Variation in penis morphology and mating ability in the acorn barnacle, *Semibalanus balanoides*

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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.

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1. 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 (Trouve et al., 1999; Scharer and Wedekind, 2001; Scharer and Ladurner, 2003; Tan et al., 2004; Lorenzi et al., 2005; Brauer et al., 2007).

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, 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 et al., 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 et al., 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

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reaches a maximum for the brief mating season in early November, after which it is cast off with the next molt (Crisp and Patel, 1958; reviewed in Barnes, 1992). 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 et al., 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 (Wu et al., 1977; Wethey, 1984). 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.

S. 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.

2. Methods

2.1. Penis morphology

In November 2004, I collected *Semibalanus balanoides* from 2 sites in Long Island, NY. The wave exposed site, Shinnecock Inlet Jetty (40°50′23.19 N, 72°28′24.80 W) faces the open Atlantic Ocean. The protected site, Shinnecock County Park (40°50′35.64 N, 72°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 et al., 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 0.01 mm, 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:

$$\frac{1}{3}\pi h\left(r_1{}^2 + r_1 r_2 + r_2{}^2\right).$$

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. 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.

2.2. 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°50′23.19 N, 72°28′24.80 W), Shinnecock Inlet Jetty West (40°50′23.30 N, 72°28′39.00 W), Democrat Point Jetty (40°37′13.10 N, 73°18′23.30 W) and three in protected areas on Long Island Sound: Stony Brook Harbor (40°55′16.88 N, 73°08′58.04 W), Flax Pond Inlet (40°58′01.28 N, 73°08′17.00 W) and Cedar Beach Jetty (40°57′54.00 N, 73°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
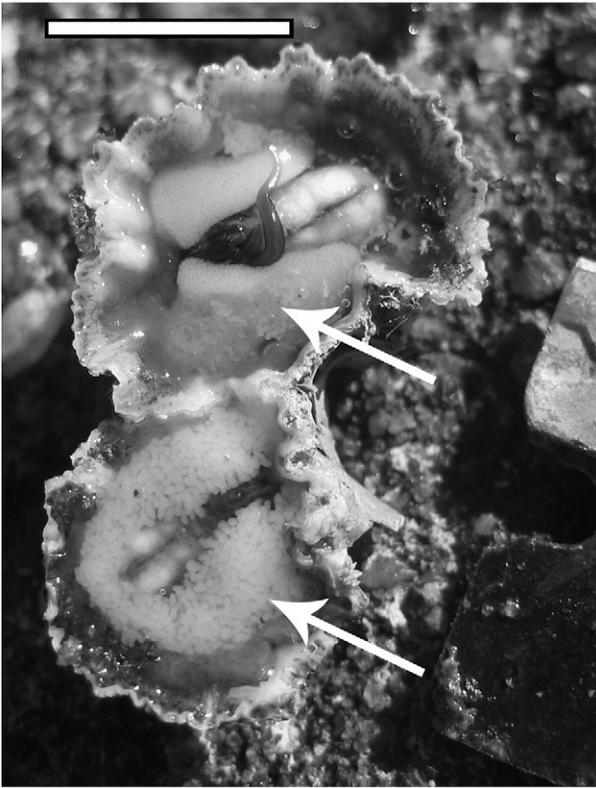
Fig. 1. A barnacle with fertilized eggs (above) and barnacle with unfertilized eggs (below). The fertilized egg masses are visible as compressed, solid pellets on either site of the body, with the penis and sperm mass are visible between them. The unfertilized egg mass is large and more diffuse, visible as globules. The scale bar is 1 cm.

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
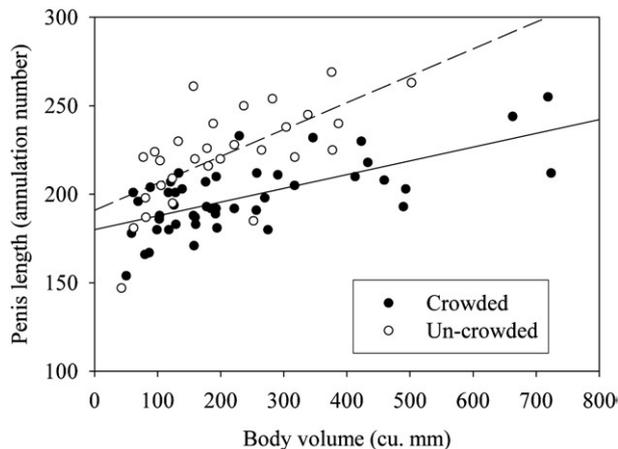
healthy adults, and there were no significant differences in body sizes or population densities between the different sites.

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.

3. Results

3.1. Penis morphology

Penises collected from barnacles in un-crowded aggregations (mean = 222.14 annulations, standard deviation = 27.0631, n = 29) were significantly longer (DF = 74, t = −4.20, P < 0.0001) than those from crowded aggregations (mean = 198.31 annulations, standard deviation = 19.8736, 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 annulations, standard deviation = 25.3824, n = 35) and the wave–exposed site, Shinnecock Inlet Jetty (mean = 210.46 annulations, standard deviation = 25.5510, 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 mm, standard deviation = 0.1861, n = 36) than at protected sites (mean diameter = 0.66 mm, standard deviation = 0.1597, 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

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.
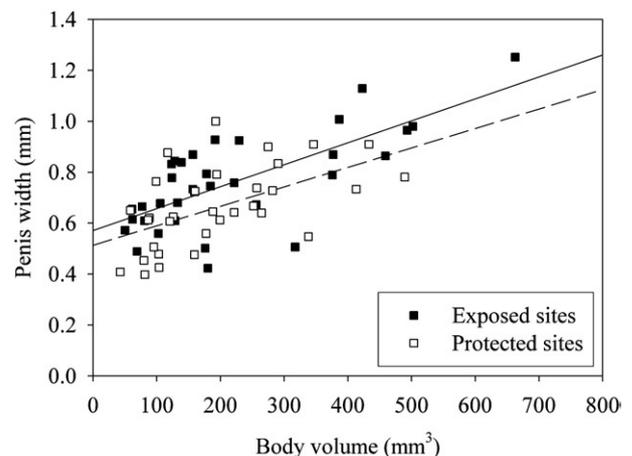

Fig. 3. Untransformed penis basal diameter (mm) plotted against body volume (mm^3) for barnacles from exposed (closed squares) and protected sites (open squares). The dashed line represents the OLS regression line for the protected site; the solid line represents OLS regression line for the exposed site.
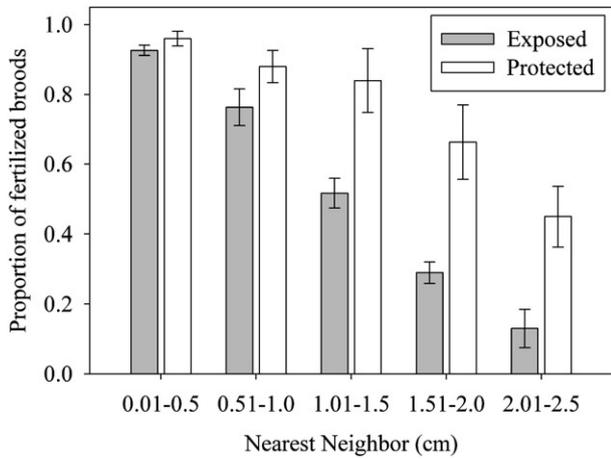
Fig. 4. Fraction of fertilized broods plotted against bins of nearest neighbor distance, comparing populations from sites exposed to waves (shaded) and protected sites (unshaded). Error bars show standard error.

diameter between crowded (mean diameter=0.68 mm, standard deviation=0.1392, n=25) and un-crowded barnacles (mean diameter= 0.66 mm, standard deviation=0.1696, 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).

3.2. 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, n=3) of broods were fertilized compared to 96% (s^2=0.000867, n=3) 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, n=3) were fertilized compared to 88% (s^2=0.004267, n=3) 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, n=3) were fertilized in the exposed sites compared to 84% (s^2=0.01680, n=3) 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, n=3) 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, n=3) were fertilized in the exposed site compared to 44% (s^2=0.0150, n=3) 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).

4. 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 (espe-

cially 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 have greater relative penis growth rates 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 to population density and wave action may be adaptive phenotypic plasticity, increasing fertilization success and ameliorating costs and risks associated with penis 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 et al., 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, 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.

Acknowledgements

References

Barnes, M., 1992. The Reproductive Periods and Condition of the Penis in Several Species of Common Cirripedes. Oceanogr. Mar. Biol. 30, 483–525.

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. J. Exp. Mar. Biol. Ecol. 27, 195–218.

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. Phenotypicall flexible sex allocation in a simultaneous hermaphrodite. Evolution 61, 216–222.

Charnov, E., 1979. Simultaneous hermaphroditism and sexual selection. Proc. Nat. Acad. Sci. USA 76, 2480–2484.

Charnov, E., 1980. Sex Allocation and local mate competition in barnacles. Mar. Biol. Lett. 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 ed. 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 Ecol. Evol. 2, 61–66.

Fisher, R., 1930. In: Bennett, J.H. (Ed.), The Genetical Theory of Natural Selection. Clarendon Press, Oxford. Variorum edition 1999.

Fischer, E., 1980. The relationship between mating system and simultaneous hermaphroditism in the coral reef fish, Hypoplectrus nigricans (Serranidae). Anim. Behav. 28, 620–633.

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. J. Exp. Mar. Biol. Ecol. 10, 243–265.

Lorenzi, M., Sella, G., Schleicherova, D., Ramella, L., 2005. Outcrossing hermaphroditic polychaete worms adjust their sex allocation to social conditions. J. Evol. Biol. 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. J. Zool. 253, 275–280.

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

Raimondi, P.T., Martin, J.E., 1991. Evidence That Mating Group–Size Affects Allocation of Reproductive Resources in a Simultaneous Hermaphrodite. Am. Nat. 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. J. Evol. Biol. 14, 942–953.

Scharer, L., Ladurner, P., 2003. Phenotypically plastic adjustment of sex allocation in a simultaneous hermaphrodite. Proc. R. Soc. Lond., B Biol. Sci. 270, 935–941.

Sokal, R., Rohlf, F., 1995. Biometry, 3rd ed. W.H. Freeman and Company, New York, NY.

Tan, G., Govedich, F., Burd, M., 2004. Social group size, potential sperm competition and reproductive investment in a hermaphroditic leech, Helobdella papillornata (Euhirudinea: Glossiphoniidae). J. Evol. Biol. 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. Can. J. Zool. 62, 1788–1795.

Wu, R., Levings, C., Randall, D., 1977. Differences in energy partition between crowded and uncrowded barnacles (Balanus balanoides Darwin). Can. J. Zool. 58, 559–566.