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Reproduction and early life history of Lottia asmi at Pigeon Point, California

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REPRODUCTION AND EARLY LIFE HISTORY OF *LOTTIA ASMI* AT PIGEON POINT, CALIFORNIA

A Thesis Presented to the Faculty

of

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In Partial Fulfillment

of the Requirements for the Degree

Master of Science in Marine Science

By

Katherine S. Muhs

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CERTIFICATION OF APPROVAL

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ABSTRACT

REPRODUCTION AND EARLY LIFE HISTORY OF *LOTTIA ASMI* AT PIGEON POINT, CALIFORNIA

by Katherine S. Muhs

Lottia asmi is an unusual limpet of the family Acmaeidae as it often resides upon the gastropod *Tegula funebralis*. Little is known of the biology of this small limpet. The reproduction, growth and metamorphic cues of *L. asmi* were studied through field sampling and laboratory experiments. *L. asmi* spawned from March through November. Sexes remained determinate throughout the winter. Adults invested up to 50% of body mass to gonads. Juveniles grew quickly and reached reproductive maturity at 4.5 mm shell length, which corresponded to an age estimated at 8.1 months.

Veligers metamorphosed in the presence of the coralline alga *Calliarthron tuberculosum*, live *T. funebralis* and live *M. californianus*. Results indicated settling veligers may increase survival by cueing to specific substrata, including those substrata they will live upon as adults.

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Chapter One

REPRODUCTION OF *LOTTIA ASMI*

INTRODUCTION

The suite of life history traits exhibited by any marine invertebrate species is closely tied to the environment in which it lives. Of the 16 species of limpets of the family Acmaeidae that inhabit rocky shores along the California coast, most live attached to rocky substrata, although a few have formed associations with living organisms (Lindberg 1981). One form of *Collisella digitalis*, for example, lives on shells of the stalked barnacle *Pollicipes polymerus* (Lindberg 1981). *Lottia instabilis* occurs on holdfasts and stipes of the brown algae *Laminaria dentigera* and *Pterygophera californica* (Lindberg 1981). *Notoacmea depicta* primarily occurs on the eel grass *Zostera marina*, while *N. paleacea* occurs only on the surf grass *Phyllospadix* (Choat and Black 1979, Lindberg 1981). In most cases, these substrata serve as living space and as a food source for the limpets.

Association with alternate substrata has been linked to variations of life history characteristics among limpet species. Choat and Black (1979) suggested that life history traits of *Acmaea insessa* are directly related to the fact that this limpet lives upon annual algae, *Egregia*. *A. insessa* reaches sexual maturity quickly (about 2.5 months after settlement), grows at seasonally constant rates to a maximum size of 13 mm, reproduces more than once and invests up to 70% of its body weight in gonads at any one

time. This strategy insures that *A. insessa* successfully settles, matures and reproduces before its host plant dies. Another example of a limpet that has accelerated development and high fecundity, and lives in an ephemeral environment is *Helcion pellucidum*, which occurs almost exclusively on laminarians (Vahl 1971).

The suite of life history characteristics exhibited by any one species can be described by any of several models. Two will be considered here. The r-K selection model describes fecundity and life span in relation to environmental predictability or stability. Dobzhansky (1950) first suggested that natural selection operates differently in the tropics than in temperate areas due to climate pulses, which are weak in the tropics and strong in temperate zones. MacArthur and Wilson (1967) developed the idea further and coined the terms "r-selection" to describe natural selection in environments conducive to rapid population growth (e.g. unpredictable environments), and "K-selection" to describe natural selection in saturated environments that favor the ability to compete and avoid predators (e.g. stable environments). R-selected populations exhibit early age at first reproduction, large clutch size, semelparity, no parental care, a large reproductive effort, small numerous offspring, low assimilation efficiency and a short generation time (Stearns 1976). In contrast, populations occupying saturated environments would be at or near carrying capacity, K, and exhibit delayed reproduction, iteroparity, small clutches, parental care, smaller reproductive effort, a few large offspring and high assimilation efficiency. Among limpets, Choat and Black (1979) linked r-selected characteristics with life upon ephemeral substrata such as annual

laminarians, and K-selection type characteristics with life upon stable substrata, such as rock benches. Vahl (1971, 1972) noted a similar relationship.

"Bet-hedging" is a model used to predict life history traits based on mortality of juveniles or adults (Stearns 1976). According to this model, high juvenile mortality favors reduced reproductive effort, greater age at first reproduction, smaller clutches, longer adult life and iteroparity. Conversely, unpredictable or heavy adult mortality would favor early, substantial investment in reproduction, large clutches and semelparity. Thus, a species inhabiting a fluctuating environment may favor reduced reproductive effort (contrary to predictions made by the r-selection model) if the environmental variability reduces survival of juveniles. Limpets that fit this model may delay reproduction until conditions allow maximization of juvenile survival (Branch 1981, Grahame and Branch 1985).

The limpet *Lottia asmi* is a prime candidate for investigating the relationship between an organism's life history strategy and its environment. *L. asmi* is a small (to 13 mm shell length), acmaeid limpet of the west coast of North America ranging from Alaska to Baja California. *L. asmi* has been reported living primarily on shells of the trochid gastropod *Tegula funebralis* (F. Test 1945, Eikenberry and Wickizer 1964, Lindberg 1990), but has also been reported living on rocks (Lindberg and Pearse 1990, Lindberg pers. comm.), on mussels (Lindberg 1981) and, in one instance, on the sponge *Halcyon* (Long 1968). *L. asmi* has been reported to occur also on *T. funebralis* shells inhabited by hermit crabs (Eikenberry and Wickizer 1964), but occurs infrequently upon this substratum along the central California coast

(personal observation). Although most of the California limpets are well-studied (A. Test 1945, Shotwell 1950, Fritchman 1961a, b, c, Kessel 1964, Abbott et al 1968, Kenny 1969, Wolcott 1973, Choat and Black 1979, Koppen et al 1996) little is known about the biology of *L. asmi*. Fritchman (1961b) reported briefly on its temporal reproductive patterns, but did not study them in depth. Given that *L. asmi* associates commonly and strongly with another gastropod, it would be valuable to learn if its life history traits are similar to those species that inhabit more permanently available rocky substrata, or if they are more like limpets that live epizoically on less stable substrata.

The purpose of this study was to identify certain life history features of *L. asmi* at Pigeon Point, CA. Goals were to 1) track reproductive condition throughout the year and identify timing of spawning events and senescence, 2) estimate mean numbers of eggs produced by females (per unit body weight), 3) determine size at reproductive maturity and estimate age at maturity, 4) determine sex ratio, 5) estimate growth rates from size-frequency distribution data and measurements of tagged individuals, and 6) compare results with similar information for other members of the family Acmaeidae occupying stable and unstable or ephemeral substrata.

METHODS

Study Site

Observations and sampling were carried out intertidally at the north side of Pigeon Point (37 degrees, 12 minutes, 20 seconds North; 122 degrees, 24 minutes West), San Mateo County, California. The study area was approximately 300 m x 100 m. Pigeon Point has been previously described by López-Gamundí (1993), and consists of a pebbly mudstone bench interspersed with areas of boulder fields. All arise from a bottom of gravel and/or sand. *T. funebralis*, *L. asmi*, fleshy red algae and coralline algae are abundant.

Shell Length, Height and Width Regressions

Limpet shells can be measured in a variety of dimensions including shell length, width or height. The dorsal tip of the shell is the shell's apex. When viewed from the side, the shell's opening is curved, beginning at the anterior base, rising to the shell curve's apex, then descending to the posterior base. Thus, two height measurements can be obtained (Figure 1). Maximum height is the measurement of the line from the shell apex to a horizontal line joining the shell's anterior-posterior base. Minimum height is the measurement of the line between the shell apex and the shell curve apex.

To determine if shell lengths can be used exclusively to describe limpet size, regression analyses were performed on length to maximum height, length to minimum height and length to width relationships. The shell of each limpet collected (n = 289) for assessment of reproductive condition or size-frequency analysis was measured to the nearest 0.01 mm with Vernier calipers and included in the regression analyses.

Reproductive Condition, Fecundity, Size at Maturity and Sex Ratio

To determine reproductive condition, mean number of eggs per female (per unit body weight), size at maturity and sex ratio, 25 limpets were haphazardly collected bi-monthly from Pigeon Point. Limpets were preserved in ethanol and returned to the lab for inspection. Shell lengths of *L. asmi* were measured with Vernier calipers to the nearest 0.01 mm. Animals were removed from their shells and weighed (wet weight) to the nearest 1.0 mg.

Gonad condition was assessed using a variation of the method used by Fritchman (1961a). Each individual was scored as "0" (indeterminate; sex cannot be identified), "1" (presence of eggs or sperm indicating sex, but gonads small and not visible without removing the digestive gland), "2" (gonads exhibiting low turgor and slightly visible), or "3" (gonads exhibiting high turgor). For purposes of this study, turgor is defined as the plumpness

of the gonads. A decrease in the mean score for samples from any single collection date indicated a spawning event.

Gonads were dissected from the animal and weighed (wet weight) to the nearest 1.0 mg, and in females, eggs were counted. The ratio of gonad weight to total body weight served as a second index of fecundity (gonad index) (Fletcher 1987), which indicated the percent of total somatic mass (wet weight) dedicated to reproduction. A decrease in the mean gonad index for samples from any single collection date indicated a spawning event.

To determine whether males and females spawn synchronously, data for the November spawning events were further analyzed separately for males and females. Linear regressions for male gonad weight as a function of somatic weight were performed on three data sets: November 6, November 20 and November 30 sampling dates. Regressions were similarly performed for females. Spawning events were indicated if the slopes of two successive data sets were significantly different (β-test from Zar 1974, $p < 0.05$).

The smallest limpet with mature gonads and the largest without mature gonads during peak reproductive periods indicated size range at maturity. This was identified graphically and by inspection of data.

Sex ratio (M:F) was determined using the following formulae:

M = number of males/ total number of limpets x 100

F = 100 - M.

Size-Frequency Distribution and Growth

Attempts to mark individual *L. asmi* in the field were unsuccessful, so growth was estimated using growth rates of limpets held in the laboratory. These data were compared to size frequency data collected in the field. To determine size frequency distribution of the *L. asmi* population at Pigeon Point, 51 to 93 quadrats (0.025 m^2) were randomly chosen monthly (biweekly on occasion) at the northern portion of Pigeon Point, San Mateo County, California. Limpets were collected in an area separate from the size-frequency sampling area to avoid confounding size-frequency data. Quadrats were systematically searched, and the shell length, maximum height, minimum height and width of the second *L. asmi* located in each quadrat was measured to the nearest 0.01 mm with Vernier calipers. Limpets too small for identification were tallied but noted as "species unidentified." Data were used to construct size-frequency histograms for 17 consecutive months, from February 1994 to July 1995, with the exception of January and March 1995, when heavy storm surf prevented data collection. Juvenile recruitment onto *T. funebralis* was identified as a peak in the curve for the smallest size categories. Analysis of cohort structure by classical techniques was made difficult by the merging and stacking of frequencies among size classes. Thus interpretation of these data was made by visual inspection of graphs.

To measure growth of adult *L. asmi* under laboratory conditions, two studies were undertaken. One lasted from August 12 to November 30, 1995, and the other lasted from March 17 to July 5, 1996. For each study, a pool of 100 limpets were randomly collected from Pigeon Point. Collections were made on August 12, 1995 and again the following spring on March 17, 1996. From each pool, five limpets in each of 3, 4, 5, 6 and 7 mm shell length categories were randomly selected. Measurements of limpet lengths were taken with Vernier calipers to the nearest 0.01 millimeters. The limpets were then tagged. Tags were cut from plastic tape and affixed to the shell with cyanoacrylic glue. Individual limpets could be identified by their unique combination of tag shape (square or triangle), color (red, green, yellow, blue, gray or white) and placement (right or left side of shell). Each limpet was then placed in a separate plastic container with 30 to 35 *T. funebralis* as a substrate and food source. Containers were covered with screened lids, placed in a sea water bath and held at ambient sea water temperature. Sea water flowed through the lid screens and out holes drilled in the bottom of the container. Pilot studies indicated that limpets died if fresh *T. funebralis* were not provided on a regular basis. Thus, *T. funebralis* were removed, returned to the shore and replaced with recently collected *T. funebralis* on a bi-weekly basis.

To compute growth rates, limpet shell lengths were measured on days 51, 77 and 111 of each experiment. Growth rates of individual limpets were

computed as Day 111 shell length minus Day 1 shell length, divided by 111 (the number of days in the study). Results were then averaged among limpets in each size class and among limpets during each season. Shell length changes were compared using a two-factor repeated measures ANOVA with size category and season as factors. Significant results (α = 0.025) indicated that each length class grows at a different rate and that limpets grow at seasonally different rates.

Because growth rate was dependent on initial size, growth study results would be confounded if organisms were not of the same size initially. This was a concern when comparing growth between spring and fall seasons, so initial limpet size data for each season were compared using a one-tailed t-test. If the t-test indicated limpets in each size class were significantly different initially, analysis of growth rates between seasons would be invalid.

For the two-factor repeated measures ANOVA analysis, data were assumed to be normally distributed, and the variance among differences between measurements was assumed to be constant. These assumptions were not tested because F-tests are robust to violations of these assumptions (Zar 1974). A scatterplot matrix comparing pairs of independent variables (Wilkinson et al 1996) was generated in SYSTAT to assess the assumption that covariances of repeated measures were homogeneous. Matrices indicated the assumption held true. In evaluating probability levels of the analysis of variance, a conservative approach was taken by choosing

stringent alpha levels ($\alpha = 0.025$) and by using Huyhn-Feldt corrected p-values rather than p-values associated with the F-tests (Kirby 1993). Results were similar whether F-test p-values or Huyhn-Feldt corrected p-values were used.

To determine growth rates of size classes smaller than 3 mm, growth of seven newly settled laboratory-raised limpets (see Chapter 2) was noted after metamorphosis. These juveniles were maintained for 66 days in 25 ml fingerbowls with the coralline alga *C. tuberculosum* as a substrate. Shell length measurements were made periodically using an ocular micrometer in an Olympus dissecting microscope at 40x magnification. Mean growth rates were calculated from these data and used in calculations to estimate age at reproductive maturity.

Age at reproductive maturity was estimated by summing growth rates for juvenile limpets and those raised in the laboratory for all size classes up to and including the smallest class in which mature gonads were observed. Where data for certain size classes were missing, data for the next largest size class were used as a conservative substitution (Sutherland 1970).

RESULTS

Shell Length, Height and Width Regressions

Maximum height, minimum height and width all varied consistently with shell length (Figure 2). Shell width exhibited the closest relationship ($p<0.001$). Minimum height had the least close relationship to shell length ($p<0.001$). The relationship of maximum height to length was intermediary ($p<0.001$). Length, therefore, is an accurate predictor of maximum height, minimum height and width, and can be used to describe limpet size.

Reproductive Condition, Fecundity, Size at Maturity and Sex Ratio

Specimens collected on January 28, 1994 indicated limpets were recovering from a period of senescence (Figure 3). Of 14 specimens, 10 were qualitatively scored as 0 (of indeterminate sex) and 4 were scored as 1. All limpets of identifiable sex were females. On this date the mean (± standard error) gonad index was 0.09 (± 0.02) for all determinate samples. By February 23, males were identifiable. Mean qualitative score for both sexes combined was 1 (± 0) and gonad index had increased to 0.24 (± 0.03). By May 11, the qualitative index for combined sexes had increased to 2.9 (± 0.1) and gonad index reached 0.31 (± 0.02).

By the end of May 1994, gonads in almost all samples collected were qualitatively scored as 3, indicating complete gametogenesis throughout the

population (Figure 3). Maximum mean gonad index observed for both sexes combined was 0.32 (± 0.02). Beginning in June, gonad indices began a gradual decline, and reached a minimum of 0.21 (± 0.03) on August 19. The index increased during the following two-and-a-half weeks to 0.32 (± 0.02), dropped slightly in September, increased again by October 8 to 0.31 (± 0.02), then dropped sharply by October 24 to a minimum for the year of 0.20 (± 0.02). In November the index increased again to 0.33 (± 0.02), then declined to a low of 0.21 (± 0.02) on November 30. The greatest gonad index observed was 0.50 in a male with a shell length of 6.07 mm and a somatic wet weight of 16 mg.

Quantitative gonad indices and qualitative assessments corresponded throughout the study period and indicated protracted spawning (Figure 3). A spawning event may have occurred in March 1994. A gradual decline in gonad index from May through August indicated ongoing spawning throughout the summer. An additional spawning event occurred in October and a final one in November. In each case, minimum body weight contributed by gonads ranged from 18 to 21 percent.

Number of eggs in females with gonads exhibiting high turgor ranged from 1.0×10^2 to 3.8×10^3 (Table 1). The female with the most eggs weighed 42 mg (total somatic wet weight) and her shell was 7.87 mm in length. Number of eggs per mg of gonad weight ranged from 46.5 to 316. The average (± standard deviation or s.d.) number of eggs per mg of gonad weight was 175.1 (± 140.6).

Limpets reach sexual maturity at approximately 4.5 to 5.0 mm length (Figure 4). The smallest *L. asmi* with gonads exhibiting high turgor (scored as 3) had a shell length of 4.57 mm.

Results of β-tests indicated sexes did not spawn simultaneously during November 1994 (Figure 5). The slope of the regression line for November 30, 1994 male gonad weight as a function of somatic weight was significantly different from November 20 data, indicating spawning had occurred ($t = 2.837$, $p = 0.01$). Inspection of similar graphs for females indicated spawning may have occurred between November 6 and November 20. The β-test, however, indicated spawning did not likely occur among females ($t = 1.541$, $p = 0.169$).

Inspection of a graph of gonad index as a function of shell length indicated no relationship between the two (Figure 6); therefore, larger females did not necessarily devote a larger percentage of somatic weight to gonad development than did smaller females.

Sex ratio of limpets collected for reproductive condition throughout the study period was 1.5:1.0 M:F. In comparison, sex ratio of the subset of limpets collected during the early part of this study, from January 1994 through May 1994, was 1.1:1 M:F.

Size-Frequency Distribution and Growth

The smallest size category of limpets observed on *T. funebralis* in the field was 1.00-1.49 mm (Figure 7). One limpet of this size was observed during June 1994, October 1994, January 1995, and June 1995. Limpets in the next largest size category, 1.50-1.99 mm, were observed on *T. funebralis* during the months of May, July and September, 1994 and during January, February and June 1995. The largest limpet recorded at any time was 8.84 mm in length and was observed once, during July 1994. A second limpet in the same size category (8.50 to 8.99 mm) was observed in October 1994. Its shell measured 8.33 mm in length.

Inspection of size-frequency histograms (Figure 7) revealed that growth of juveniles was relatively rapid. Limpets in the 4.0 - 4.49 mm size class in June 1994 appear to represent the modal size class. Shell lengths ranged from 4.01 to 4.47 mm with a mean of 4.17 (± 0.13) mm. In July the modal class had shifted to the next size class, 4.50 - 4.99 mm. Shell lengths at this time ranged from 4.52 to 4.98 mm with an average of 4.74 (± 0.16). Similarly, in September limpets in the 3.0 - 3.49 mm length class appeared to represent the modal size class. Shell lengths ranged from 3.05 to 3.43 mm with a mean of 3.22 (± 0.17). In October the mode had shifted to the 3.50 - 3.99 mm size class. Limpets ranged from 3.61 to 3.84 mm with a mean of 3.68 (± 0.09). Growth of larger limpets was difficult to analyze and is open to subjective interpretation.

Growth rates measured in the laboratory varied between seasons (Figure 8). Rates were higher during the fall than during the spring. This trend was seen in all but the largest limpets, where growth slowed and in some cases, was not measurable.

Mean daily growth rates measured in the laboratory varied among length classes (Figure 9). Overall, daily growth rates were inversely proportional to initial shell length. During the fall study the 3 mm size class grew an average of 0.02 (± 0.00) mm/day; the 4 mm class grew 0.01 (± 0.00) mm/day; the 5 mm class grew 0.01 (± 0.00) mm/day; the 6 mm class grew 0.00 (± 0.00) mm/day; and the 7 mm class grew 0.00 (± 0.00) mm/day. During the spring study, a similar trend was observed, although daily growth was less than that observed in the fall. The 3 mm size class grew an average of 0.01 (± 0.00) mm/day; the 4 mm class grew 0.01 (± 0.00) mm/day; the 5 mm class grew 0.01 (± 0.00) mm/day; the 6 mm class grew 0.00 (± 0.00) mm/day; and the 7 mm class grew - 0.01 (± 0.01) mm/day.

The two-factor repeated measures ANOVA showed that differences in shell growth varied among size classes and between seasons (Appendix Table 1). A significant difference in overall shell length changes was observed when data from all limpets were pooled. In addition, a significant difference in shell length changes between fall and spring (Figure 8) and among size classes (Figure 9) was observed.

Results of the t-test comparing shell length among limpets within each size category indicated that mean initial length of limpets in the spring were not significantly greater than the mean initial length of fall limpets ($p = 0.27$); thus, the data were comparable (Appendix Table 2).

Growth rate of juveniles was greatest initially, then slowed as the limpets grew larger (Figure 10). Ten days after metamorphosis, the mean limpet length was 0.325 mm (± 0.035). After 23 days, they were 0.738 (± 0.159) mm; after 34 days they were 0.875 (± 0.115) mm; after 42 days they were 1.005 (± 0.231) mm; and after 66 days they averaged 1.104 (± 0.07) mm. Thus, on average, limpets one mm in length were estimated to be approximately 42 days old (post-metamorphosis).

L. asmi was estimated to reach reproductive maturity within its first year. Approximately 42 days passed before laboratory-raised juveniles reached 1.00 mm in length (Figure 10). Growth rates of 1 mm and 2 mm size class limpets were not measured, but 3 mm size class rates, which would be theoretically slower, can be substituted to make a conservative estimate (Sutherland 1970). At 0.02 mm/day, it would take limpets 100 days to grow 2 mm. It would take them another 50 days to reach 4 mm at 0.02 mm/day, and another 50 days to grow another 0.5 mm at 0.01 mm/day. Summing these numbers leads to an estimate of 242 days to reach 4.5 mm, the size at first maturity (Appendix Table 3). Thus it can be concluded that based on juvenile

growth rates measured in the laboratory, mature limpets were approximately 8.1 months of age.

Discussion

L. asmi is a fast-growing limpet that likely matures during its first year of life. Females can produce a relatively great number of large eggs suggesting lecithotrophy. Although its reproductive cycle varies from year to year, this species is reproductively active during most of the year. Spawning does not appear to be always synchronous between males and females, nor does it appear to be synchronous within each sex.

Contrary to data reported by Fritchman (1961a), *L. asmi* in this study exhibited a protracted spawning pattern. Gonad indices and qualitative gonad assessment indicated a possible small spawning event during March. These data may represent a true spawning event, but may also reflect the fact that some limpet gonads examined had not completed gametogenesis after the previous winter senescence. Thus the drop likely represents an artifact of the large standard deviation in the gonad index (s.d. = 0.12 before spawning and 0.12 after spawning), indicating intrapopulation variation. Beginning in late May, limpets entered a prolonged spawning period lasting through the summer, followed by a rapid redevelopment of gonads in late August and early September. Another spawning event occurred in October followed by rapid redevelopment. A final spawning occurred during November. After the last spawning, gonads slowly redeveloped throughout the winter. This observed pattern alters the biannual spawning regime reported by Fritchman

(1961a) and is similar to that of other acmaeid limpets along the central California coast (A. Test 1945, Fritchman 1961a, b, c, Homna 1995).

Perhaps the most striking variation in reproductive cycle is the lack of reproductive senescence during this study. Fritchman (1961a) reported *L. asmi* entered a period of senescence after the fall spawning, and that gonads did not enter gametogenesis until January or February. Limpets at Pigeon Point underwent senescence during the winter of 1993-1994, but retained full gonads throughout the winter of 1994-1995.

This lack of reproductive senescence may be related to water temperature. Sea surface temperatures during the first week of October 1994 were similar to those during 1992 and to those reported by Fritchman (1961b). On October 8, however, temperatures dropped and, throughout the winter, remained one to three degrees Celsius colder than previous winters (Appendix Figure 1). Although direct cause-and-effect evidence does not exist as an explanation for the presence of gonads from October 1994 through January 1995, the severe drop in sea surface temperature is intriguing to note. Temperature has been identified as a possible spawning cue or reproductive regulator for other limpet species (Fritchman 1961a, b, c, Underwood 1974, Picken 1980). Of particular interest is *Patella vulgata*, which, like *L. asmi*, undergoes sexual senescence during part of the year. In an extensive study of the reproduction of this British species, Orton et al (1956) found that the start of maturation occurred earlier in locations with colder water temperatures.

The reliability of these results is strengthened by the use of two different gonad indices. The indices indicated similar reproductive conditions throughout the study period, and both methods revealed each spawning event. Although these methods have their limitations (Parry 1982a, Orton et al 1956) the agreement between them indicated the data were reliable to assess and to track spawning events and to estimate fecundity.

The fact that limpets did not appear to spawn synchronously is not surprising. Synchronous spawning benefits those species that broadcast spawn once each reproductive season (Picken 1980). During synchronous spawning, gametes of the population are released at once and are assured an equal chance of completing fertilization. The efficacy of this strategy is affected by currents and wave action (Lasiak 1990). Protracted asynchronous spawning among population members (males and females), on the other hand, facilitates successful settlement by increasing the number of "batches" of larvae over time. It also allows genetic variation in the progeny (Lasiak 1990). Asynchronous protracted spawning is common among Acmaeidae (Fritchman 1961a, b, c, Branch 1981) and may have adaptive significance given the dynamic nature of the intertidal environment. On the other hand, *L. asmi*, being the smallest limpet along the California coast, may have to increase its reproductive effort just to maintain its population. Protracted synchronous spawning among population members would ensure a steady supply of recruits.

The asynchronous spawning between the sexes observed during this study is somewhat troubling. If gametes of both sexes are not in the water at the same time, no fertilization can result, resulting in wastage. However, on November 20, for example, although male gametes increased mass on average, gametes of some individuals were scored as '1'. It appears, therefore, that some spawning by males had occurred.

Size-frequency histograms proved inadequate for quantitatively assessing cohort growth within the Pigeon Point population. The merging and stacking of adult size-frequencies made it difficult to analyze cohort structure. This stacking is a result of the protracted spawning season throughout the spring, summer and fall months, making identification of cohorts impossible (Creese 1981). When this study was begun, the single report in the literature regarding *L. asmi* reproductive cycles (Fritchman 1961a) indicated *L. asmi* spawned twice yearly, once in spring and again during the fall. Had this pattern held true, this species would have been a prime candidate for growth analysis using size-frequency histograms.

Growth of certain size classes was evident, however, during some months, and can be roughly estimated by tracking shifts in modal points (Seapy 1966). In June 1994 the 4.0 - 4.49 mm size class clearly represents the mode for the data set. The mean length for this class was 4.17 mm. One month later, on July 10 the mode had shifted to the 4.50 - 4.99 mm size class, with a mean length of 4.74. A similar pattern can be traced again during late

summer for the 3.00 - 3.49 mm size class. The mode for this class in September shifted to the next half-millimeter size class in October, representing a change in mean length from 3.20 to 3.68. These patterns can be interpreted to indicate cohort growth at a rate of approximately 0.57 mm per month from June to July and 0.49 mm from September to October. These results are greater than, but similar to growth rates observed during the laboratory growth study, which occurred from August to November and again from March to June the following year.

Growth of *L. asmi* is inversely proportional to size. An inverse relationship between growth rate and size is not uncommon among limpets (Kenny 1969, Sutherland 1970, Parry 1978, Picken 1980, Phillips 1981, Fletcher 1987) but is not always exhibited (Frank 1965). In *L. asmi*, early growth is exceptionally rapid and may indicate selective pressure to increase size or reach sexual maturity quickly, traits that have been theorized to relate to expanding populations (Stearns 1976) or obligate associations with annual algae (Choat and Black 1979). The selective pressure on *L. asmi* may be due to the dynamic nature of this species' substrate, *T. funebralis*.

Seasonal growth rate has been related to gametogenesis or food supply (Frank 1965, Seapy 1966, Sutherland 1970, Parry 1978). Increases in *L. asmi* shell length were greater during the fall than during the spring, when gonads are redeveloping after senescence and little energy for shell formation is available. During the fall, however, gonads were fully

developed; thus, greater energy is potentially available for shell formation (Parry 1982a, b). Seapy (1966) found growth rates in *Acmaea limatula* increased rapidly after spawning periods in April and October, and attributed the growth spurts to a minimal energy expenditure for gonadal build-up. *Notoacmaea scutum* also exhibits seasonal growth, which was positively correlated with the standing crop of macroalgae (Phillips 1981) rather than reproductive cycle. The relationship between growth rates of *L. asmi* in the field and food supply remains to be investigated.

Erosion is a factor in growth of mollusks and must be considered when evaluating age as a function of shell size (G. Cailliet, pers. comm.). In addition, poor organism health or slowed growth resulting from artificial conditions are a consideration during lab studies (Morse et al 1979, Morse and Morse 1984, P. Raimondi, pers. comm.). The proportions of "natural" growth and lab artifact contributing to the decrease in growth rate in these data, however, cannot be ascertained here. It would be presumptive to assume laboratory growth rates measured here are equivalent to growth rates in natural conditions. They do, however, provide a conservative growth rate estimate.

Compared to rock dwelling species (Frank 1965, Seapy 1966, Giesel 1969, Sutherland 1970, Phillips 1981, Creese and Ballantine 1983), *L. asmi* exhibits an accelerated growth rate and relatively large reproductive output. The life history traits of *L. asmi* most closely resemble those of limpets that

live epizoically or stenotypically upon other marine species. *Helcion pellucidum* of Great Britain, for example, lives epiphytically upon various species of macroalgae, primarily laminarians. It recruits at a modal size of 2.5 mm, becomes sexually mature at 5 to 8 mm length (depending on subspecies) and grows 10 to 12 mm during its first year. Breeding occurs throughout the year and larval life lasts a few weeks (Graham and Fretter 1947, Vahl 1971). Along the central California coast, *Notoacmaea insessa*, which is epiphytic upon *Egregia*, grows quickly and invests up to 70% of its somatic weight to gonads (Fritchman 1961b, Choat & Black 1979, Lindberg 1981). Choat and Black (1979) attributed this species' fast growth and high fecundity to the annual life-cycle of the alga upon which it lives.

Because *L. asmi* is a broadcast spawner, survivorship of larvae increases with an increase in the number of eggs released. Eggs appeared to be large (averaging 200 microns in diameter) and lecithotrophic. The greatest number of eggs observed in a female was 3,800, which is much fewer than other larger limpets. In *Lottia instabilis*, for example, up to 53,000 eggs have been observed (Homna 1995). Number of eggs a female *L. asmi* can produce is limited due to the small size of this species, and therefore, the size of *L. asmi* may constrain its reproductive capability and thus its population abundance. *L. asmi* does increase fecundity as body size increases, similar to other limpets (Branch 1974, Creese 1980, Homna 1995). This allows smaller limpets to contribute at least some gametes during spawning. Fecundity

limitations of this small species may also be offset, at least in part, by rapid growth to maturity and multiple yearly spawnings.

There may be substantial export of *L. asmi* larvae away from Pigeon Point as well as import of larvae from other sites up or down the coast. This is evidenced by the fact that recruitment followed most but not all spawning events by about one to two months. Spawning events occurred during March, May, September, October and November of 1994. From the size-frequency histograms, recruitment was evident during June and October of 1994 and January 1995. Import and export of larvae is advantageous to marine invertebrate species as it contributes to gene-pool mixing, extends the range of the breeding population and may directly influence community structure (Roughgarden et al 1985, Roughgarden et al 1988, Possingham and Roughgarden 1990). Size-frequency histograms may also reflect recruitment of other limpet species, as recruits were not identified to species and juveniles of other limpet species are known to occur on *T. funebralis* (Brewer 1975). Additional recruitment may have occurred but may have not been detected, as *T. funebralis* shells were inspected visually for recruits using a magnifying lens, but due to constraints of working in the field, were not examined microscopically.

L. asmi could be considered an r-selected organism or a bet-hedger. If *L. asmi* were a bet hedger, one could predict that irregular heavy adult mortality would be a greater influence than environmental variability

resulting in reduced juvenile survival (Stearns 1976). *L. asmi* does experience periodic massive disturbance, resulting in adult mortality. Winter storm waves toss *T. funebralis* about, thereby dislodging its *L. asmi* associates (personal observation). Those that cannot regain purchase on an appropriate substrate perish. If this disturbance were frequent enough or traumatic enough to the population, it may become an important factor in natural selection. Rapid growth to reproductive maturity may then evolve, allowing recruits to spawn before the approach of the next storm season. On the other hand, *L. asmi* is r-selected by definition. It produces a large amount of eggs relative to its body weight and grows to maturity more quickly than many limpets in the family Acmaeidae. Its accelerated growth rate may allow newly settled juveniles to reach sexual maturity and reproduce before winter approaches and gonads senesce. R-selection is not a predictor of a particular suite of environmental influences, except to say that it is favored in unpredictable environments resulting in rapid population growth.

To *L. asmi*, *T. funebralis* may represent a semi-unpredictable environment because of its susceptibility to be dislodged by storm waves. R-selection characteristics in *L. asmi* may also be a consequence of this species' small size, which may be dictated by other factors, such as interspecific competition, that subsequently led it to take up residence on another organism. This would account for the abundance of this limpet despite the reproductive constraints resulting from its size.

Although *L. asmi* is no longer considered to be a stenotypic species, it does occur commonly and in great abundance upon *T. funebralis*. Life upon this gastropod may relieve this small limpet of pressures imposed by sessile organisms (i.e., space constraints) or by interspecific competition for food or other resources. For the life history traits observed in *L. asmi* to have evolved, those pressures must have been sufficiently strong to result in natural selection for those traits.

Chapter Two

METAMORPHOSIS OF *LOTTIA ASMI* VELIGERS IN LABORATORY CULTURE

INTRODUCTION

Classic rocky shore ecological theory states that planktonic larvae of intertidal invertebrates wash onto shore and settle randomly; those that settle and undergo metamorphosis in a suitable place survive (e.g. Colman 1933; also see reviews by Morse 1990, Pawlik 1992). In the last few decades, descriptions of larval settlement and metamorphosis in response to specific cues have grown common in the literature. While it is still generally accepted that environmental signals (illumination, physical texture, salinity, etc.) play a role in larval settlement and metamorphosis (Crisp 1974), it is becoming apparent that species-specific cues play a major role in inducing larval settlement and metamorphosis of some species (Henderson and Lucas 1971, Switzer-Dunlop and Hadfield 1977, Heslinga 1981, Sebens 1983, Morse et al 1988, Pawlik 1992).

Cues that induce settlement and metamorphosis often relate to trophic requirements or presence of conspecific adults. Juveniles or adults of some species such as nudibranchs, chitons and abalone have specific food requirements, and the larvae may settle only if food organisms are present (Barnes and Gonor 1973, Steneck 1982, Hadfield 1986, Chia and Koss 1988). Other invertebrates such as the sand dollar *Dendraster excentricus*

(Highsmith 1982), the oyster *Crassostrea* sp. (Bonar et al 1990) and the polychaete *Phragmatopoma californica* (Jensen and Morse 1984) settle in response to the presence of conspecific adults. For sessile organisms, gregarious settlement guarantees that other adults will be present for reproduction (Pawlik 1992).

A variety of intertidal organisms are induced to settle and metamorphose when they contact coralline algae. These include tubeworms (Gee 1965), abalone (Morse and Morse 1984), the limpet *Acmaea testudinalis* (Steneck 1982), asteroids (Henderson and Lucas 1971, Barker 1977), and chitons (Barnes and Gonor 1973, Rumrill and Cameron 1983) among others. These species may rely upon coralline algae as a food source for juveniles, for cover or for refuge from predators, but may not associate with coralline algae as adults.

Marine invertebrate larvae may also settle and metamorphose in response to specific cues if they live stenotypically or epizoically upon a specific substratum. Gomez (1973) reported that the barnacle *Balanus galeatus* settled only upon certain species of gorgonians, and metamorphosed individuals cannot survive in the absence of this substratum. Other species of barnacles settle and metamorphose only on whales, sea turtles, or other marine vertebrates (Crisp 1974). Few settling assays have been performed, however, on such stenotypic or epizoic species.

In California, several acmaeid limpets live stenotypically upon certain species of algae (Branch 1981, Lindberg 1981) and one species, *L. asmi*, was originally reported to live stenotypically upon the gastropod *Tegula funebralis* (Test, A. 1945, Test, F. 1945). Later researchers reported *L. asmi* on

Mytilus californianus, rocks and *T. funebralis* shells inhabited by *Pagurus* (Eikenberry and Wickizer 1964, Lindberg 1981, Lindberg and Pearse 1990). The specific nature of the relationship between *L. asmi* and its biotic substratum (e.g., trophic, commensal, etc.) is unclear. No studies have been conducted to determine if any species-specific cues for metamorphosis exist for this limpet species or for any other California acmaeid.

In order to perform experiments that address questions of metamorphosis, a reliable source of veligers is required. Because veligers are not available commercially, the best way is for the experimenter to provide her own; no protocol exists, however, for spawning and raising *L. asmi* veligers in the laboratory. Spawning methods used in the past on other acmaeid limpets include the "thermal shock" method (Kessel 1964, Proctor 1968), in which limpets are placed in a water bath, then gradually warmed until limpets spawn, and the "teabag" or "stripping" method (Strathmann 1987), which involves dissection of gonads to free gametes.

The objectives of this study were to 1) develop a reliable protocol for spawning *L. asmi* and raising veligers to competency, 2) identify substrata that induce metamorphosis of *L. asmi*, and 3) determine the role, if any, that texture plays in inducing metamorphosis.

METHODS

Artificial Spawning

Spawning experiments were performed at Moss Landing Marine Laboratories, Moss Landing, CA. For all spawning trials, glassware was scrubbed in hot tap water, scrubbed in cold tap water, then rinsed with filtered sea water. All sea water used was filtered to 5μ with a filter bag obtained from Long Marine Laboratory in Santa Cruz, California.

To obtain gametes for artificial fertilization, three methods were attempted and evaluated based on three criteria: presence of gametes, quantity of gametes, and viability of zygotes. The first method was the thermal shock method used by Kessel (1964) with *Acmaea testudinalis* and by Proctor (1968) with *Notoacmea insessa*. Limpets were collected haphazardly from Pigeon Point and held overnight in the laboratory in flowing sea water at 16.5 ± 1.0°C. The following morning they were placed in individual containers and, over the following two hours, allowed to warm gradually to between 19°C and 23°C in indirect sunlight until spawning occurred. The "teabag" method (Strathmann 1987) was also attempted. An incision was made in the gonad of a limpet and the animal agitated in one of two 1000 ml beakers of sea water (one for males and one for females). Five to ten ml of sperm suspension was added to the eggs for fertilization.

In the third method, thirty to fifty adult *L. asmi* were haphazardly collected from Pigeon Point and returned to the laboratory. Five to eight limpets were placed in each of several clean 1000 ml beakers filled with 900 ml filtered sea water of ambient temperature (16 ± 1.0°C). Beakers were placed in a seawater bath and allowed to sit overnight. The next morning, trochophore larvae were present. The larvae were siphoned into clean 1000 ml beakers (200 to 300 larvae per beaker), which were filled with filtered sea water. Each beaker was placed in the water bath. During larval development, air was gently bubbled into each beaker to keep larvae from settling on the bottom where they were susceptible to bacterial infection and subsequent attack by ciliates.

Developing larvae were observed under a dissecting microscope and a compound microscope hourly the first day, and daily thereafter. Larval health was assessed and careful drawings of each development stage were made. A timetable of development was constructed for comparison to other limpet species.

Veliger Settling Experiments

To test the hypothesis that *L. asmi* larvae metamorphose only in the presence of specific substrata, two settling assay experiments were performed at Moss Landing Marine Laboratories' facility in Moss Landing, CA during

August and September, 1996. An additional experiment was performed in June 1997.

A series of pilot tests was performed to identify the age at which *L. asmi* veligers were competent to settle and metamorphose. Results indicated that larvae were competent to settle at 9 to 10 days of age. The onset of metamorphosis could be identified by the absence of a velum and a spreading of the eyespots indicating head development. Results of one experiment also indicated that a large percentage of larvae had metamorphosed in the presence of *C. tuberculosum* (mean = 43.3%, standard error or s.e. = 0.61%).

Experiment 1 was performed on 10-day-old larvae. Twenty larvae were pipetted into each of fifteen 10 ml fingerbowls. Five of the fingerbowls contained small (2.5 cm long) pieces of live *Calliarthron tuberculosum*, arranged so they covered most of the bottom of the fingerbowl, and five bowls contained one live *Tegula funebralis*. Five control fingerbowls contained only filtered sea water and larvae. A watchglass was placed over each container to prevent contamination from splashing water and to prevent *T. funebralis* from escaping. After 41 hours, larvae were observed and counted under an Olympus dissecting microscope at a magnification of 40x. Number of metamorphosing and non-metamorphosing larvae in each replicate was recorded.

Experiment 2 was performed on 9-day-old larvae and was designed to test whether or not veligers metamorphose in the presence of additional substrata. Twenty fingerbowls were set up similar to the first experiment. Treatments included live *C. tuberculosum*; dried *C. tuberculosum*, which had been collected and dried in the sun for 6 weeks; granite pebbles (approximately 5 cm diameter) and live *T. funebralis*. The dried *C. tuberculosum* were included in the experiment to address the question, do the alga's textural properties cue metamorphosis? Again, five control fingerbowls contained only filtered sea water and larvae. After 41 hours, larvae were observed and counted under an Olympus dissecting microscope at a magnification of 40x. Number of metamorphosing and non-metamorphosing larvae in each replicate was recorded.

A third experiment (Experiment 3) was conducted to identify a biotic substrate that would not cue metamorphosis. It was also designed to test whether or not *M. californianus*, another of *L. asmi*'s reported substrata (Lindberg 1981), would cue metamorphosis, and to see if larvae would metamorphose on *T. funebralis* shells in the absence of a live snail. Treatments for this experiment included the fleshy red alga *Mastocarpus hardeni* (a common and abundant red alga), empty *T. funebralis* shells, and live *M. californianus*. Live *C. tuberculosum* was also included, and fingerbowls containing filtered sea water and veligers served as additional controls. Because few larvae were available for this experiment, treatments

and the control were replicated three times, and fifteen larvae were placed in each replicate. After 41 hours, larvae were observed and counted under an Olympus dissecting microscope at a magnification of 40x. Number of metamorphosing and non-metamorphosing larvae in each replicate were recorded.

Data for all three experiments were analyzed using a log-likelihood ratio chi square, or G-test (Zar 1974) to test for significant differences among treatments and controls. The α level was set at 0.025. A significant difference indicated that the frequency with which larvae began metamorphosis differed among treatments. In cases where statistical significance was detected, analyses were repeated using pairwise comparisons to determine if the response frequency between treatment pairs differed. In such cases, probability levels were divided by the number of comparisons made to reduce the probability of making a type I error.

RESULTS

Artificial Spawning

Of the three methods attempted, the method that worked best was to collect adults, place a few in beakers and allow to sit overnight. This simple method produced the greatest number of gametes resulting in viable larvae. This method usually provided several thousand trochophores that could be aliquotted to additional beakers. Mortality was significant during development, but if initial trochophore numbers were great, then the number of healthy veligers at the end of 10 days was sufficient for experimentation. Bubbling air into each beaker increased survivorship, and larvae developed best in densities of 200 to 300 per beaker.

Spawning after treatment with thermal shock was sporadic at best. Spawning trials resulted in only one sex spawning or in polyspermy when males and females were placed in the same beaker. Only in one case did heat stress and subsequent artificial fertilization result in successful fertilization. Fertilized gametes developed to the 16-cell stage before they expired. Thus, this method did not meet the criteria as it did not provide adequate numbers of viable zygotes.

"Stripping" gametes did not work. *L. asmi* eggs are surrounded by the thin, membranous chorion, and during natural spawning the chorion is removed and fertilization is facilitated. Eggs that have been "stripped" retain the chorion and fertilization is prevented. In gonads dissected from males,

few sperm can free themselves from the mucousy mass of gonadal tissue to reach an egg. Thus, this method resulted in few zygotes and failed to meet the criteria.

L. asmi developed in a manner typical for acmaeid limpets (Table 1). Unfertilized eggs were approximately 200 microns in diameter. Although similar in size, fertilized eggs could be discerned by the presence of a fertilization membrane. Cleavage appeared spiral and within 15 to 20 minutes of fertilization, the cell began dividing and reached the 4-cell stage. Within 30 minutes, the embryos reached the 8-cell stage, and by 53 minutes they reached the 32-cell stage. About an hour and a half after fertilization cilia of the prototroch became evident, the apical tuft began to develop and embryos exhibited polar elongation, indicating the onset of gastrulation (Smith 1935). Within 12 hours, fully developed trochophores could be seen swimming. Within 23 hours the trochophores began to change to pre-torsional veligers and the protoconch began to develop. Torsion was complete within 37 hours and by 68 hours (nearly 3 days), larvae were exhibiting alternating creeping and swimming behavior. The eyespots, tentacles and operculum were also present. Nine to ten days after fertilization the larvae were competent to metamorphose. Larvae did not feed during this period.

The timetable of development for *L. asmi* appears more accelerated than that for *Acmaea testudinalis*, from the western North Atlantic (Kessel

1964). It also appears more accelerated than that for *Lottia digitalis* raised at Friday Harbor, Washington (Koppen et al 1996), and for *Patella vulgata* of the eastern North Atlantic (Smith 1935, Dodd 1957) (Table 2).

Veliger Settling Experiments

Results indicated a strong response to *C. tuberculosum* and *M. californianus*, a strong but lesser response to *T. funebralis*, and a minimal response to empty *T. funebralis* shells, *M. hardeni* and bare rocks. No larvae settled in the presence of dried *C. tuberculosum*.

In Experiment 1, larval settling response varied significantly among treatments and the control ($G = 109$, $p < 0.001$) (Figure 11). Larvae responded most strongly (mean ± s.e.) to the presence of *C. tuberculosum* (59.0% ± 3.1%) and less strongly to *T. funebralis* (38.0% ± 2.8%). No larvae showed signs of metamorphosis in the controls (0.0% ± 0.0%). A posteriori pairwise comparisons indicated that the frequency of larvae that metamorphosed on *C. tuberculosum* was significantly different than *T. funebralis* ($G = 8.89$, $p = 0.003$, p critical = 0.008).

In Experiment 2, *L. asmi* larvae did not respond to dried *C. tuberculosum* (0.0% ± 0.0%), responded weakly to scrubbed rocks (5.0% ± 2.0%) and responded strongly to the presence of *C. tuberculosum* (35.0% ± 3.26%) (Figure 12). Again, no larvae showed signs of metamorphosis in the controls (0.0% ± 0.0%). A log-likelihood ratio chi square indicated that

settling response varied among treatments ($G = 90.87$, $p < 0.001$). A posteriori pairwise comparisons indicated that the frequency of larvae metamorphosing in the presence of *C. tuberculosum* was significantly different than that for rocks ($G = 30.97$, $p = 2.6 \times 10^{-8}$, p critical = 0.006). A second a posteriori comparison indicated there was no significant difference between dried *C. tuberculosum* and rocks ($G = 7.06$, $p = 0.007$, p critical = 0.006). Because no larvae metamorphosed in the presence of dried *C. tuberculosum*, results for dried *C. tuberculosum* were equivalent to those for controls.

Results from Experiment 3 indicated larvae responded weakly (mean ± s.e.) in the presence of *Mastocarpus hardeni* (13.3% ± 1.7%) and *T. funebralis* shells (13.3% ± 3.85%) but strongly to the presence of *M. californianus* (40.0% ± 1.7%) and *C. tuberculosum* (53.3% ± 1.7%). No larvae showed signs of metamorphosis in the controls (0.0% ± 0.0%) (Figure 13). A log-likelihood ratio chi square indicated that settling response varied among treatments ($G = 54.55$, $p < 0.001$). A posteriori pairwise comparisons indicated that the frequency of larvae metamorphosing in the presence of *M. californianus* was not significantly different than that for *C. tuberculosum* ($G = 1.61$, $p = 0.204$), but was significant between *M. californianus* and *T. funebralis* shell ($G = 8.47$, $p = 0.004$, p critical = 0.005). Additional a posteriori testing indicated that frequency of larvae that metamorphosed on *M. hardeni*

was significantly different than the frequency that metamorphosed in controls ($G = 8.75$, $p = 0.003$, p critical $= 0.005$).

DISCUSSION

The reasons are unclear for the success of the spawning method used during this study. Limpets may have spawned due to stress. Some limpets were not attached to a substrate but landed upside-down when they were placed in the test beaker. (Of these, some had managed to right themselves and climb toward the top of the beaker, where they subsequently spawned.) A second source of stress may have originated from a decreased oxygen concentration in the water; dissolved oxygen, however, was not measured. The limpets may have also spawned in response to prolonged submersion. Spawning trials were further hampered by the fact that sexes cannot be identified in this species without dissection.

L. asmi veligers metamorphose in the presence of the substrate upon which adults commonly occur, as well as in the presence of habitats in which the adults do not occur. *L. asmi* veligers metamorphose in the presence of *C. tuberculosum* and *M. californianus*, and to a lesser degree, to live *T. funebralis*. A small percentage of larvae metamorphosed in the presence of *M. hardeni*, empty *T. funebralis* shells and rocks. No response was observed to dried *C. tuberculosum*.

The strong response to live *C. tuberculosum* is not surprising, as the importance of coralline algae to settling larvae of many marine phyla is well documented (Gee 1965, Henderson and Lucas 1971, Barnes and Gonor 1973,

Steneck 1982, Rumrill and Cameron 1983). Similar assays by Morse et al (1979) and Morse and Morse (1984) indicated that *Haliotis* veligers respond strongly to the presence of crustose or foliose algal species in the family Corallinaceae (Morse 1991). The agent that induced metamorphosis was identified as a group of small molecules that contained peptides (Morse and Morse 1984, Morse et al 1984). In addition, Morse et al (1979) and Morse and Morse (1984) established that *Haliotis* veligers must make physical contact with the algae to initiate metamorphosis, and the process is irreversible. In the experiments presented here, larvae may not have made physical contact with substrata and this may account for the disparity between expected and observed results. Nonetheless, the importance of coralline algae to *L. asmi* veligers has been established.

Evidence from additional studies confirmed that coralline algae beds are important habitats for additional marine invertebrates. These include the tubeworm *Spirorbis rupestris* (Gee 1965), the chitons *Tonicella lineata* (Barnes and Gonor 1973), *Mopalia muscosa* (Morse et al 1979) and *Katharina tunicata* (Rumrill and Cameron 1983), the asteroids *Acanthaster planci* (Henderson and Lucas 1971) and *Stichaster australis* (Barker 1977), the soft coral *Alcyonium siderium* (Sebens 1983), the scleractinian coral *Agaricia humilis* and two congenerics (Morse et al 1988), the gastropod *Trochus niloticus* (Heslinga 1981) and the limpet *Acmaea testudinalis* (Steneck 1982). Coralline algae beds provide these species shelter, refuge from predators and

in some cases, a source of food (Steneck 1982). For *L. asmi*, which often resides on *T. funebralis* shells, coralline beds may offer refuge to young limpets during the storm season while older *L. asmi* risk being dislodged from *T. funebralis* as the snails get tossed about by storm waves. The fact that *L. asmi* veligers did not respond to the dried *C. tuberculosum* indicates that coralline algae may provide this species something more than space or texture, and that the cue exists only on live algae. The specific nature of that cue is unknown. A group of GABA-mimicking molecules have been implicated as a species-specific cue for various marine invertebrate larvae worldwide (Morse 1992) and may cue *L. asmi* as well.

The significant responses to *T. funebralis* and *M. californianus* indicates that these organisms may be important substrata to settling *L. asmi* veligers. At Pigeon Point, newly settled juveniles can be found on the undersides of *T. funebralis* shells, near the operculum. This portion of the shell retains moisture during low tide, when *T. funebralis* clump in crevices (personal observation). Similarly, *M. californianus* beds create a cool, damp refuge during low tide, and juveniles of myriad organisms can be found beneath the mat of byssal threads (Ricketts et al 1968) as well as on the shells of *M. californianus* (personal observation). During high tide, both substrata may provide shelter and possible refuge from predation. Few studies have examined the possibility that marine larvae of species that are epibiotic upon mollusks settle and metamorphose in response to cues specific to mollusk

shells (Morse 1990, Pawlik and Hadfield 1990, Hadfield 1986). This is one of the first studies indicating that larvae of an epibiotic limpet may cue directly to the biotic substrate upon which the adults are commonly found. It remains to be seen whether the *L. asmi* larvae are responding to some chemical related to the periostracum of the *T. funebralis* and *M. californianus* shells, or to some other cue.

L. asmi larvae responded weakly to the presence of rocks as a metamorphic cue. Because the rocks used in this experiment were scrubbed and rinsed in fresh water, it is unlikely that the few larvae that did metamorphose were responding to any diatom or bacterial films present. If a small percentage of larvae in the controls had begun metamorphosis as well, then one could conclude that the larvae were stressed and metamorphosing as a "bail-out response." This, however, was not observed. On a broader scale, most of the substrata tested elicited a response, albeit a weak one. Thus, substrate specificity of *L. asmi* larvae is not as strong as has been reported for other marine invertebrate organisms, such as *Haliotis* .

Although significant numbers of *L. asmi* larvae did not metamorphose in the presence of *M. hardeni*, fleshy red macrophytes have been identified as species-specific cues for a variety of other marine invertebrate species. Veligers of the queen conch *Strombus gigas* have been induced to settle on intact *Laurencia poitei* (Siddal 1982), molecules from *Porphyra* induce metamorphosis of *Haliotis* larvae (Morse et al 1984) and

Aplysia californica have been shown to metamorphose on *Laurencia pacifica* (Kriegstein et al 1974). *Callithamnion halliae, Laurencia* sp. and *Chondrococcus hornemanni* have been implicated as species-specific cues of additional herbivorous opistobranch mollusks (Switzer-Dunlop and Hadfield 1977). Settling upon red algae may favor *L. asmi* veligers in the absence of a more suitable substratum, as veligers would not be precluded from settling and beginning metamorphosis. Subsequent chances for survival, however, may not be as great as they would have been had the larvae settled on a more favorable substrate.

The next step in this study would be to confirm the presence of metamorphosed juvenile *L. asmi* on each of the tested substrata in the field. Studies in the natural habitat have confirmed laboratory settling studies using coralline algae as a species-specific cue for other species. Morse et al (1979) reported *Haliotis rufescens* juveniles settle preferentially on crustose red algal species including *Lithothamnium, Lithophyllum* and *Hildenbrandia.* Densities of young *Haliotis* were greater by "orders of magnitude" than densities in surrounding habitats that lack these algae (Morse et al 1979). In controlled field experiments, Shepherd and Turner (1985) demonstrated that *H. laevigata* and *H. scalaris* recruit only onto coralline algae. Similar results were found for *H. rubra* (Prince et al 1987). Few studies directly demonstrate, *in situ*, the efficacy of a substance other

than coralline algae that has been identified as a settlement cue in the laboratory (Jensen and Morse 1990, Morse 1992).

If such *in situ* work were to be undertaken for *L. asmi*, two problems would have to be overcome. Most of the 16 or so limpet species along this coast cannot be identified until well after metamorphosis. One possible solution would be to use radular morphology as an identification tool. But before this can be done, careful documentation of radular changes during development of juvenile *L. asmi* is required. If radular features of limpets in the family Acmaeidae change with age as they do in *Patella vulgata* (Smith 1935), then adult radular morphology is not sufficient for identification of juveniles.

It would also be interesting to study survivorship of limpet veligers among the various substrata tested, both in the laboratory and in the field. Results of experiments presented here, and of similar work done by other researchers do not address post-metamorphosis mortality issues (see Morse 1992). Differential post-metamorphosis survival is crucial to our understanding of rocky shore ecology as it is directly related to distribution patterns of many benthic marine invertebrates (Connell 1985). Nonetheless, the strong response of *L. asmi* veligers to *T. funebralis* indicates the relationship between *L. asmi* and *T. funebralis* is ingrained into the life history of this organism.

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Table 1. Development of *Lottia asmi* veligers held in the laboratory at 16.5 ± 1.0°C. Observations were made on 12 different batches of larvae from adults spawned artificially in the laboratory.

Time	Development Stage	Notes
0 min.	fertilization	
15-20 min.	4-cell	
30 min.	8-cell	
53 min.	32-cell	
100 min.	gastrula	cilia of prototroch evident apical tuft developing shape elongated in a polar direction
<12.25 hours	trochophores	trochophores swimming
<23 hours	change to pre-torsion veligers	protoconch begins to develop
<37.25 hours	torsion	
67.75 hours	creeping behavior begins	larvae crawling and swimming; eyespots, tentacles and operculum present
9-11 days	competency	

Table 2. Comparative schedules of development stages for four species of limpets. *L. asmi* was raised at 16 ± 1.0°C, *Acmaea testudinalis* was raised at 12.1 ± 0.5°C, *Lottia digitalis* was raised at 9°C, and *Patella vulgata* was raised at 12.5 ± 0.5°C. Data for *A. testudinalis* taken from Kessel (1964). Data for *L. digitalis* taken from Koppen et al (1996). Data for *P. vulgata* taken from Smith (1935) and Dodd (1957). h = hours.

Development Stage	*L. asmi*	*A. testudinalis*	*L. digitalis*	*P. vulgata*
first cleavage		60 min.	60-90 min.	4 h
second cleavage	15-20 min.	90 min.	2-3 h	
third cleavage	30 min.	2.8 h	3-4 h	
ciliated blastula/gastrula	100 min.		14 h	21 h
trochophore	< 12 h	10-13 h	24 h	24-30 h
pre-torsional veliger	<23 h	30 h	48-72 h	48 h
torsion	<37.25 h	61 h		72-120 h
operculum	<67.75 h	62.5 h		
eyespots and tentacles developed; larva alternates between creeping and swimming	<67.75 h	85 h		

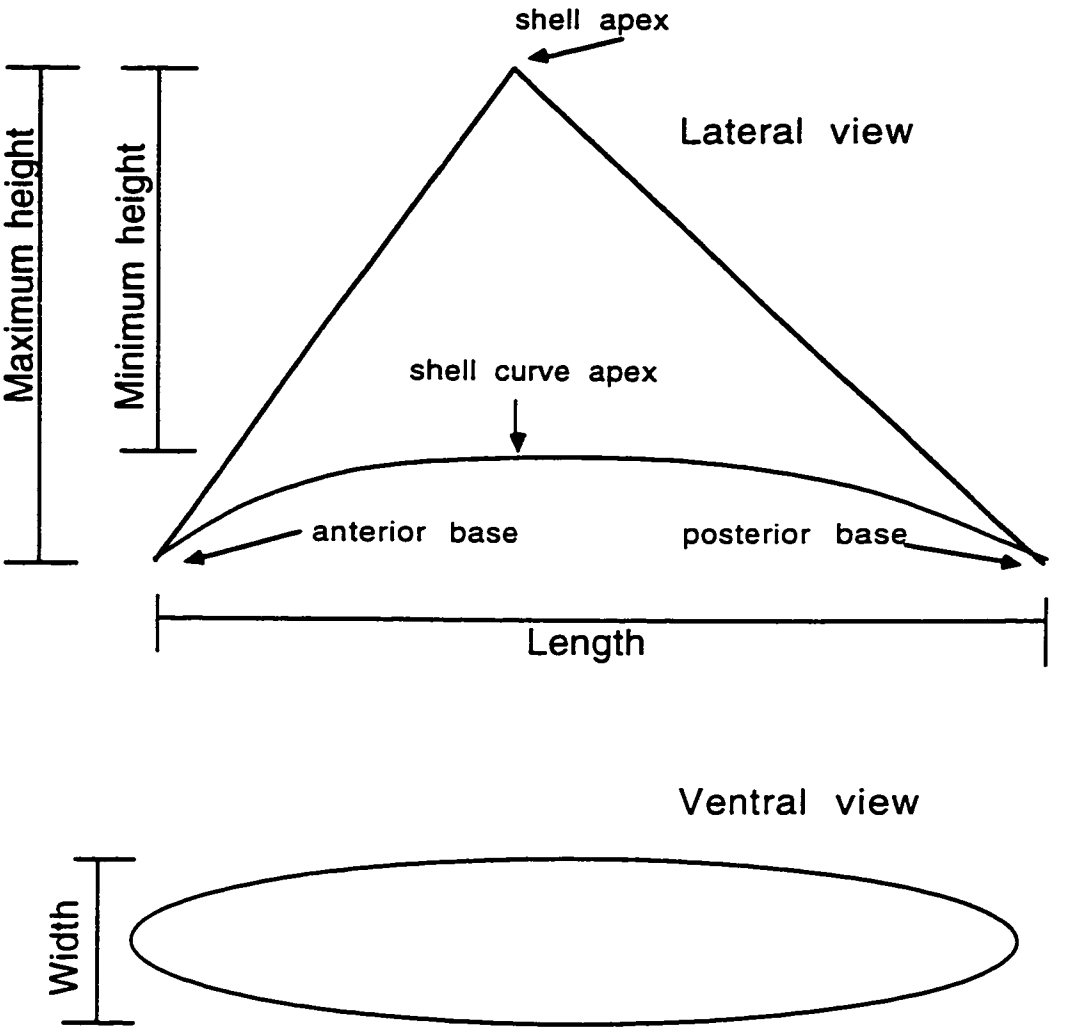


Figure 1: Dimensions measured to obtain maximum height, minimum height, length and width of *L. asmi* shells. Top figure is side view with the limpet's anterior to the left. Lower figure is ventral view.

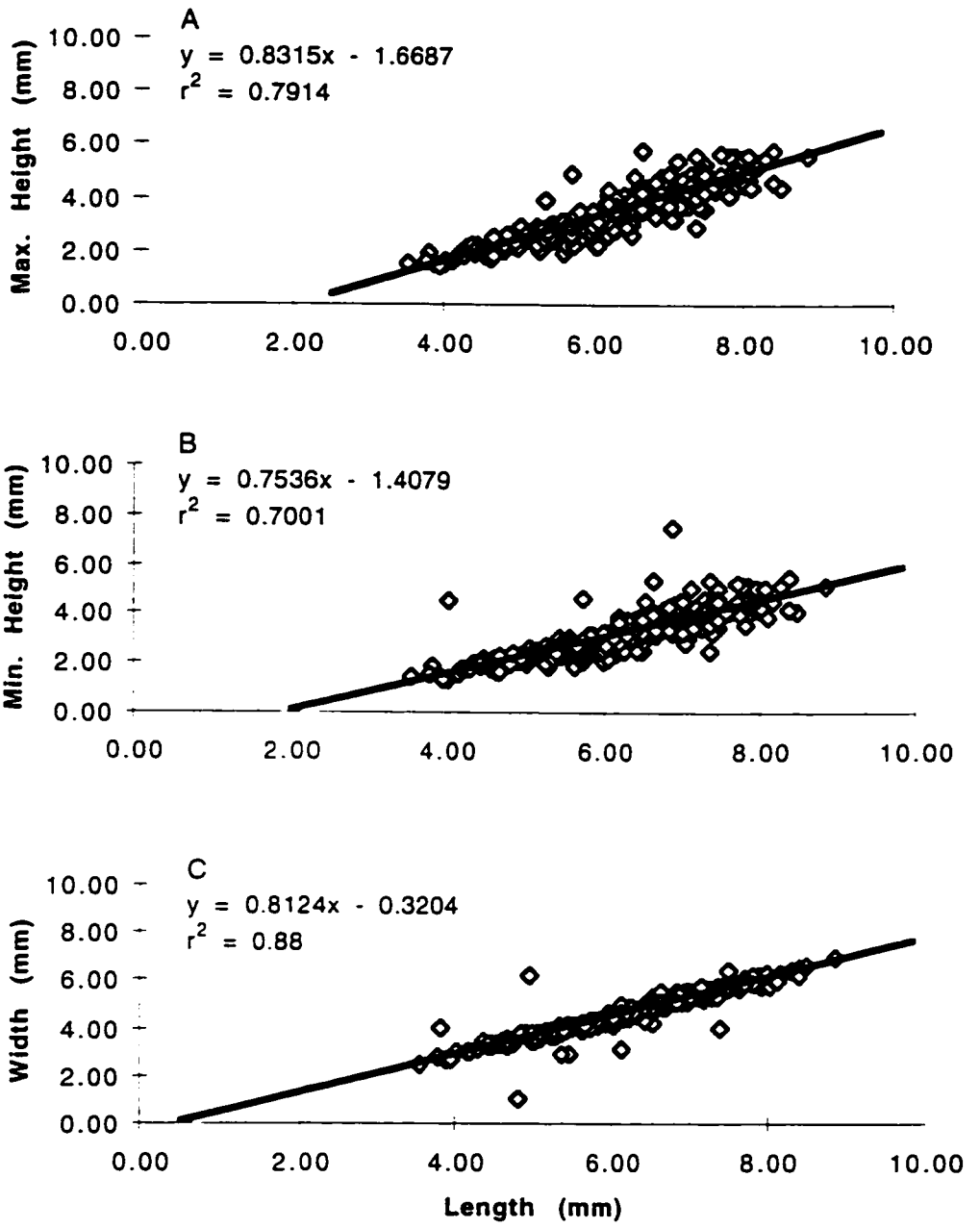


Figure 2. Regressions of maximum shell height (A), minimum shell height (B) and shell width (C) vs. shell length of *L. asmi*. Regression equations are shown for significant regressions ($p < 0.001$). N = 290.

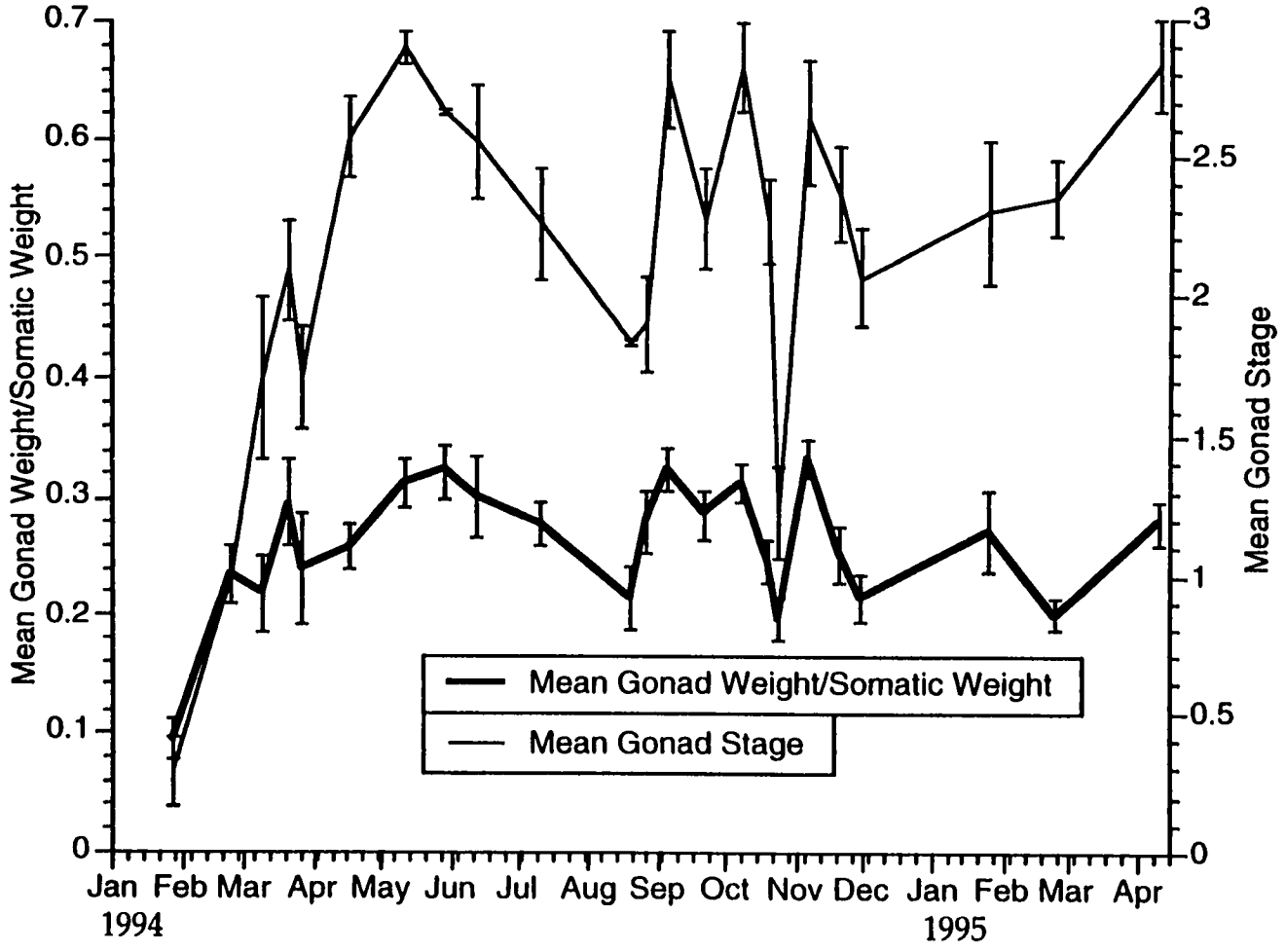


Figure 3. Gonad weight/somatic weight (gonad index) and gonad stage in adult *L. asmi*. A drop in the slope indicates a spawning event. Gonad index was derived from quantitative measurements. Gonad stage was assessed qualitatively. Scores ranged from 0 (indeterminate) to 3 (gonads visible from beneath digestive gland and exhibiting high turgor). Error bars represent standard error.

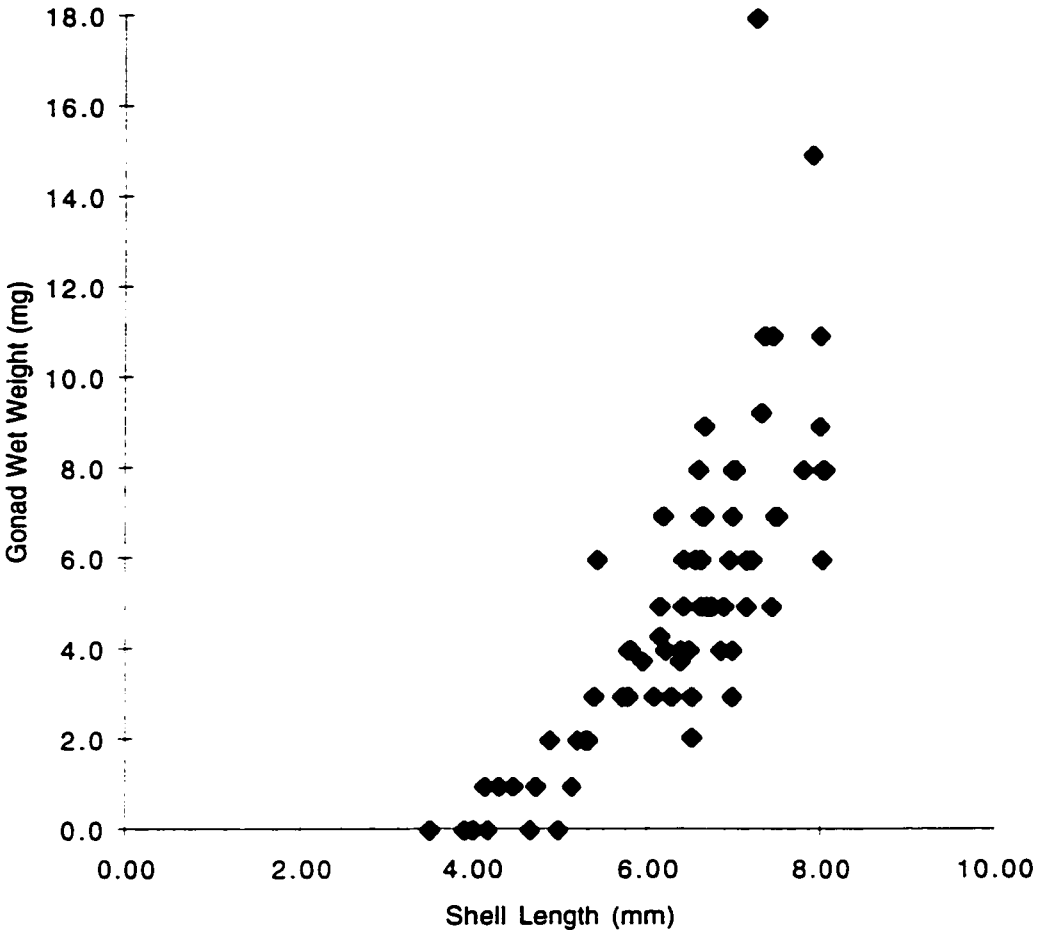


Figure 4. Relationship of gonad wet weight (mg) to shell length (mm) in male and female *L. asmi* collected during periods of reproductive maturity.

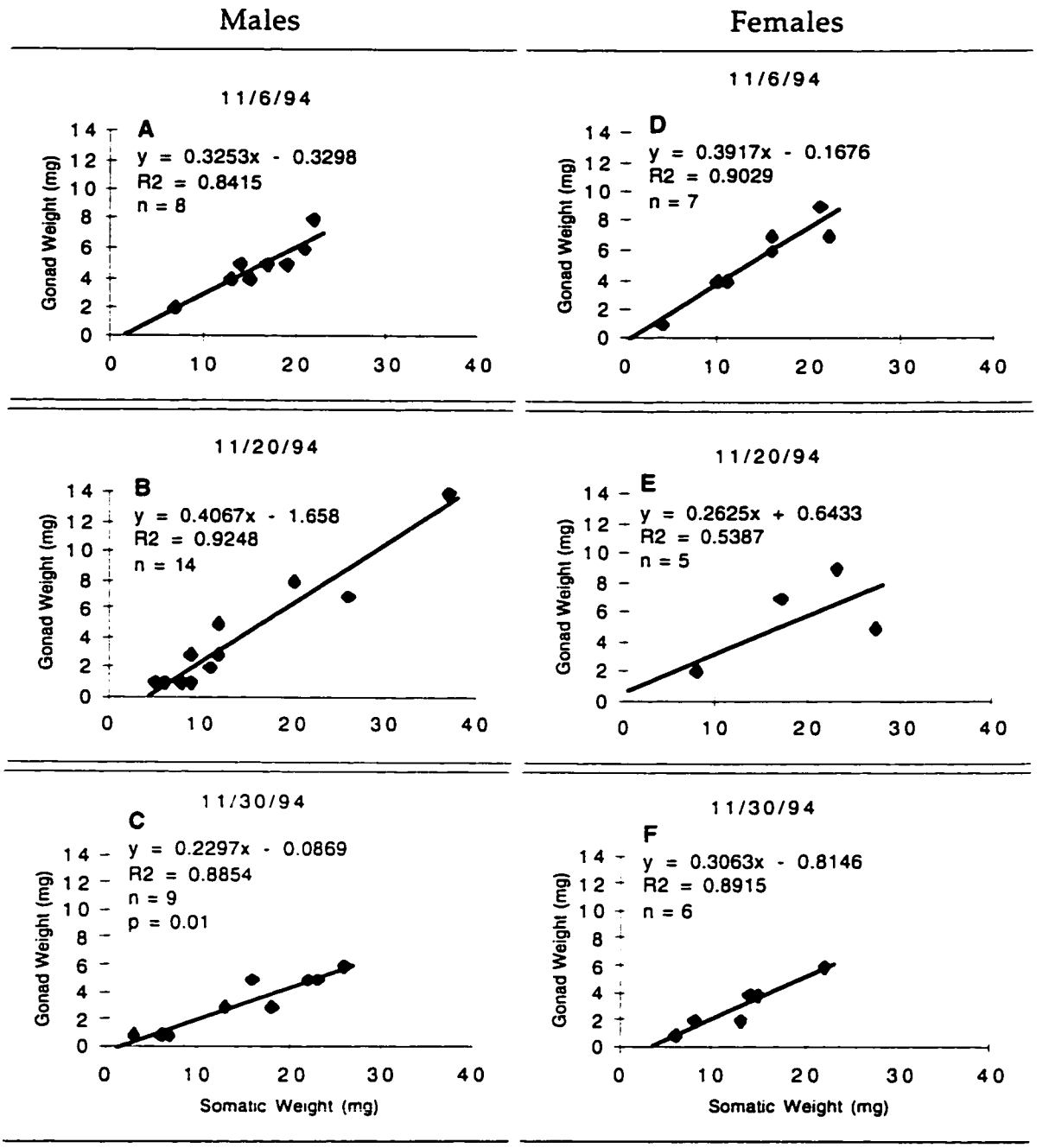


Figure 5. Relationship between gonad wet weight and somatic wet weight for male and female *L. asmi* (shown separately) during November 1994. A drop in the slope indicates a possible spawning event. A β-test to compare slopes was performed on successive slopes to verify or rule out a spawning event. The p-value is shown on graph C, the only graph whose slope was significantly different than the slope of the previous graph. All regressions were significant ($p < 0.01$) except for graph E ($p > 0.05$).

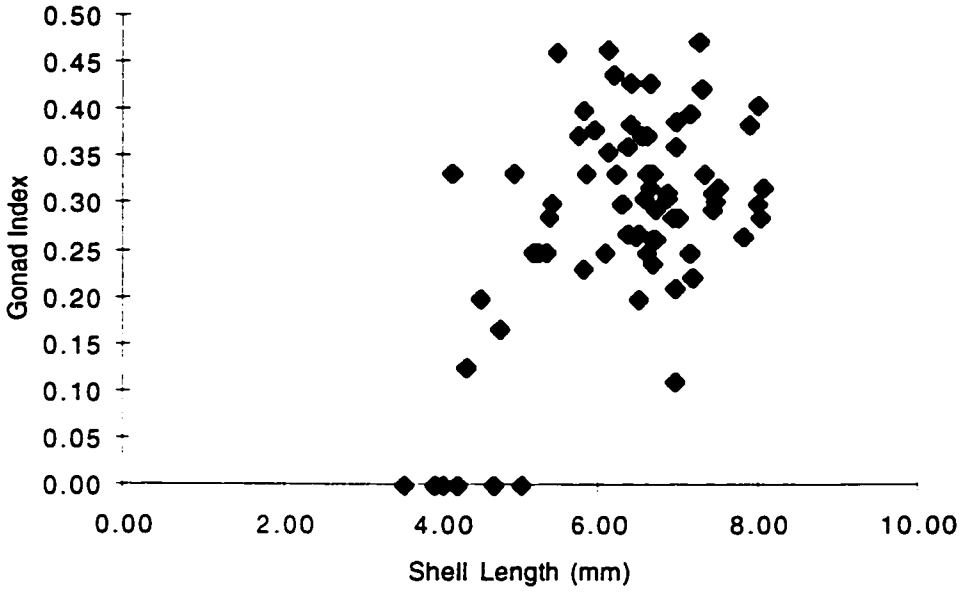


Figure 6. Gonad index as a function of shell length (mm). Data were from collection dates immediately preceding data indicating a spawning event. N = 74.

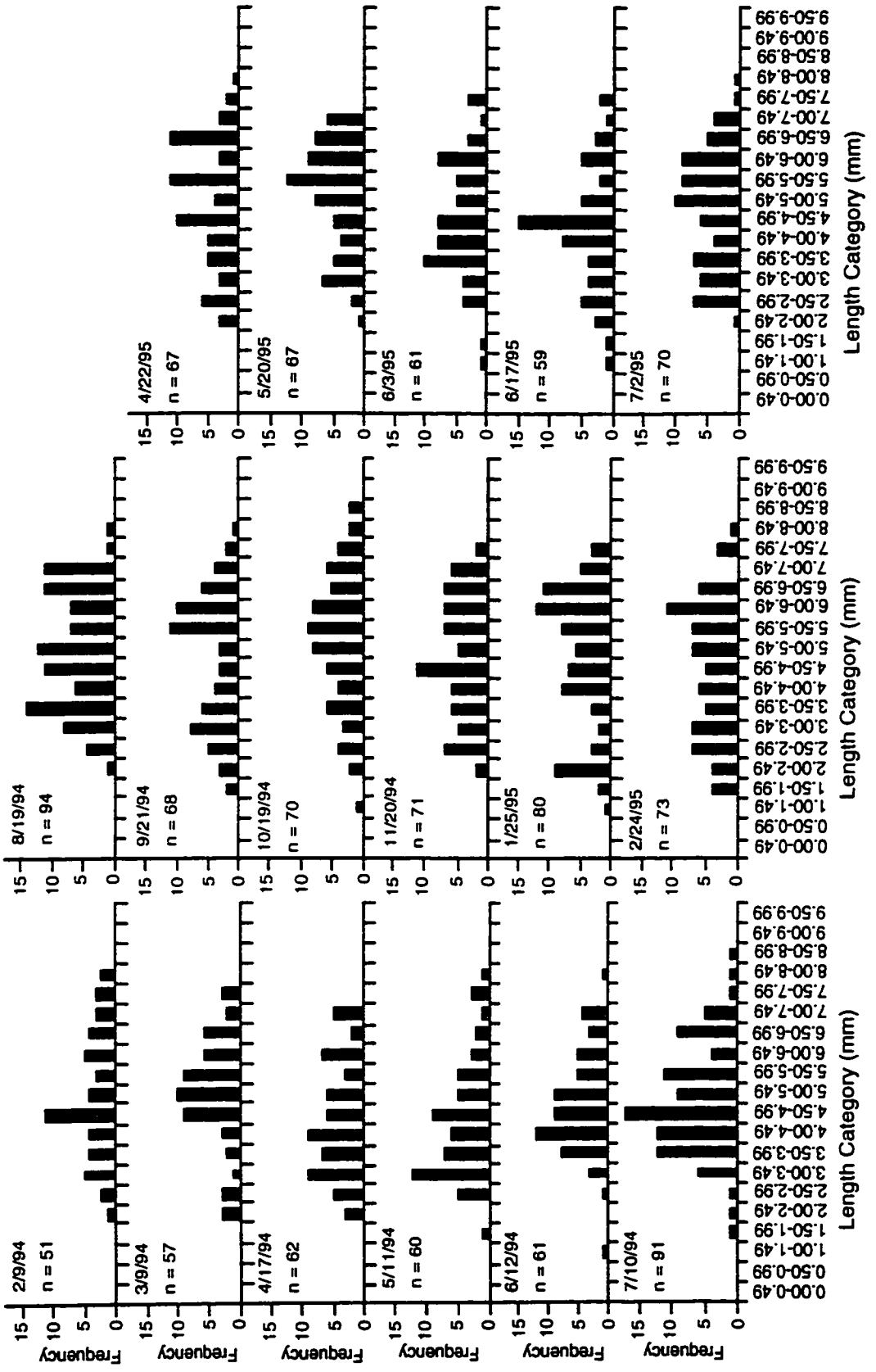


Figure 7. Size-frequency histograms of *L. asmi* at Pigeon Point from February 1994 to July 1995. Sample dates and number of samples taken are shown in upper left hand corner of each graph.

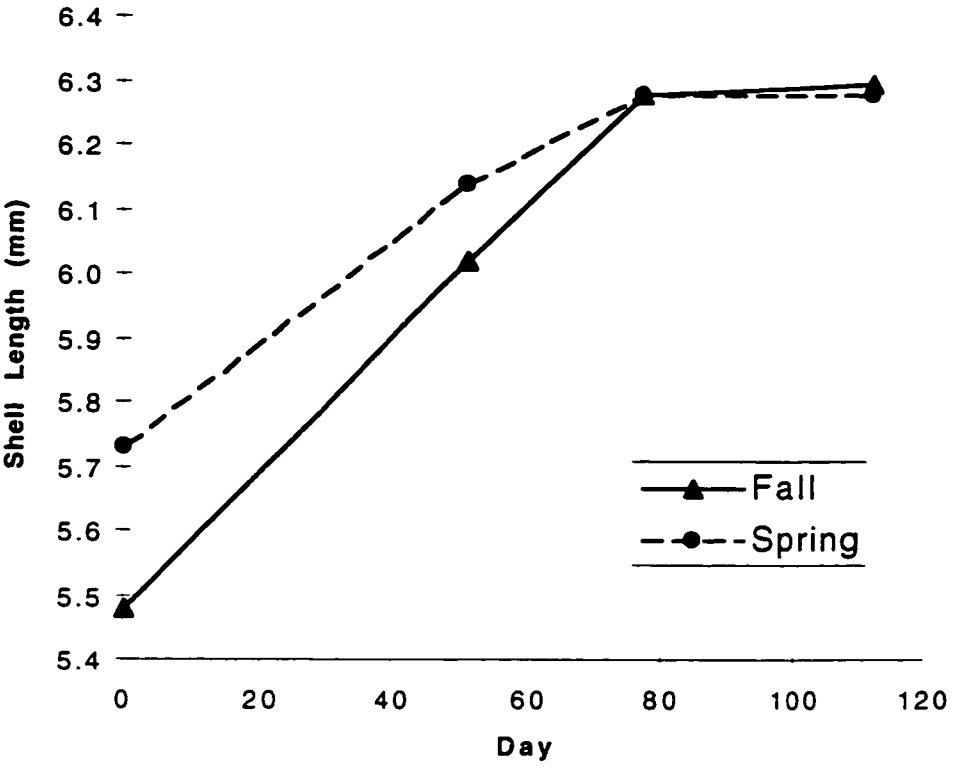


Figure 8. Significant shell length x season interactions from two-factor repeated measures ANOVA, with shell length (mm) as the repeated measure. Length data for limpets in all size classes were pooled for each season. Limpets were measured on days 1, 51, 77 and 111 of each 111-day study. N = 25 limpets in each season, $p = < 0.001$.

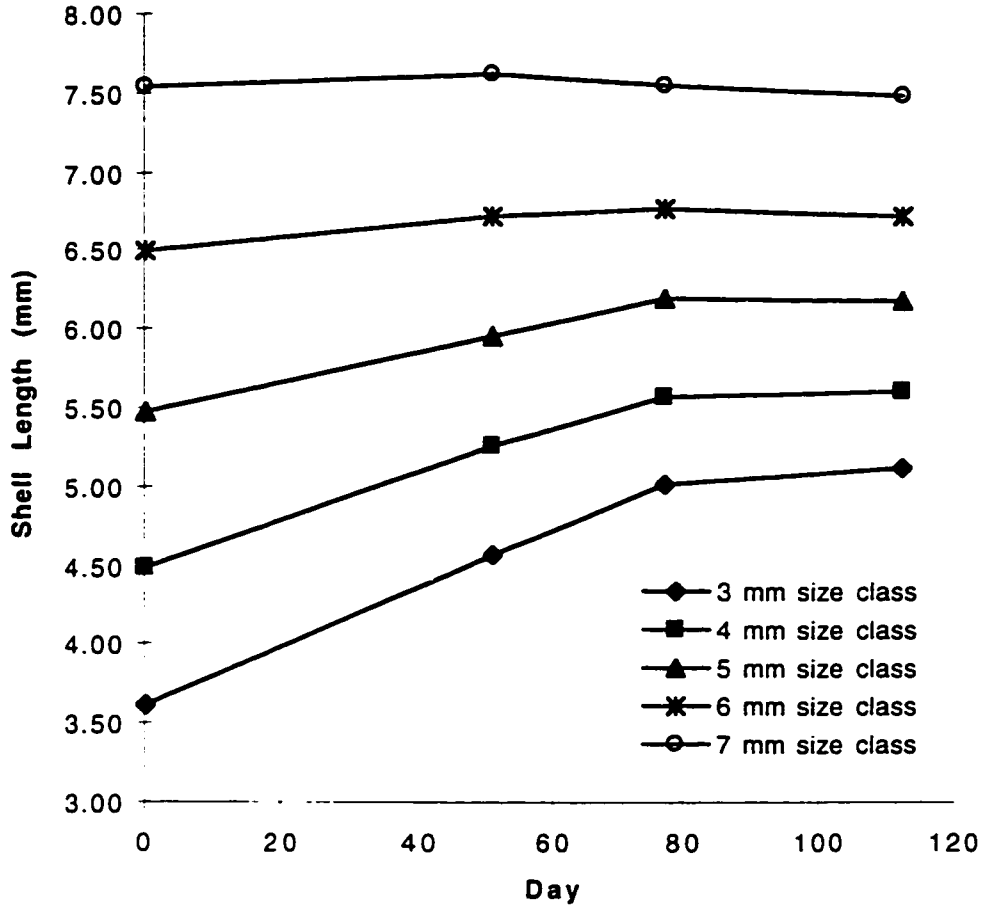


Figure 9. Significant shell length x size class interactions from two-factor repeated measures ANOVA, with shell length (mm) as the repeated measure. Data from fall and spring studies were pooled for each length class. Limpets were measured on days 1, 51, 77 and 111 of each 111-day study. N = 10 limpets in each size class, $p < 0.001$.

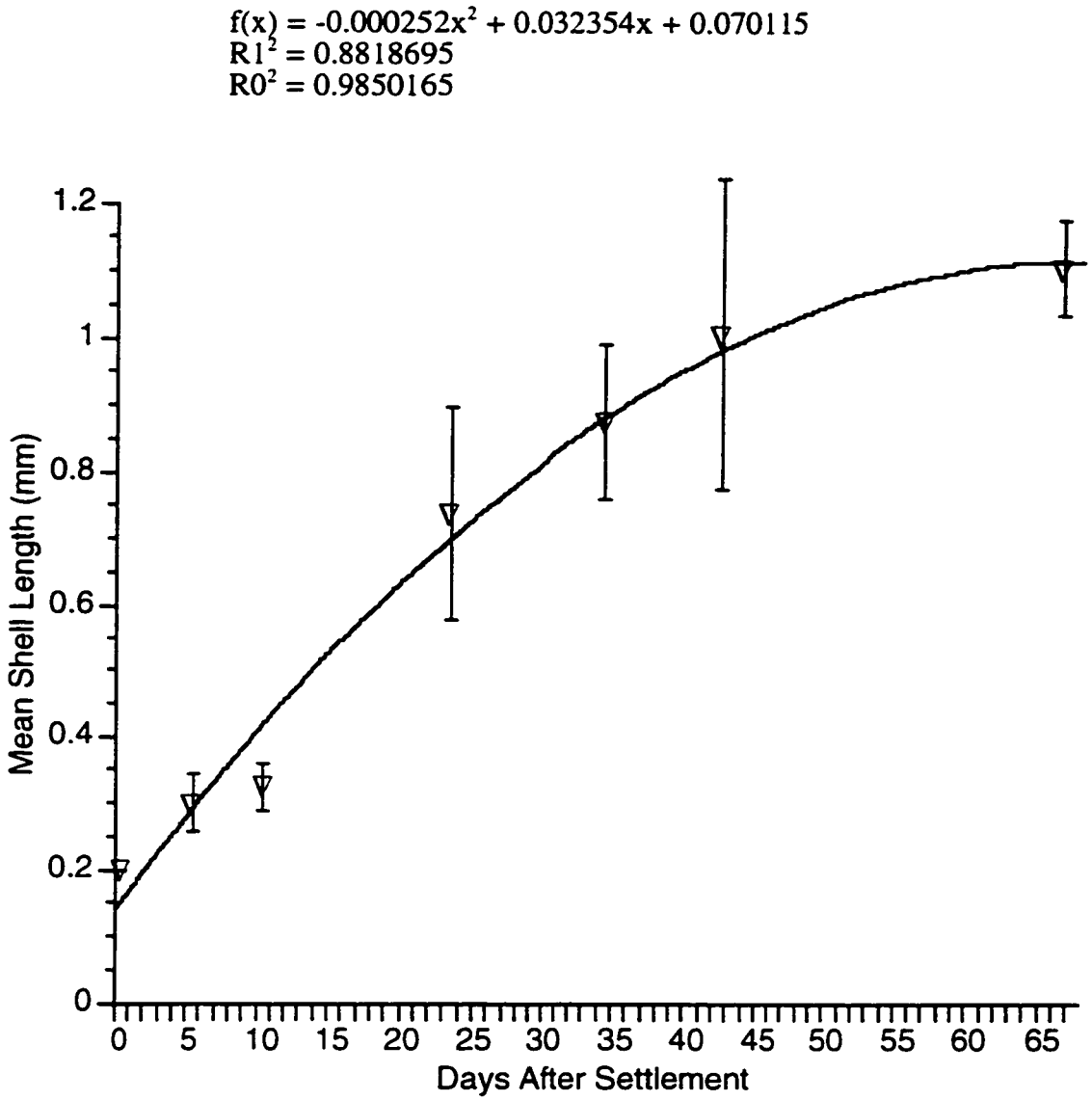


Figure 10. Growth of juvenile limpets in laboratory culture. Error bars represent one standard deviation. Size at settlement was 0.2 mm. N = 3 for day 5, n = 4 for day 10, n = 2 for day 23, n = 7 for day 34, n = 4 for day 42, and n = 5 for day 66. Regression coefficients are shown for the linear ($R0^2$) and quadratic ($R1^2$) portions of the curve.

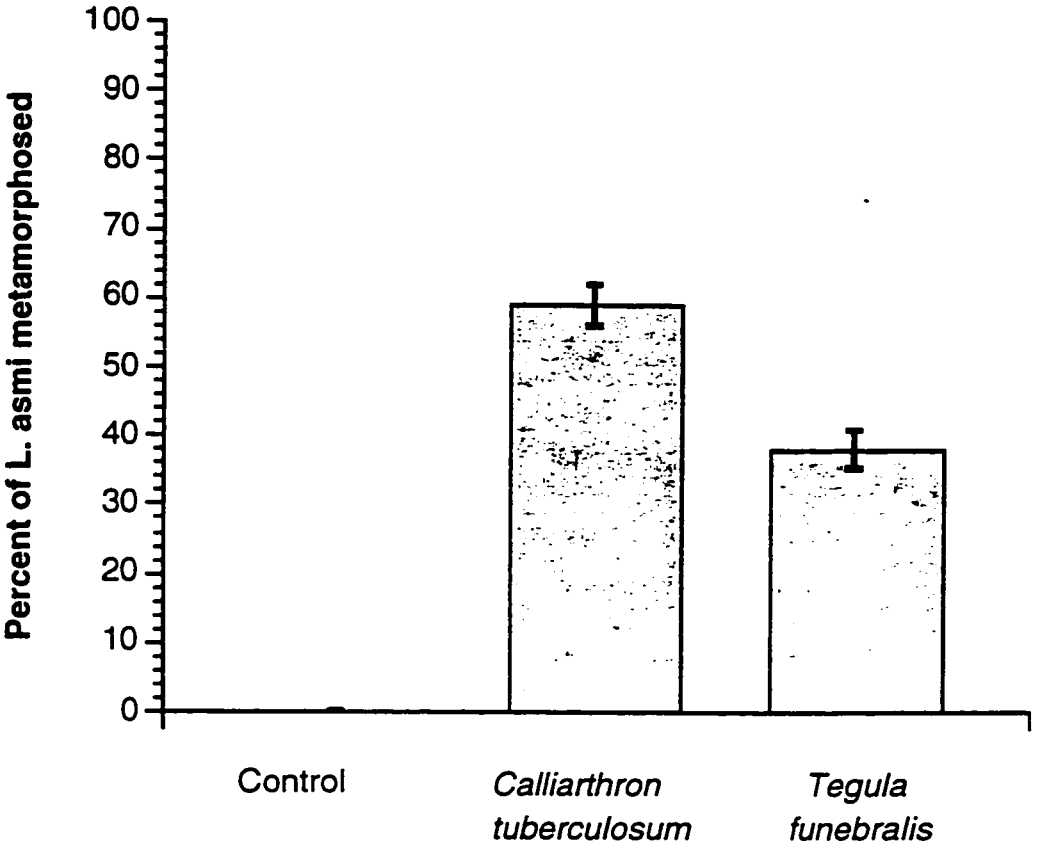


Figure 11. Mean percent of *L. asmi* veligers that settled and began metamorphosis after 41 hours in each of two substratum treatments and a control. Error bars represent standard error. N = 5 replicates per treatment. Each treatment included 20 larvae.

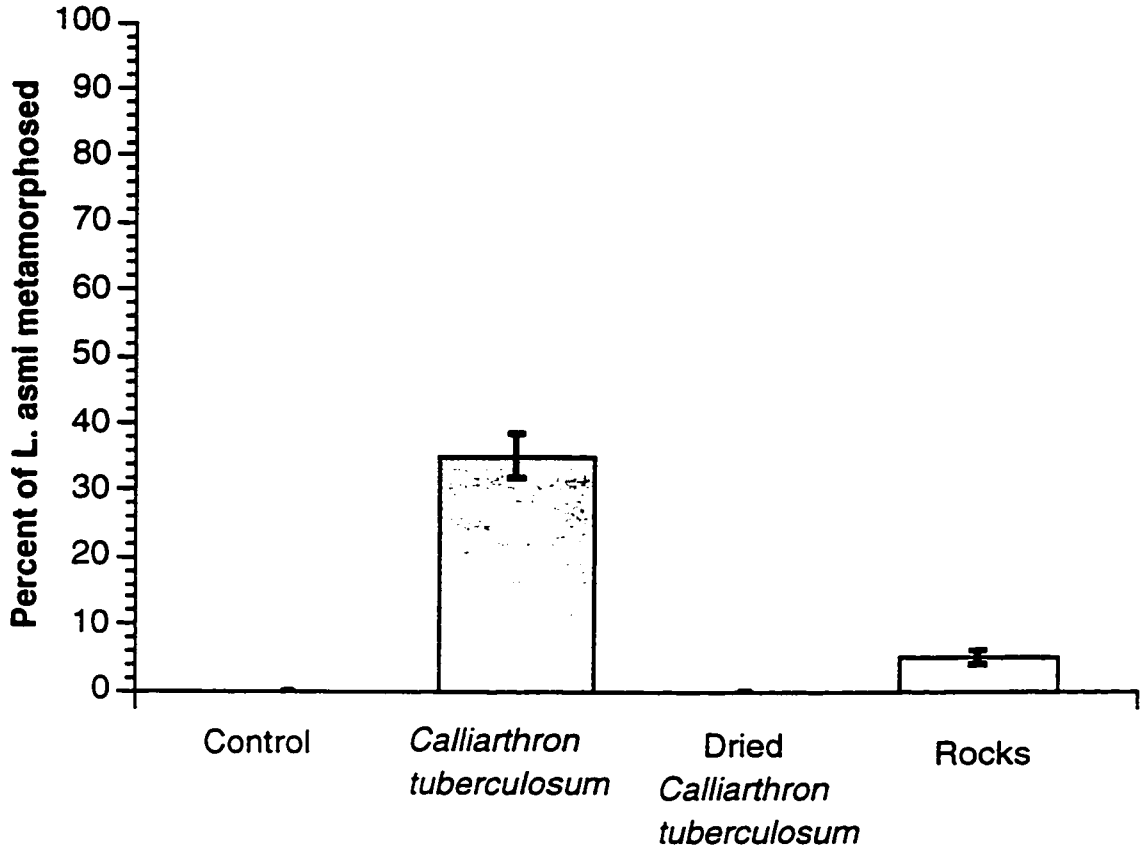


Figure 12. Mean percent of *L. asmi* veligers that settled and began metamorphosis after 41 hours in each of three substratum treatments and a control. Error bars represent standard error. N = 5 replicates per treatment. Each treatment included 20 larvae.

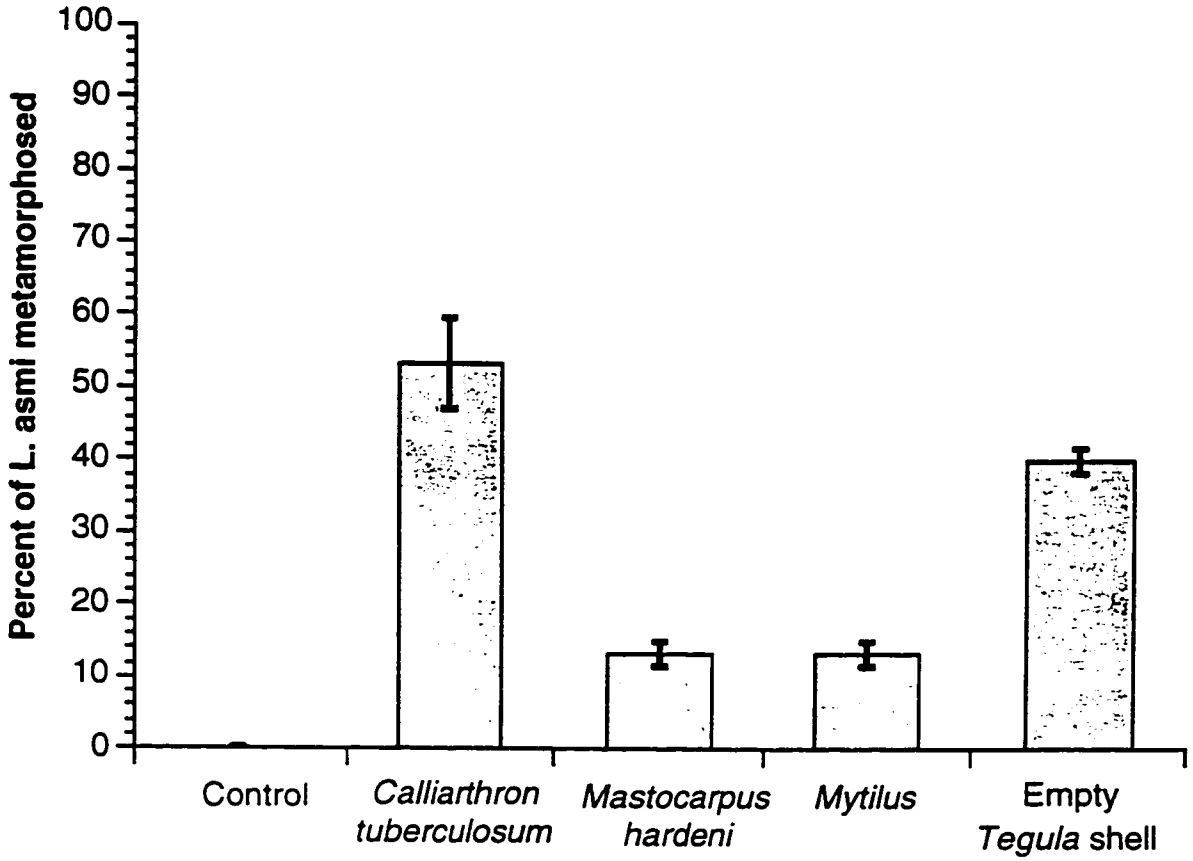


Figure 13. Mean percent of *L. asmi* veligers that settled and began metamorphosis after 41 hours in each of four substratum treatments and a control. Error bars represent standard error. N = 3 replicates per treatment. Each treatment included 15 larvae.

Appendix Table 1. Results of two-factor repeated measures ANOVA testing change in shell length of *L. asmi* held in the laboratory for 111 days during two seasons. Four shell length measurements made on each limpet, including initial length. Factors were season (fall or spring) and size class (3, 4, 5, 6, and 7 mm shell length). H-F = Huyhn-Feldt corrected p-value.

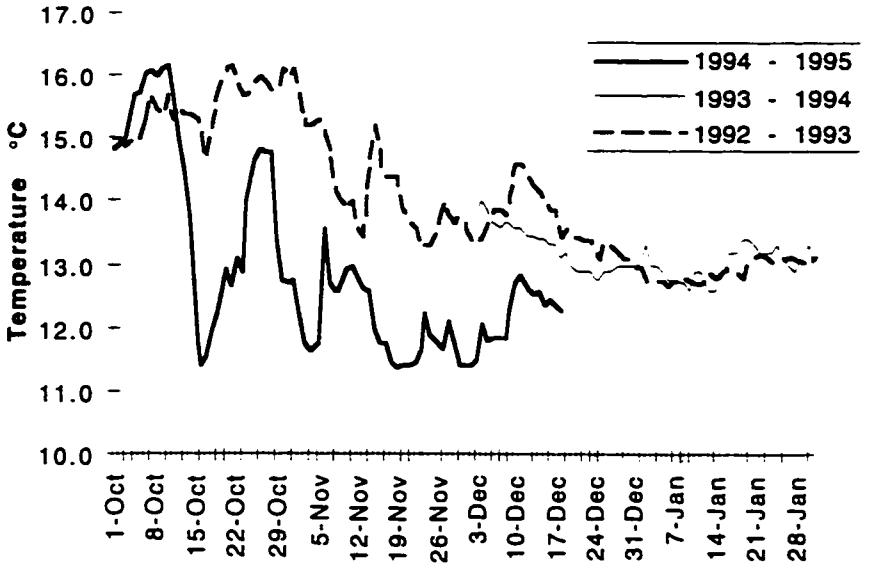
Source	SS	DF	MS	F	P	H-F
Between Subjects						
season	0.21377	1	0.21377	1.14545	0.29163	
size class	194.68	4	48.6711	260.799	0.00000	
season*size class	0.57381	4	0.14345	0.76867	0.55270	
error	6.71842	36	0.18662			
Within Subjects						
length	14.2977	3	4.76589	316.000	0.00000	0.00000
length*season	0.74988	3	0.24996	16.56124	0.00000	0.00001
length*size class	8.38681	12	0.69890	46.30637	0.00000	0.00000
length*season* size class	0.13614	12	0.01134	0.75167	0.69802	0.65424
error	1.63004	108	0.01509			

Appendix Table 2. Results of one-tailed t-test comparing the difference in mean initial shell length of limpets during fall and spring laboratory growth studies.

	Fall	Spring
mean initial length	5.4762	5.7304
variance	2.1032	1.9610
pooled variance	2.0386	
degrees of freedom	44	
t-statistic	-0.6013	
p (one-tail)	0.2754	
t-critical (one-tail)	1.6802	

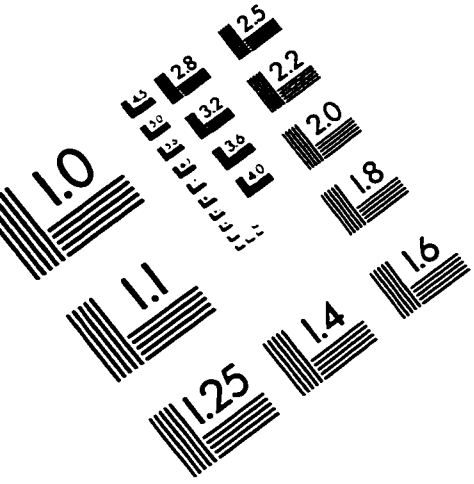
Appendix Table 3. Estimate of age at maturity for *L. asmi* based on growth rates observed in the laboratory for each size class shown. Age at size for 1 mm to 3 mm size classes was conservatively figured using growth rates for 3 mm limpets. Estimated age does not include time spent as plankton. Method modeled after Sutherland (1970).

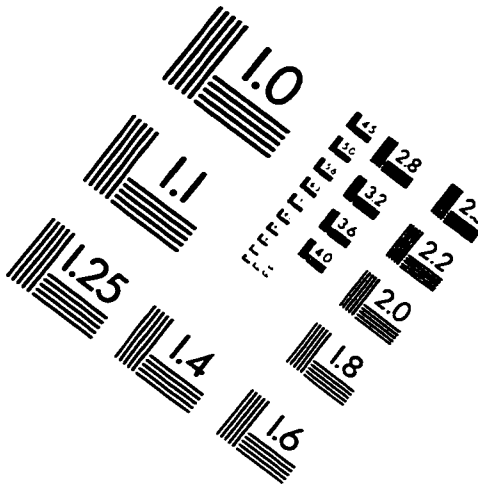
Settlement to 1 mm *(growth rate measured in laboratory = 0.02 mm/day)*	1 mm to 3 mm *(growth rate unknown)*	3 mm to 4 mm *(growth rate measured in laboratory = 0.02 mm/day)*	4 mm to 4.5 mm *(growth rate measured in laboratory = 0.01mm/day)*	Total Time to reach 4.5 mm
Time Estimate				
1 mm x 1 day/0.02 mm = 42 days	2 mm x 1 day/0.02 mm = 133 days	1 mm x 1 day/0.02 mm = 67 days	0.5 mm x 1 day/0.01 mm = 55 days	242 days

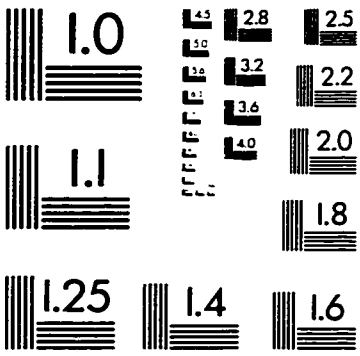


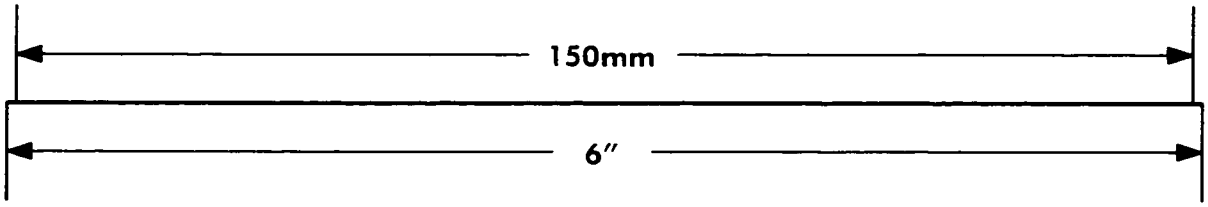
Appendix Figure 1. Daily mean sea surface temperatures for October 1 through January 31 recorded in 1992-1993, 1993-1994 and 1994-1995 winter seasons by buoy 46012 located near Half Moon Bay, CA (37.39° N, 122.73° W) and maintained by National Data Buoy Center.

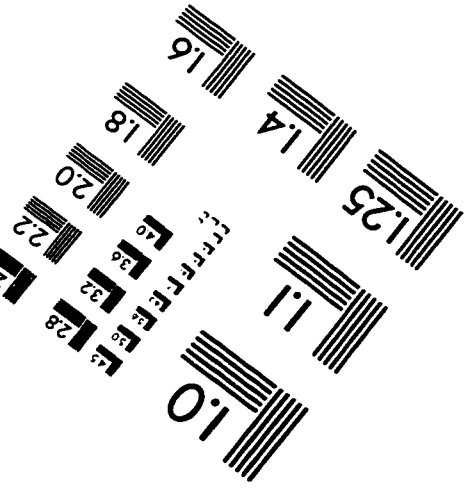
IMAGE EVALUATION
TEST TARGET (QA-3)

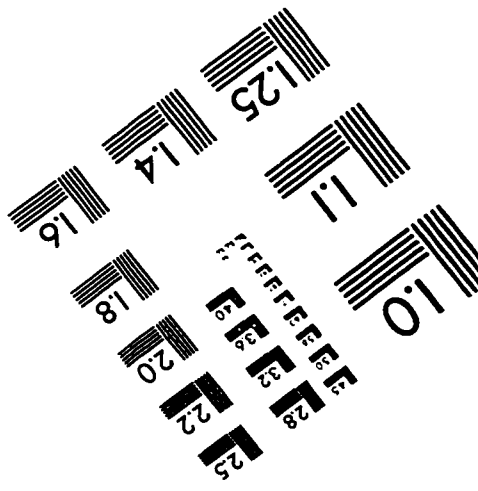












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