.99	0.3202
Error	461	155.40	0.34		

Variable	DF	Parameter estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.602	0.041	14.65	<0.0001
Number	1	-0.005	0.005	-1.00	0.3202

Female mass as a function of number of mates

R^2= 0.0000

Source	DF	SS	MS	F Value	Pr > F
Model	1	0.029	0.029	0.02	0.9006
Error	461	866.30	1.88		

Variable	DF	Parameter estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.966	0.097	20.23	<0.0001
Number	1	0.0015	0.0118	0.13	0.9006

Male mass as a function of body volume

R^2= 0.6035

Source	DF	SS	MS	F Value	Pr > F
Model	1	205.69	205.69	704.67	<0.0001
Error	463	135.14	0.292		

Variable	DF	Parameter estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-4.494	0.203	-22.11	<0.0001
Volume	1	0.887	0.033	26.55	<0.0001

Female Mass as a function of body volume

R^2= 0.6648

Source	DF	SS	MS	F Value	Pr > F
Model	1	509.77	509.77	918.43	<0.0001
Error	463	256.99	0.555		

Variable	DF	Parameter estimate	Standard Error	t Value	Pr > |t|
Intercept	1	-6.481	0.280	-23.12	<0.0001
Volume	1	1.397	0.046	30.31	<0.0001

Table 2: Analyses of Covariance

Results of two-way ANCOVAs for *Semibalanus balanoides*

Variation in *q* with factors of number of mates and aggregation density, with test volume as covariate

Source	DF	SS	MS	F Value	Pr > F
Volume	1	0.5697	0.5697	43.91	< 0.0001
Number	1	0.0248	0.0248	1.91	0.1676
Density	1	0.0002	0.0002	0.01	0.9129
Number*Density	1	0.0457	0.0457	3.52	0.0616

Variation in male mass with factors of number of mates and aggregation density, with test volume as covariate

Source	DF	SS	MS	F Value	Pr > F
Volume	1	64.1772	64.1772	275.10	< 0.0001
Number	1	0.0047	0.0047	0.02	0.8876
Density	1	1.1027	1.1027	4.73	0.0305
Number*Density	1	0.2467	0.2467	1.06	0.3047

Variation in female mass with factors of number of mates and aggregation density , with test volume as covariate

Source	DF	SS	MS	F Value	Pr > F
Volume	1	107.8812	107.8812	503.75	< 0.0001
Number	1	1.2443	1.2443	5.81	0.0166
Density	1	1.009	1.009	4.71	0.0308
Number*Density	1	0.0069	0.0069	0.03	0.8580

Results of two-way ANCOVAs for *Balanus glandula*

Variation in *q* with factors of number of mates and aggregation density, with test volume as covariate

Source	DF	SS	MS	F Value	Pr > F
Volume	1	1.8581	1.8581	112.47	< 0.0001
Number	1	0.0120	0.0120	0.73	0.3942
Density	1	0.0449	0.0449	2.72	0.1005
Number*Density	1	0.0577	0.0577	3.50	0.0626

Variation in male mass with factors of site, number of mates and aggregation density, with test volume as covariate

Source	DF	SS	MS	F Value	Pr > F
Volume	1	45.6126	45.6126	352.94	< 0.0001
Site	5	20.6600	4.1320	31.97	< 0.0001
Number	1	0.4558	0.4558	3.53	0.0616
Site*Number	5	1.6808	0.3361	2.6	0.0258
Density	1	1.4301	1.4301	11.07	0.001
Site*Density	5	0.5693	0.1138	0.88	0.4944
Number*Density	1	0.2206	0.2206	1.71	0.1926
Site*Num.*Dens.	5	0.7759	0.1551	1.2	0.3093

Variation in female mass with factors of number of mates and aggregation density, with test volume as covariate

Source	DF	SS	MS	F Value	Pr > F
Volume	1	273.3302	273.3302	645.43	< 0.0001
Number	1	0.0015	0.0015	0.00	0.9528
Density	1	0.0159	0.0159	0.04	0.8462
Number*Density	1	0.2957	0.2957	0.70	0.4041

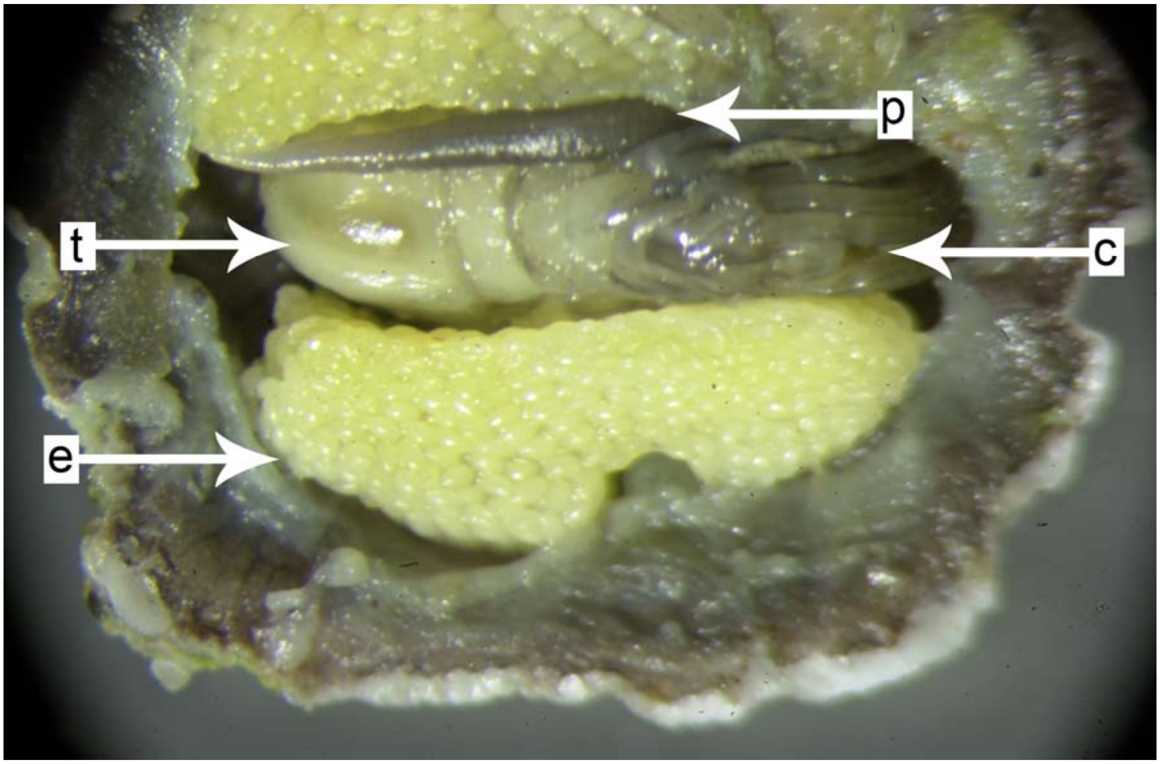

Fig. 1: An acorn barnacle, *Semibalanus balanoides*, removed from a rock and viewed from below. The basal membrane has been removed. This barnacle has been fertilized, as can be seen from the pelletized egg mass with individual embryos visible. The arrows are labeled as follows: "p" indicates the penis; "c" indicates the point of attachment of the penis, between the feeding cirri (the base of the penis is folded under the cirri); "t" indicates the portion of the soma that holds the testes (this individual was collected post-mating, so the testes are greatly reduced); "e" indicates the pellet of fertilized embryos.

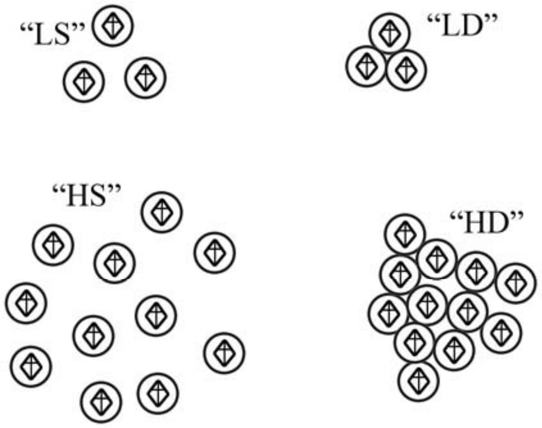

Fig. 2: Schematic showing the arrangement of an experimental plot. LS is the low number-sparsely aggregated treatment, LD is low number-densely aggregated treatment, HS is high number-sparsely aggregated treatment and HD is high number-densely aggregated treatment. Each plot contained one of each treatment, in a haphazardly assigned position. The individuals in a given treatment were distant enough from those of other treatments to prevent extra mating.

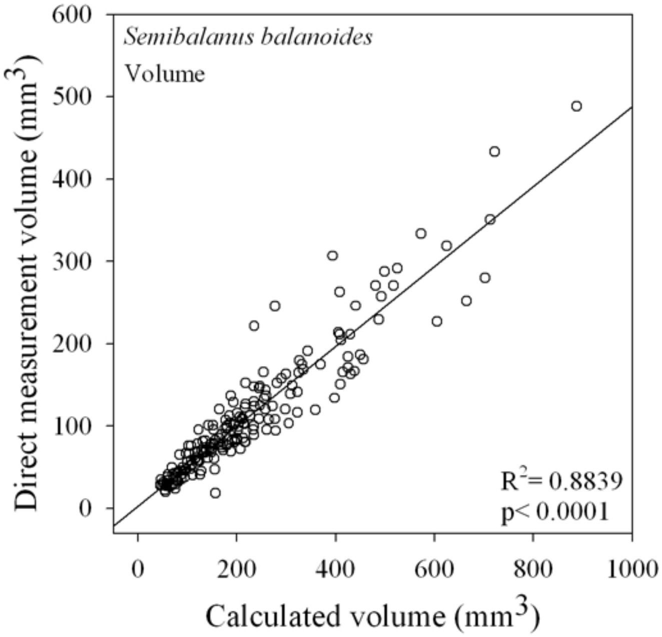

Fig. 3: Internal test volume measured directly with wax regressed against test volume calculated as a truncated cone. Raw data shown; analysis was performed on log-transformed data.

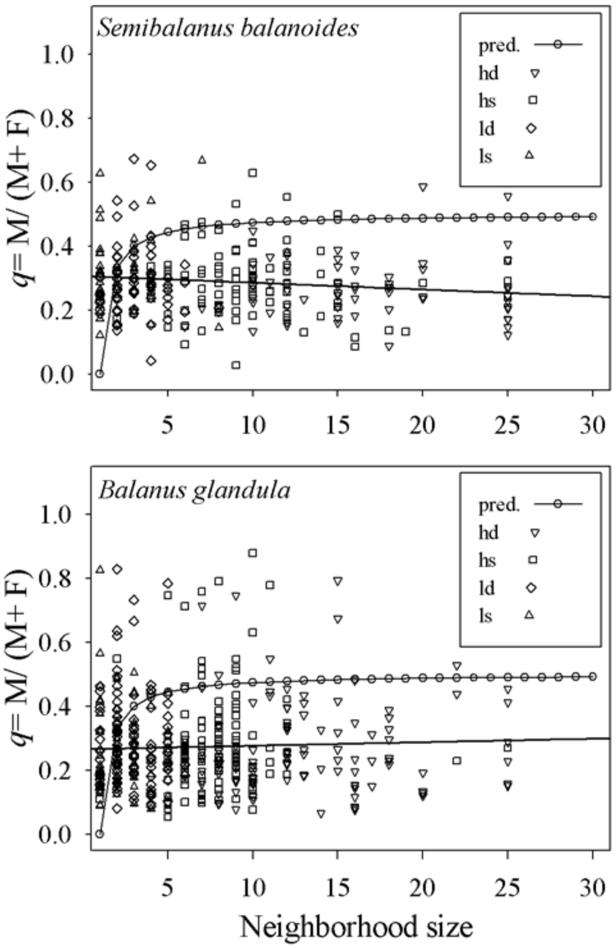

Fig. 4: Relative allocation to male function (q= M/ (M+ F)) plotted against the number of neighbors within penis reach of the focal barnacle (2.5 cm for *Semibalanus balanoides*, 3.0 cm for *Balanus glandula*). The values predicted by Charnov's sex allocation model (1980) are shown as circles connected by lines. The other symbols represent each of the four experimental treatments. Raw data shown, with regression line constructed using non-transformed values of q; statistical analyses were performed on arcsine square root transformed data.

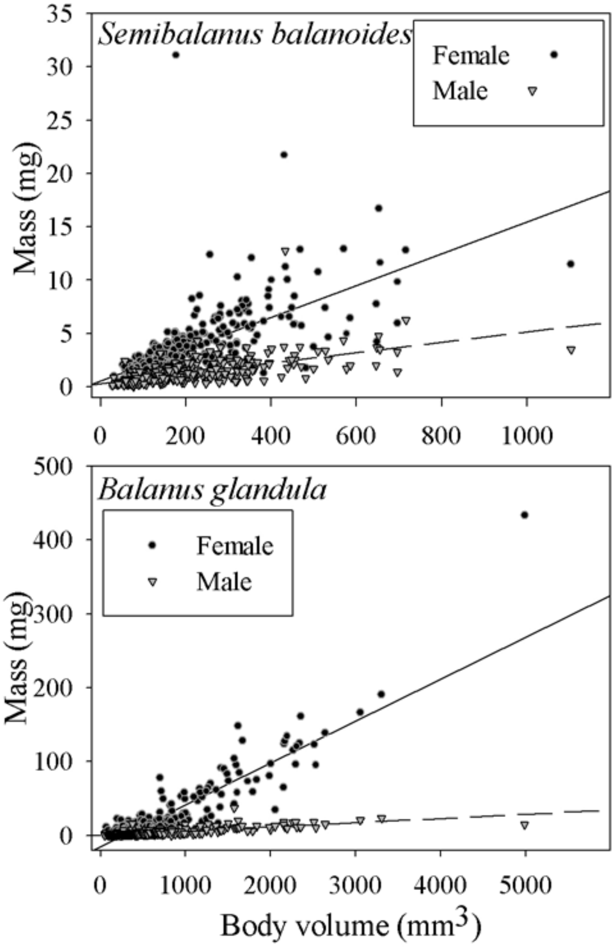

Fig. 5: Sex specific mass plotted against body volume. Female data points are shown as dark circles with a solid regression line. Male data points are shown as light triangles with a dashed regression line. Raw data are shown, although analyses were performed on log-transformed data.

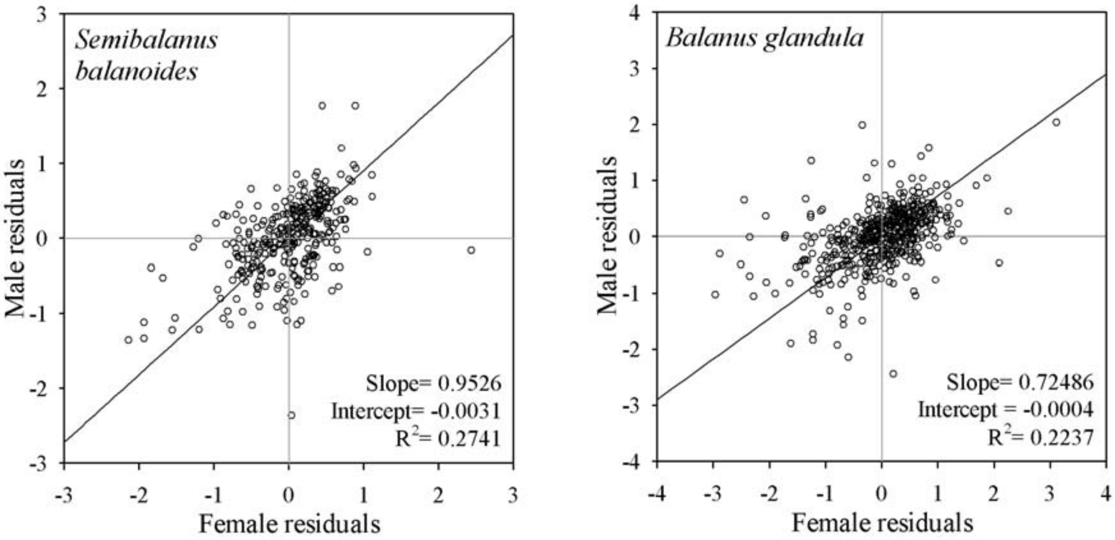

Fig. 6: Residual variation of male mass and female mass (against body volume) plotted against each other for both *S. balanoides* and *B. glandula*. The trend line shows the RMA regression line.

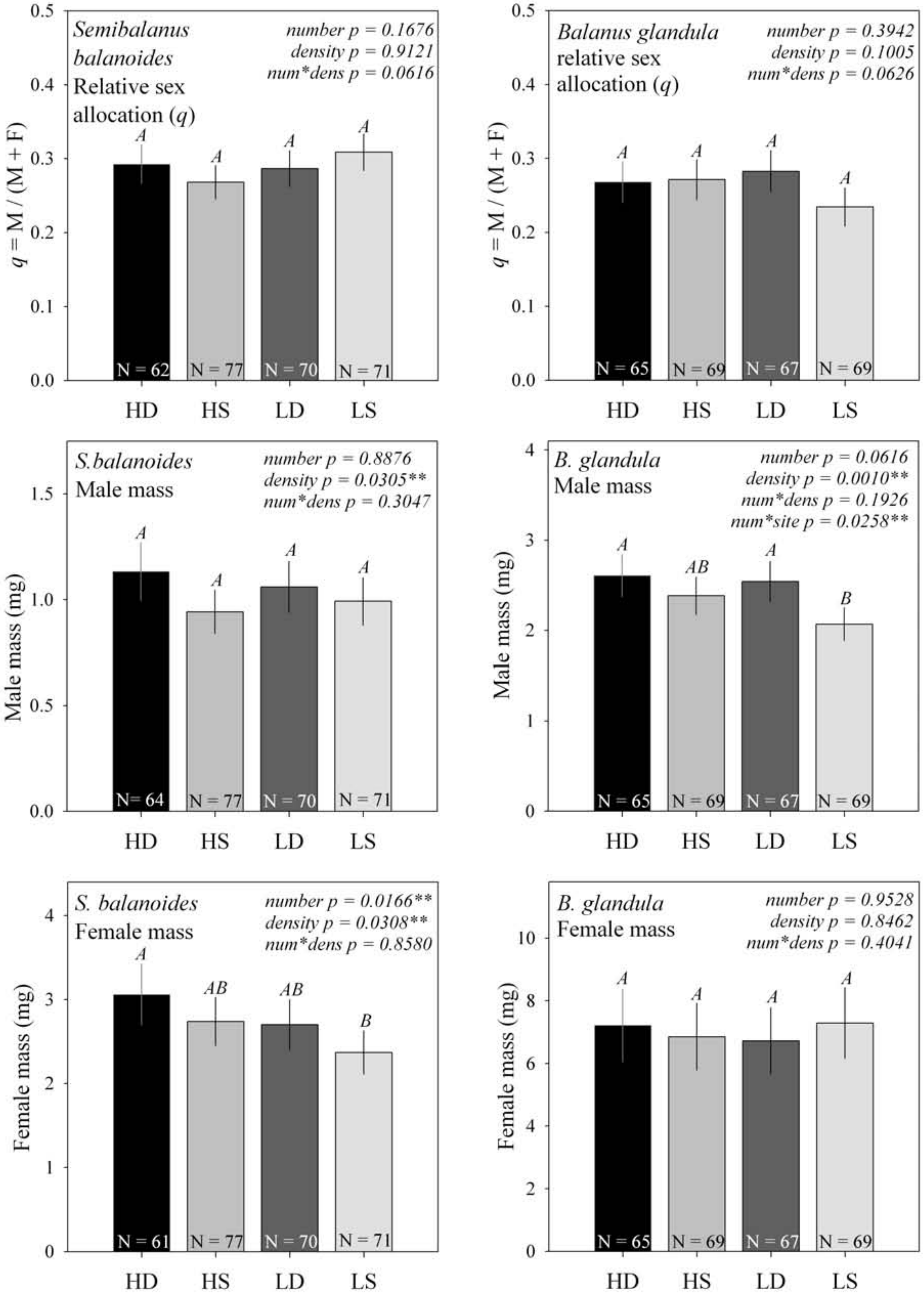
Semibalanus balanoides
Relative sex allocation (q)
number p = 0.1676
density p = 0.9121
num*dens p = 0.0616
q = M / (M + F)
0.0
0.1
0.2
0.3
0.4
0.5
A
A
A
A
N = 62
N = 77
N = 70
N = 71
HD
HS
LD
LS
Balanus glandula
relative sex allocation (q)
number p = 0.3942
density p = 0.1005
num*dens p = 0.0626
q = M / (M + F)
0.0
0.1
0.2
0.3
0.4
0.5
A
A
A
A
N = 65
N = 69
N = 67
N = 69
HD
HS
LD
LS
S.balanoides
Male mass
number p = 0.8876
density p = 0.0305**
num*dens p = 0.3047
Male mass (mg)
0.0
0.5
1.0
1.5
A
A
A
A
N= 64
N = 77
N = 70
N = 71
HD
HS
LD
LS
B. glandula
Male mass
number p = 0.0616
density p = 0.0010**
num*dens p = 0.1926
num*site p = 0.0258**
Male mass (mg)
0
1
2
3
4
A
AB
A
B
N = 65
N = 69
N = 67
N = 69
HD
HS
LD
LS
S. balanoides
Female mass
number p = 0.0166**
density p = 0.0308**
num*dens p = 0.8580
Female mass (mg)
0
1
2
3
4
A
AB
AB
B
N = 61
N = 77
N = 70
N = 71
HD
HS
LD
LS
B. glandula
Female mass
number p = 0.9528
density p = 0.8462
num*dens p = 0.4041
Female mass (mg)
0
2
4
6
8
10
A
A
A
A
N = 65
N = 69
N = 67
N = 69
HD
HS
LD
LS

Fig. 7 (preceding page): Plots showing the back-transformed least square means of *q* (above), male mass (center) and female mass (lower) of each treatment for *Semibalanus balanoides* (left) and *Balanus glandula* (right). P values listed in the upper right corner of each graph indicate significance of mate number, crowding density and the interactions between them as reported by two-way ANCOVA (and the site by mate number interaction for male function of *B. glandula*). Bars labeled with the same letter are not significantly different from each other according the Tukey-Kramer post hoc test; for example, the bars labeled *A* are not different than others labeled with an *A*; bars labeled *AB* are not different from bars labeled *A* and bars labeled *B*. Error bars show 95% confidence intervals. Sample size for each treatment is noted in each bar.

References

Anderson, D. T. 1994. *Barnacles: Structure, Function, Development and Evolution*. Chapman and Hall, New York.

Angeloni, L., J. W. Bradbury, and R. S. Burton. 2003. Multiple mating, paternity, and body size in a simultaneous hermaphrodite, *Aplysia californica*. Behavioral Ecology **14**:554-560.

Arnold, S. 1994. Bateman's Principle and the measurement of sexual selection in plants and animals. American Naturalist **144**:s126-s149.

Barnes, H., and M. Barnes. 1968. Egg numbers, metabolic efficiency of egg production and fecundity; local and regional variations in a number of common cirripedes. Journal of Experimental Marine Biology and Ecology **2**:135-153.

Barnes, H., M. Barnes, and W. Klepal. 1977. Studies on the reproduction of cirripedes. I. Introduction: copulation, release of the oocytes and formation of the egg lamellae. Journal of Experimental Marine Biology and Ecology **27**:195-218.

Barnes, H., and H. T. Powell. 1950. The development, general morphology, and subsequent elimination of barnacle populations after heavy initial settlement Journal of Animal Ecology **19**:175-179.

Barnes, M. 1992. The reproductive periods and condition of the penis in several species of common cirripedes. Oceanography and Marine Biology **30**:483-525.

Bateman, A. 1948. Intra-sexual selection in *Drosophila*. Heredity **2**:349-368.

Berglund, A. 1986. Sex change by a polychaete: effects of social and reproductive costs. Ecology **67**:837-845.

Bertness, M., S. Gaines, and S. Yeh. 1998. Making mountains out of barnacles: the dynamics of acorn barnacle hummocking. Ecology **79**:1382-1394.

Bohonak, A. J., and K. van der Linde. 2004. RMA: Software for Reduced Major Axis Regression, Java version. *in*. http://www.kimvdlinde.com/professional/rma.html. .

Chaine, A., and L. Angeloni. 2005. Size-dependent mating and gender choice in a simultaneous hermaphrodite, *Bulla gouldiana*. Behavioral Ecology and Sociobiology **59**:58-68.

Charnov, E. 1980. Sex allocation and local mate competition in barnacles. Marine Biology Letters **1**:269-272.

Charnov, E. 1996. Sperm competition and sex allocation in simultaneous hermaphrodites. Evolutionary Ecology **10**:457-462.

Charnov, E. L. 1979. Simultaneous hermaphroditism and sexual selection. Proceedings of the National Academy of Sciences USA **76**:2480-2484.

Charnov, E. L. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton NJ.

Connell, J. 1961. Effects of competition, predation by *Thais lapillis*, and other factors on natural populations of the barnacle, *Balanus balanoides*. Ecological monographs **31**:61-104.

Crisp, D. J., and H. Barnes. 1954. The orientation and distribution of barnacles at settlement with particular reference to surface contour. Journal of Animal Ecology **23**:142-162.

Darwin, C. 1854. *The Balanidae (or Sessile Cirripedes); the Verrucidae, etc.: A Monograph of the Sub-class Cirripedia*. The Ray Society, London, UK.

Fischer, E. 1980. The relationship between mating system and simultaneous hermaphroditism in the coral reef fish, *Hypoplectrus nigricans* (Serranidae). Animal Behaviour **28**:620-633.

Ghiselin, M. T. 1969. The evolution of hermaphroditism among animals. Quarterly Review of Biology **44**:189-208.

Greeff, J. M., and N. K. Michiels. 1999. Sperm digestion and reciprocal sperm transfer can drive hermaphrodite sex allocation to equality. American Naturalist **153**:421-430.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science **156**:477-488.

Heath, D. J. 1977. Simultaneous hermaphroditism: cost and benefit. Journal of Theoretical Biology **81**:151-155.

Heath, D. J. 1979. Brooding and the evolution of hermaphroditism. Journal of Theoretical Biology **81**:151-155.

Hoch, J. M. 2008. Variation in penis morphology and mating ability in the barnacle, *Semibalanus balanoides*. Journal of Experimental Marine Biology and Ecology **359**:126-130.

Hoch, J. M. 2009. Adaptive plasticity of the penis in a simultaneous hermaphrodite. Evolution **63**: 1946-1953.

Klepal, W. 1990. The fundamentals of insemination in cirripedes. Oceanography and Marine Biology **28**:353-379.

Klinkhamer, P. G. L., T. J. de Jong, and H. Metz. 1997. Sex and size in cosexual plants. Trends in Ecology and Evolution **12**:260-265.

McCartney, M. 1997. Sex allocation and male fitness gain in a colonial, hermaphroditic marine invertebrate. Evolution **51**:127-140.

Morgan, M. 1994. Models of sexual selection in hermaphrodites, especially plants. American Naturalist **144**:s100-s125.

Munn, E. A., W. Klepal, and H. Barnes. 1974. The fine structure and possible function of the sensory setae of the penis of *Balanus balanoides* (L.). Journal of Experimental Marine Biology and Ecology **14**:89-98.

Murata, A., M. Imafuku, and N. Abe. 2001. Copulation by the barnacle *Tetraclita japonica* under natural conditions. Journal of Zoology **253**:275-280.

Neufeld, C., and A. Palmer. 2008. Precisely proportioned: intertidal barnacles alter penis form to suit coastal wave action. Proceedings of the Royal Society B: Biological Sciences **275**:1081-1087.

Norton, C. G., A. F. Johnson, and R. L. Mueller. 2008. Relative size influences gender role in the freshwater hermaphroditic snail, *Helisoma trivolus*. Behavioral Ecology **19**:1122-1127.

Peterson, C. W., and E. A. Fischer. 1996. Intraspecific variation in sex allocation in a simultaneous hermaphrodite: The effect of individual size. Evolution **50**:636-645.

Raimondi, P. T., and J. E. Martin. 1991. Evidence that mating group-size affects allocation of reproductive resources in a simultaneous hermaphrodite. American Naturalist **138**:1206-1217.

Scharer, L. 2009. Tests of sex allocation theory in simultaneously hermaphroditic animals. Evolution **63**: 1377-1405.

Scharer, L., L. M. Karlsson, M. Christen, and C. Wedekind. 2001. Size-dependent sex allocation in a simultaneous hermaphrodite parasite. Journal of Evolutionary Biology **14**:55-67.

Scharer, L., and P. Ladurner. 2003. Phenotypically plastic adjustment of sex allocation in a simultaneous hermaphrodite. Proceedings of the Royal Society of London B: Biological Sciences **270**:935-941.

Scharer, L., P. Ladurner, and R. M. Rieger. 2004. Bigger testes do work more: experimental evidence that testis size reflects testicular cell proliferation activity in the marine invertebrate, the free-living flatworm *Macrostomum* sp. Behavioral Ecology and Sociobiology **56**:420-425.

Scharer, L., P. Sandner, and N. K. Michiels. 2005. Trade-off between male and female allocation in the simultaneously hermaphroditic flatworm *Macrostomum* sp. Journal of Evolutionary Biology **18**:396-404.

Scharer, L., and C. Wedekind. 2001. Social situation, sperm competition and sex allocation in a simultaneous hermaphrodite parasite, the cestode *Schostocephalus solidus*. Journal of Evolutionary Biology **14**:942-953.

Sokal, R. R., and F. J. Rohlf. 1995. *Biometry, third edition*. W.H. Freeman and Company, New York.

Strathmann, M. 1987. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. University of Washington Press, Seattle.

Stubbings, H. 1975. *Balanus balanoides.* Liverpool University Press, Liverpool.

Tan, G., F. Govedich, and M. Burd. 2004. Social group size, potential sperm competition and reproductive investment in a hermaphroditic leech, *Helobdella papillornata* (Euhirudinea: Glossiphoniidae). Journal of Evolutionary Biology **17**:574-580.

Trouve, S., J. Jourdane, F. Renaud, P. Durand, and S. Morand. 1999. Adaptive sex allocation in a simultaneous hermaphrodite. Evolution **53**:1599-1604.

Vizoso, D. B., and L. Sharer. 2007. Resource dependent sex allocation in a simultaneous hermaphrodite. Journal of Evolutionary Biology **20**:1046-1055.

Yund, P. O. 1998. The effect of sperm competition on male gain curves in a colonial marine invertebrate. Ecology **79**:328-339.

Yusa, Y. 1996. The effects of body size on mating features in a field population of the hermaphroditic sea hare, *Aplysia kurodai* Baba, 1937 (Gastropoda: Opisthobranchia). Journal of Molluscan Studies **62**:381-386.

Yusa, Y. 2008. Size-dependent sex allocation and sexual selection in *Aplysia kurodai*, a hermaphrodite with nonreciprocal mating. Invertebrate Biology **127**:291-298.

Chapter 7 - Conclusions

In *Semibalanus balanoides*, penis length (as measured by number of annulations) is phenotypically plastic. Penises are longer in groups where the shells of neighbors are not in contact. This is not a response to neighbor distance or number of neighbors, but density alone. Penis thickness (measured as diameter at the base) is also phenotypically plastic, with barnacles from wave exposed sites growing penises with greater diameter. This plasticity confers fitness advantage and the thinner penises in sheltered sites are capable of stretching to greater distances than the thicker penises from exposed sites. The thicker penises from exposed sites seem to be better able to resist perturbation by wave action, but sacrifice the ability to stretch to distant mates. There may also be differing thresholds for hiding from waves between barnacles from exposed and protected sites. Overall, barnacles are better able to reach mates at greater distances in protected sites than in wave exposed sites.

Balanus glandula shows similar patterns of plasticity of functional morphology. They grow thicker penises (measured as diameter at the base) in wave exposed sites, but are limited from reaching neighbors at greater distances. This phenotypic plasticity has been previously documented for *B. glandula* (Neufeld and Palmer 2008). Penis length (measured in millimeters) does not vary with mate number, mate distance or mate density, but the number of annulations may (this work is forthcoming).

Sex allocation in both species of barnacle does not explicitly meet the predictions of Charnov's (1980) model. The relative allocation to male function (*q*; the sum of male specific investment divided by the sum to total reproductive investment) does not increase with increased levels of competition (whether gauged by barnacles as mate number, mate distance or mate density). This may be because there is not a direct tradeoff between male and female function. Instead, as measured by residuals of male and female sex allocation as a function of body size, allocation between sex roles is positively correlated. Allocation to male function does show a response to increased competition: barnacles in dense mating groups (with the tests of neighbors touching) have greater allocation to male mass. Female specific allocation is size-specific and seems to be limited by body size more than anything else. This is largely similar to size-specific sex allocation as observed in simultaneously hermaphroditic coral reef fish (Peterson and Fischer 1996) and sea hares (Angeloni et al. 2002, Angeloni 2003).

There remain many exciting opportunities for future research in the field of sex allocation and reproduction in barnacles. Fitness gain by barnacles strategically allocating to the sex roles would provide a fruitful measurement to test the optimality of sex allocation. In *Semibalanus balanoides*, the paternity of brooded embryos could be identified with microsatellite alleles. This could then be used to estimate fitness gain by individual functional males. The fitness of functional females is more easily calculated, by counts of the eggs within its brood. Comparisons of sex allocation patterns between barnacle species that vary for average mating group size may also be an instructive area of research. Phenotypic plasticity may only account for a small proportion of variation in sex allocation ratio in an individual hermaphrodite. Fixed differences in sex allocation between species may be a result of differing average population density or ability to reach mates. Finally, as populations expand, Allee effects at range margins, owing to an inability of barnacles to reach mates with their penises, may result in limiting the speed of migration.

References

Angeloni, L. 2003. Sexual selection in a simultaneous hermaphrodite with hypodermic insemination: body size, allocation to sexual roles and paternity. Animal Behaviour **66**:417-426.

Angeloni, L., J. W. Bradbury, and E. L. Charnov. 2002. Body size and sex allocation in simultaneously hermaphroditic animals. Behavioral Ecology **13**.

Charnov, E. 1980. Sex Allocation and local mate competition in barnacles. Marine Biology Letters **1**:269-272.

Neufeld, C. and A. R. Palmer. 2008. Precisely proportioned: intertidal barnacles alter penis form to suit coastal wave action. Proceedings of the Royal Society B: Biological Sciences **275**:1081-1087.

Peterson, C. W. and E. A. Fischer. 1996. Intraspecific variation in sex allocation in a simultaneous hermaphrodite: The effect of individual size. Evolution **50**:636-645.

References

Arsenault, D. J., K. B. Marchinko, and A. R. Palmer. 2001. Precise tuning of barnacle leg length to coastal wave action. Proceedings of the Royal Society B: Biological Sciences **268**:2149-2154.

Anderson, D. T. 1994. *Barnacles: Structure, Function, Development and Evolution*. Chapman and Hall, New York.

Angeloni, L. 2003. Sexual selection in a simultaneous hermaphrodite with hypodermic insemination: body size, allocation to sexual roles and paternity. Animal Behaviour **66**:417-426.

Angeloni, L., J. W. Bradbury, and E. L. Charnov. 2002. Body size and sex allocation in simultaneously hermaphroditic animals. Behavioral Ecology **13**.

Angeloni, L., J. W. Bradbury, and R. S. Burton. 2003. Multiple mating, paternity, and body size in a simultaneous hermaphrodite, *Aplysia californica*. Behavioral Ecology **14**:554-560.

Arnold, S. 1994. Bateman's Principle and the measurement of sexual selection in plants and animals. American Naturalist **144**:s126-s149.

Arsenault, D. J., K. B. Marchinko, and A. R. Palmer. 2001. Precise tuning of barnacle leg length to coastal wave action. Proceedings of the Royal Society B: Biological Sciences **268**:2149-2154.

Barnes, H. and H. T. Powell. 1950. The development, general morphology, and subsequent elimination of barnacle populations after heavy initial settlement Journal of Animal Ecology **19**:175-179.

Barnes, H. and M. Barnes. 1982. Effect of turbulence on the feeding and molting of the cirripede *Balanus balanoides* (L) given and algal diet. Journal of Experimental Marine Biology and Ecology **65**:163-172.

Barnes, H., and M. Barnes. 1968. Egg numbers, metabolic efficiency of egg production and fecundity; local and regional variations in a number of common cirripedes. Journal of Experimental Marine Biology and Ecology **2**:135-153.

Barnes, H., M. Barnes, and W. Klepal. 1977. Studies on the reproduction of cirripedes. I. Introduction: copulation, release of the oocytes and formation of the egg lamellae. Journal of Experimental Marine Biology and Ecology **27**:195-218.

Barnes, M. 1992. The reproductive periods and condition of the penis in several species of common cirripedes. Oceanography and Marine Biology **30**:483-525.

Bateman, A. J. 1948. Intra-sexual selection in *Drosophila*. Heredity **2**:349-368.

Berglund, A. 1986. Sex change by a polychaete: effects of social and reproductive costs. Ecology **67**:837-845.

Bertness, M., S. Gaines, and S. Yeh. 1998. Making mountains out of barnacles: the dynamics of acorn barnacle hummocking. Ecology **79**:1382-1394.

Bohonak, A. J. and K. van der Linde. 2004. RMA: Software for Reduced Major Axis Regression, Java version. http://www.kimvdlinde.com/professional/rma.html.

Brauer, V., Scharer, L., Michiels, N., 2007. Phenotypically flexible sex allocation in a simultaneous hermaphrodite. Evolution **61:** 216-222.

Chaine, A., and L. Angeloni. 2005. Size-dependent mating and gender choice in a simultaneous hermaphrodite, *Bulla gouldiana*. Behavioral Ecology and Sociobiology **59**:58-68.

Charnov, E. 1980. Sex allocation and local mate competition in barnacles. Marine Biology

Letters **1**:269-272.

Charnov, E. 1982. *The Theory of Sex Allocation*. Princeton University Press, Princeton, NJ.

Charnov, E. 1996. Sperm competition and sex allocation in simultaneous hermaphrodites. Evolutionary Ecology **10**:457-462.

Charnov, E. L. 1979. Simultaneous hermaphroditism and sexual selection. Proceedings of the National Academy of Sciences USA **76**:2480-2484.

Cody, R. P. and J. K. Smith. 2006. *Applied Statistics and the SAS Programming Language. 5th edition*. Pearson Prentice Hall, Upper Saddle River, NJ.

Cody, R. P. and J. K. Smith. 1997. *Applied Statistics and the SAS Programming Language. 4th edition*. Pearson Prentice Hall, Upper Saddle River, NJ.

Connell, J. 1961. Effects of competition, predation by *Thais lapillis*, and other factors on natural populations of the barnacle, *Balanus balanoides*. Ecological monographs **31**:61-104.

Crisp, D. J. and A. J. Southward. 1961. Different types of cirral activity of barnacles. Philosophical Transactions of the Royal Society of London B **243**:271-307.

Crisp, D. J. and B. S. Patel. 1958. Relation between brooding and ecdysis in cirripedes. Nature **181**:1078-1079.

Crisp, D. J. and H. Barnes. 1954. The orientation and distribution of barnacles at settlement with particular reference to surface contour. Journal of Animal Ecology **23**:142-162.

Crisp, D. J. and MacLean. 1990. The relationship between the dimensions of the cirral net, the beat frequency and the size and age of the animal in *Balanus balanoides* and *Eliminius modestus*. Journal of the Marine Biological association of the UK **70**:505-514.

Darwin, C. 1854. *The Balanidae (or Sessile Cirripedes); the Verrucidae, etc.: A Monograph* *of the Sub-class Cirripedia*. The Ray Society, London, UK.

Darwin, C. 1854. *A monograph on the sub-class Cirripedia, with figures of all the species. The Balanidæ, (or sessile cirripedes); the Verrucidæ, etc. etc. etc.* The Ray Society, London.

Denny, M. W. 1987. Life in the maelstrom: The biomechanics of wave-swept rocky shores. Trends in Ecology and Evolution **2**:61-66.

Fischer, E. 1980. The relationship between mating system and simultaneous hermaphroditism in the coral reef fish, *Hypoplectrus nigricans* (Serranidae). Animal Behaviour **28**:620-633.

Fisher, R. A. 1930. *The Genetical Theory of Natural Selection*. Clarendon Press, Oxford.

Ghiselin, M. T. 1969. The evolution of hermaphroditism among animals. Quarterly Review of Biology **44**:189-208.

Greeff, J. M., and N. K. Michiels. 1999. Sperm digestion and reciprocal sperm transfer can drive hermaphrodite sex allocation to equality. American Naturalist **153**:421-430.

Hamilton, W. D. 1967. Extraordinary sex ratios. Science **156**:477-488.

Heath, D. J. 1977. Simultaneous hermaphroditism: cost and benefit. Journal of Theoretical Biology **81**:151-155.

Heath, D. J. 1979. Brooding and the evolution of hermaphroditism. Journal of Theoretical Biology **81**:151-155.

Hoch, J. M. 2008. Variation in penis morphology and mating ability in the barnacle, *Semibalanus balanoides*. Journal of Experimental Marine Biology and Ecology **359**:126-130.

Hoch, J. M. 2009. Adaptive plasticity of the penis in a simultaneous hermaphrodite. Evolution

63: 1946-1953.

Klepal, W. 1990. The fundamentals of insemination in cirripedes. Oceanography and Marine Biology **28**:353-379.

Klepal, W., H. Barnes, and E. A. Munn. 1972. The morphology and histology of the cirripede penis. Journal of Experimental Marine Biology and Ecology 10:243-265.

Klepal, W., H. Barnes, and M. Barnes. 1975. Variability in the 'loss' of the penis in *Balanus balanoides* (L.) under natural and experimental conditions and histology of degeneration. Pages 275-286 *in* Proc. 9th European Marine Biological Symposium. Aberdeen University Press.

Klepal, W., H. Barnes, and M. Barnes. 1977. Studies on reproduction in cirripedes. VI. Passage of spermatozoa into the oviducal sac and closure of pores. Journal of Experimental Marine Biology and Ecology **27**:289-304.

Klinkhamer, P. G. L., T. J. de Jong, and H. Metz. 1997. Sex and size in cosexual plants. Trends in Ecology and Evolution **12**:260-265.

Lorenzi, M., Sella, G., Schleicherova, D., Ramella, L., 2005. Outcrossing hermaphroditic polychaete worms adjust their sex allocation to social conditions. Journal of Evolutionary Biology **18:** 1341-1347.

Marchinko, K. B. 2003. Dramatic phenotypic plasticity in barnacle legs (*Balanus glandula* Darwin): Magnitude, age dependence and speed of response. Evolution **57**:1281-1290.

Marchinko, K. B. and A. R. Palmer. 2003. Feeding in flow extremes: dependence of cirrus form on wave-exposure in four barnacle species. Zoology **106**:127-141.

McCartney, M. 1997. Sex allocation and male fitness gain in a colonial, hermaphroditic marine invertebrate. Evolution **51**:127-140.

Miller, L. P. 2007. Feeding in extreme flows: behavior compensates for mechanical constraints in barnacle cirri. Marine Ecology-Progress Series **349**:227-234.

Morgan, M. 1994. Models of sexual selection in hermaphrodites, especially plants. American Naturalist **144**:s100-s125.

Munn, E. A., W. Klepal, and H. Barnes. 1974. The fine structure and possible function of the sensory setae of the penis of *Balanus balanoides* (L.). Journal of Experimental Marine Biology and Ecology **14**:89-98.

Murata, A., M. Imafuku, and N. Abe. 2001. Copulation by the barnacle *Tetraclita japonica* under natural conditions. Journal of Zoology **253**:275-280.

Neufeld, C. and A. Palmer. 2008. Precisely proportioned: intertidal barnacles alter penis form to suit coastal wave action. Proceedings of the Royal Society B: Biological Sciences **275**:1081-1087.

Norton, C. G., A. F. Johnson, and R. L. Mueller. 2008. Relative size influences gender role in the freshwater hermaphroditic snail, *Helisoma trivolus*. Behavioral Ecology **19**:1122-1127.

Pentcheff, N. 1991. Resistance to crushing from wave-borne debris in the barnacle *Balanus glandula*. Journal of Crustacean Biology **110**:399-408.

Peterson, C. W. and E. A. Fischer. 1996. Intraspecific variation in sex allocation in a simultaneous hermaphrodite: The effect of individual size. Evolution **50**:636-645.

Quinn, G. P., and M. J. Keough. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge.

Raimondi, P. T. and J. E. Martin. 1991. Evidence that mating group-size affects allocation of reproductive resources in a simultaneous hermaphrodite. American Naturalist

138:1206-1217.

Rasband, W. S. 1997-2007. ImageJ. US National Institute of Health, http://rsb.info.nih.gov/ij/, Bethesda MD.

Scharer, L. 2009. Tests of sex allocation theory in simultaneously hermaphroditic animals. Evolution **63**: 1377-1405.

Scharer, L., and C. Wedekind. 2001. Social situation, sperm competition and sex allocation in a simultaneous hermaphrodite parasite, the cestode *Schostocephalus solidus*. Journal of Evolutionary Biology **14**:942-953.

Scharer, L., and P. Ladurner. 2003. Phenotypically plastic adjustment of sex allocation in a simultaneous hermaphrodite. Proceedings of the Royal Society of London B: Biological Sciences **270**:935-941.

Scharer, L., L. M. Karlsson, M. Christen, and C. Wedekind. 2001. Size-dependent sex allocation in a simultaneous hermaphrodite parasite. Journal of Evolutionary Biology **14**:55-67.

Scharer, L., P. Ladurner, and R. M. Rieger. 2004. Bigger testes do work more: experimental evidence that testis size reflects testicular cell proliferation activity in the marine invertebrate, the free-living flatworm *Macrostomum* sp. Behavioral Ecology and Sociobiology **56**:420-425.

Scharer, L., P. Sandner, and N. K. Michiels. 2005. Trade-off between male and female allocation in the simultaneously hermaphroditic flatworm *Macrostomum* sp. Journal of Evolutionary Biology **18**:396-404.

Sokal, R. R. and F. J. Rohlf. 1995. *Biometry, third edition*. W. H. Freeman and Company, New York.

Strathmann, M. 1987. *Reproduction and Development of Marine Invertebrates of the Northern Pacific Coast*. University of Washington Press, Seattle.

Stubbings, H. 1975. *Balanus balanoides*. Liverpool University Press, Liverpool.

Tan, G., F. Govedich, and M. Burd. 2004. Social group size, potential sperm competition and reproductive investment in a hermaphroditic leech, *Helobdella papillornata* (Euhirudinea: Glossiphoniidae). Journal of Evolutionary Biology **17**:574-580.

Thompson, T. L. and E. P. Glenn. 1994. Plaster standards to measure water motion. Limnology and Oceanography **39**:1768-1779.

Trouve, S., J. Jourdane, F. Renaud, P. Durand, and S. Morand. 1999. Adaptive sex allocation in a simultaneous hermaphrodite. Evolution **53**:1599-1604.

Vizoso, D. B., and L. Sharer. 2007. Resource dependent sex allocation in a simultaneous hermaphrodite. Journal of Evolutionary Biology **20**:1046-1055.

Vogel, S. 2003. *Comparitve Biomechanics: Life's Physical World*. Princeton University Press, Princeton, NJ.

Wethey, D. S. 1984. Effects of crowding on fecundity in barnacles: *Semibalanus (Balanus) balanoides*, *Balanus glandula* and *Chthamalus dalli*. Canadian Journal of Zoology **62**:1788-1795.

Wu, R., C. Levings, and D. Randall. 1977. Differences in energy partition between crowded and uncrowded barnacles (*Balanus balanoides* Darwin). Canadian Journal of Zoology **58**:559-566.

Yund, P. O. 1998. The effect of sperm competition on male gain curves in a colonial marine invertebrate. Ecology **79**:328-339.

Yusa, Y. 1996. The effects of body size on mating features in a field population of the hermaphroditic sea hare, *Aplysia kurodai* Baba, 1937 (Gastropoda: Opisthobranchia). Journal of Molluscan Studies **62**:381-386.

Yusa, Y. 2008. Size-dependent sex allocation and sexual selection in *Aplysia kurodai*, a hermaphrodite with nonreciprocal mating. Invertebrate Biology **127**:291-298.