Life history of *Littorina scutulata* and *L. plena*, sibling gastropod species with planktotrophic larvae

Paul A. Hohenlohe[a]

Friday Harbor Laboratories, University of Washington, Friday Harbor, Washington 98250, USA

Abstract. The intertidal, sibling species *Littorina scutulata* and *L. plena* (Gastropoda, Prosobranchia) are sympatric throughout most of their ranges along the Pacific coast of North America. Both species release disc-shaped, planktonic egg capsules from which planktotrophic veliger larvae hatch. Here I review existing data and present new observations on these species' life history, including age at first reproduction, spawning season, maximum fecundity rates, capsule morphology, egg size and number, pre-hatching development, larval growth at three food concentrations, potential settlement cues, planktonic period, and protoconch size. Previous classification of egg capsule morphologies used to distinguish the species is inaccurate; instead, capsules can be categorized into three types of which each species may produce two. Females of *L. scutulata* produced capsules with either two rims of unequal diameter or one rim, while females of *L. plena* produced capsules with one rim or two rims of nearly equal diameter. Females of each species spawned sporadically from early spring to early fall in Puget Sound. Larvae of *L. plena* hatched one day earlier than those of *L. scutulata*, and both species grew fastest in the laboratory at intermediate food concentrations. Larvae metamorphosed in the presence of a variety of materials collected from their adult habitat, including conspecific adults, algae, rocks, and barnacle tests. This is the first report of planktotrophic larvae in this genus metamorphosing in the laboratory. The total planktonic period of 8 larvae of *L. scutulata* raised in the laboratory was 37–70 days, and a single larva of *L. plena* metamorphosed after 62 days. Protoconch diameter of shells collected from the field was 256–436 μm and did not differ significantly between the species. Previous allozyme and mitochondrial DNA work has suggested high levels of genetic variability in both species and greater genetic population structure in *L. plena*, despite the long spawning season and long-lived larvae in both species. The interspecific life history differences described here appear insufficient to produce consistent differences in gene flow patterns.

Additional key words: development, dispersal, gene flow, metamorphosis, larval settlement

Thorson (1946, 1950) classified modes of development among marine invertebrates into several types depending on their nutritional mode and site of development. Planktotrophic larvae feed as they develop, while lecithotrophic larvae depend on maternal yolk in the egg for nutrition. Planktotrophic larvae are typically planktonic, developing in the water column, while lecithotrophic larvae may be planktonic or benthic, developing in attached egg masses or brooded by a parent. With a few qualifications, these major categories remain useful (Jablonski & Lutz 1983; Levin & Bridges 1995). The marine gastropod genus *Littorina*

FÉRUSSAC 1822 exhibits three modes of larval development among its 19 species, and these modes can be mapped onto a cladogram derived from independent morphological and molecular characters (Reid 1996). Females in the 9 basal members of the clade release planktonic egg capsules containing one or several eggs that develop into planktotrophic veliger larvae. Lecithotrophic development probably arose once in the genus and is currently found in 9 species that lay benthic egg masses, from which crawl-away juveniles hatch (Reid 1990, 1996). From these lecithotrophic ancestors a third mode of development evolved in the polymorphic species *L. saxatilis*, in which developing offspring are brooded in the pallial oviduct until the juvenile stage. Adults of *Littorina* species are ecologically similar, so this genus is a potential model system for test-

[a] Present address: Corvallis Forestry Sciences Laboratory, USDA Forest Service, Corvallis, Oregon 97331, USA. E-mail: phohenlohe@fs.fed.us

ing the hypothesis that gene flow among populations should be higher in species with planktonic larvae because of their greater dispersal capacity (Scheltema 1971, 1986a; Crisp 1978; Jablonski & Lutz 1983).

The sibling species *L. scutulata* (GOULD 1849) and *L. plena* (GOULD 1849), which are sympatric over most of their ranges along the Pacific coast of North America, have planktotrophic larvae. However, these two species appear to have high levels of genetic variation among populations, similar to that found in some of the benthic-developing congeners (Ward 1990). In addition, Ward's (1990) recalculation of allozyme data from Mastro et al. (1982) and DNA work on the mitochondrial cytochrome b gene (Kyle & Boulding 2000) suggest that *L. plena* has greater genetic differentiation of populations, and hence more restricted gene flow, than *L. scutulata*. Among the numerous factors that could influence gene flow (Scheltema 1986b; Palumbi 1994) are basic life history traits (length and timing of spawning season, number of eggs per capsule, capsule morphology) and length of planktonic period, including both precompetent (pre- and posthatching) and competent periods. Here I investigate these factors using a combination of laboratory studies of larval growth and field collections of larvae and adults, in order to determine whether a difference in life history could contribute to a difference in population genetic structure between these species.

I also clarify the egg capsule morphologies characteristic of each species. Murray (1979) and Mastro et al. (1982) described diagnostic egg capsule morphologies for *L. scutulata* and *L. plena*, which had previously been regarded as a single species. Females of *L. scutulata* produced transparent egg capsules with two unequal rims (Fig. 1A). Murray (1979) included in this category capsules shaped like saucers with only one rim (Fig. 1B), described by Buckland-Nicks et al. (1973). Reid (1996) also considered both of these capsule types to be *L. scutulata*, with the diameter of the larger rim ranging 700–1000 μm. Murray (1979) described capsules of *L. plena* as slightly larger with two equal rims (Fig. 1C), about 1100 μm in diameter, usually with 17–32 eggs in each.

No one to date has reported raising planktotrophic larvae of any species of *Littorina* through metamorphosis, but there is indirect evidence of the planktonic development period following hatching. Buckland-Nicks et al. (1973) raised veligers, probably of *L. scutulata*, for 25 days after hatching to a size of 300–360 μm. Reid (1996) measured protoconch sizes of 240 μm in "poorly preserved available specimens" of *L. scutulata* and 300 μm in *L. plena*, suggesting that the larvae of Buckland-Nicks et al. (1973) may have been competent to settle at 25 days post-hatching. Chow

(1989) observed a peak of settlement of *L. plena* in fall and winter in California, occurring several months after the peak spawning in spring and summer in the same region (Chow 1987).

Methods

Egg capsule morphology

Initially, the egg capsule descriptions of Murray (1979) and Reid (1996) were used to identify capsules to species. All capsules of type A or B (Fig. 1) were classified as *Littorina scutulata*, until it was discovered that this distinction was inaccurate. Henceforth all females that spawned in the laboratory were identified to species, if possible, using a combination of independent characters including tentacle coloration, shell morphology, and shell color pattern (Hohenlohe & Boulding 2001). Data from the laboratory work described below, except where noted, relied on egg capsules produced by independently identified females. In order to determine the capsule types produced by each species, capsule shape, outer diameter, and number of eggs were recorded in several of the experiments described below.

Spawning season

Periodically during the spring and summer of 1997 through 2000, snails were collected from the west side of San Juan Island, Washington, where both *L. scutulata* and *L. plena* are found in roughly equal numbers. They were brought into the laboratory and submerged in filtered seawater, either together in a large container or separately in culture wells. Snails were kept for at least a week, and production of egg capsules recorded.

Periodic plankton samples were also taken from April through August in 1997 and 1998, and April through June in 1999, in San Juan Channel, Washington, at rising or high tide. A total of 2.9 m³ of the top 10 m were sampled with a net (250-μm mesh) drawn vertically through the water column, collecting egg capsules but not hatched veligers of the two species. However, the adjacent shores yielded *L. scutulata* almost exclusively, so these data provide evidence only for the spawning season in *L. scutulata;* very few egg capsules of *L. plena* were found.

Fecundity

Females of *L. scutulata* (n = 31) were collected from 3 sites on San Juan Island, Washington, and kept submerged with conspecific males in filtered seawater in separate culture wells for 3 months. Snails were fed *ad libitum* with varying proportions of cultured mi-
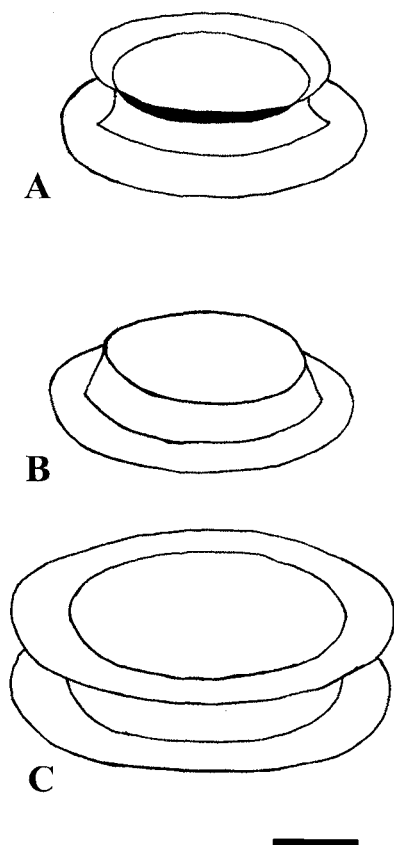
Fig. 1. Three egg capsule morphologies in *Littorina scutulata* and *Littorina plena*. Type A has two rims of unequal diameter, type B has only one rim, and type C has two rims of nearly equal diameter. Females of *L. scutulata* produce types A and B, and those of *L. plena* produce types B and C. Scale bar, ~200 μm.

croalgae (*Isochrysis galbana* and *Rhodomonas lens*), which settled to the bottom of the culture wells where they were eaten by the snails, and egg capsule production was recorded periodically. Capsule morphology, outer capsule diameter, and number of eggs per capsule were recorded on a sample of capsules from each spawning event.

Larval development

Capsules of both species were spawned simultaneously in July 1999 by females submerged in seawater in separate culture wells. Capsules were maintained at ambient seawater temperature (12–14°C), and timing of prehatching development was recorded for 32 eggs of *L. scutulata* and 57 eggs of *L. plena*.

One brood of egg capsules from each species was split into 4 treatments and fed with the microalgae *I. galbana* and *R. lens* at the following concentrations: minimal food (5 × 10² cells ml⁻¹), low (5 × 10³ cells ml⁻¹), medium (5 × 10⁴ cells ml⁻¹), and high (5 × 10⁵

cells ml⁻¹). As cells of *R. lens* are larger than those of *I. galbana,* treatments actually contained more of the former species by volume. For each treatment, ~50 larvae were kept in 100 ml of unstirred filtered seawater at ambient seawater temperature (12–14°C). Water was changed and replenished with food every 3–4 days. Shell lengths of a sample of up to 9 larvae were measured every 6–8 days. Since the larval shells were virtually planispiral, shell length here means the maximum dimension of the shell—the distance from the outer aperture lip to the opposite side of the last whorl (Fig. 6). Using regression analyses on data from each treatment, 4 different growth models were tested to determine which best explained the relationship between time and size of larvae: linear, natural logarithmic, cube-root, and fifth-root growth equations.

Planktonic period

To determine planktonic period, I attempted to induce settlement by larvae from the growth experiments described above, as well as larvae raised under similar conditions and fed high concentrations (10⁵–10⁶ cells ml⁻¹) of *I. galbana* and *R. lens.* Materials with potential settlement cues were collected from the intertidal and introduced periodically as early as 12 days post-hatching. These materials included conspecific adults, rocks, empty barnacle tests, and algae.

Protoconch size

Snails of both species were collected from two protected sites in southern Puget Sound and one on San Juan Island. Only small specimens were chosen in order to collect the least degraded shells. As small individuals can be difficult to identify using morphological criteria (Hohenlohe & Boulding 2001), only males were used and were identified using penis morphology (Murray 1979). The soft tissue was removed and the shells prepared in a variety of ways, adapted from Hickman (1995a). Methods included soaking in a dilute bleach solution, cleaning in a sonicator, and etching for 1–10 min in dilute hydrochloric acid, all followed by drying with 95% ethanol. Specimens were then mounted on SEM stubs with conductive carbon adhesive, sputter-coated, and viewed in a Jeol JSM-35 scanning electron microscope. Protoconch diameters were measured on micrographs taken from an apical view.

Results

Egg capsule morphology

Three egg capsule types were produced by these species (Fig. 1). Type A capsules resembled those pic-

tured for *Littorina scutulata* by Murray (1979) and Mastro et al. (1982). They had two rims of unequal diameter, with the smaller rim often somewhat upturned (Fig. 1A). The diameter of the smaller rim was 57–83% (mean = 61%) of the diameter of the larger rim. Type B capsules resembled the capsule shown in Buckland-Nicks et al. (1973). They had only one rim, and the diameter of the opposite side was 55–73% (mean = 64%) of the diameter of the rim. Type C capsules had two rims of nearly equal diameter (smaller diameter greater than 90% of the larger) and resem-

bled capsules shown for *L. plena* by Murray (1979) and Mastro et al. (1982).

Females of *L. scutulata* produced capsules of type A and/or type B (Fig. 1). Only one type was produced during a single spawning event, but 20 of the 39 females kept in the laboratory for 3 months produced capsules of both types, sometimes within a week. Of the remainder, 17 females produced only type A capsules and 2 females produced only type B capsules. Egg capsules were 337–976 μm in outer diameter and contained 1–11 eggs, typically 2–4 (mean = 2.7; Fig.
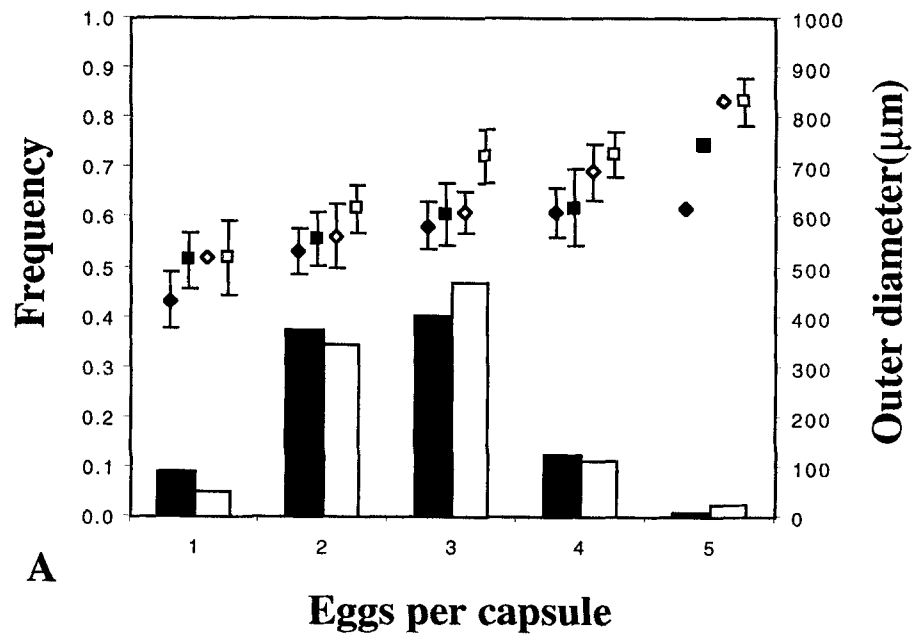

Fig. 2. Number of eggs per capsule and outer capsule diameters. **A.** *L. scutulata.* Bars represent frequencies of capsules (both types A and B) with 1–5 eggs, spawned in laboratory (filled bars; total n = 11,421) or collected in plankton samples (open bars; total n = 84). Points above represent mean outer diameter of capsules in each class from the laboratory (type A: filled diamonds; type B: filled squares) and from the plankton (type A: open diamonds; type B: open squares). Error bars represent standard deviation. Sample size for the points is 1–143. **B.** *L. plena.* Number of eggs and diameter of type B (open squares) and type C (filled circles) capsules spawned in the laboratory. Shown for comparison are type B capsules spawned in the lab by *L. scutulata* (filled rectangles).
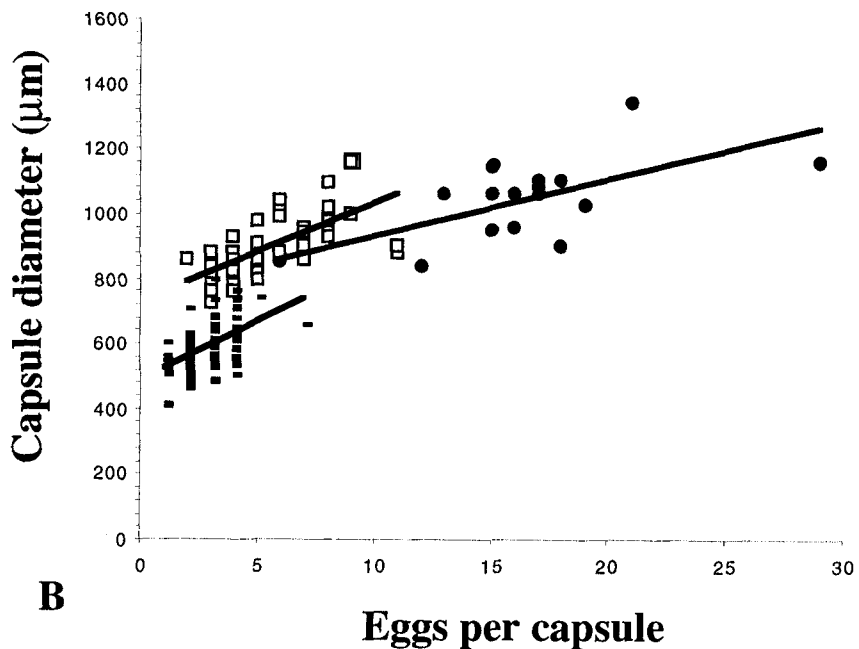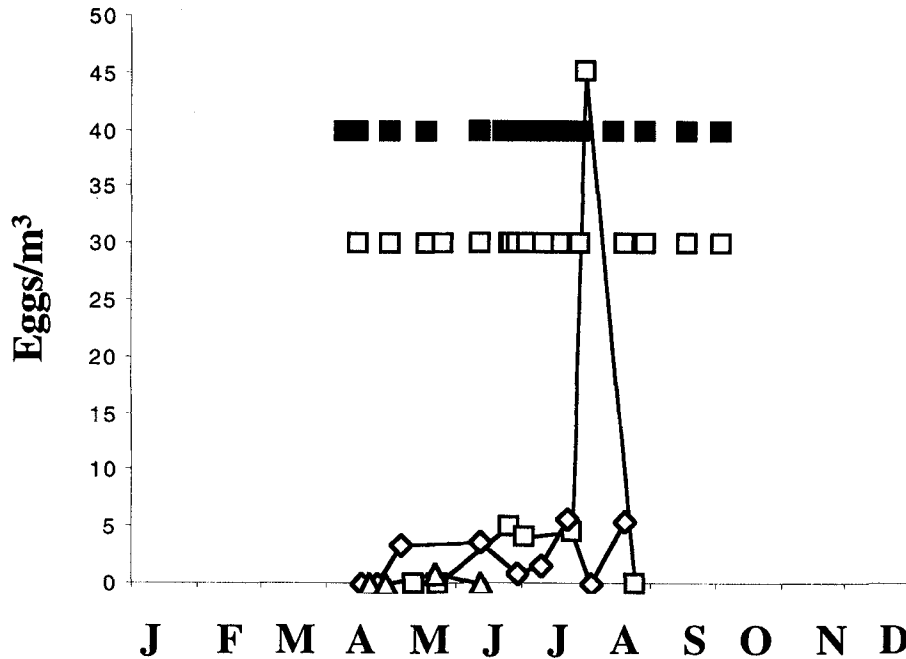
Fig. 3. Spawning season of *L. scutulata* and *L. plena* in Puget Sound. Symbols connected by lines represent densities of eggs of *L. scutulata* in capsules from plankton samples in 1997 (squares), 1998 (diamonds), and 1999 (triangles). Unconnected symbols represent spawning trials in which at least 50 females of both species were brought into the laboratory and kept for at least 1 week. Shown are spawning events for *L. plena* (filled squares) and *L. scutulata* (open squares).

2A). Outer diameter was significantly correlated with number of eggs in the capsule (r = .703; p < .001). Plankton-collected capsules were larger than lab-spawned, and type B capsules were larger than type A capsules (ANCOVA; p < .001 for both factors). However, diameters overlapped broadly across both of these factors (Fig. 2A). No seasonal pattern was detected in the frequency of the two capsule types, either in the lab or in plankton samples.

Females of *L. plena* produced capsules of either type B or type C (Fig. 1), except for one female that produced capsules of both types, one type in each of two separate spawning events. No females of this species were kept in the laboratory for longer than 2 weeks. At least 22 females identified as *L. plena* by tentacle coloration and discrete shell characters produced only type B capsules, and 7 females produced only type C capsules. The two types differed in number of eggs and outer diameter (Fig. 2B). Type B capsules were 730–1160 μm in outer diameter and contained 2–11 eggs (mean = 5.0). Type C capsules were 841–1340 μm in outer diameter and contained 6–47 eggs (mean = 19.0). Within both types, the number of eggs per capsule was significantly correlated with capsule diameter (type B: Y = 725.7 + 30.4X, r = .71, p < .001; type C: Y = 756.8 + 17.4X, r = .682, p = .001), though the slopes from these two regression analyses differ significantly (Student's t test, p < .001; Zar 1984). Again, no seasonal pattern was detected in the production of the two capsule types.

Within type B capsules, capsules of *L. plena* tended to be larger and have more eggs than those of *L. scu-*

tulata. The slopes of the two lines shown in Fig. 2B do not differ (Student's t test; p > .5), but the intercepts differ significantly (p < .001). Thus the discriminant function Y = 693 + 15E, where E is the number of eggs, correctly classified 98.6% of the 211 type B capsules shown in Fig. 2B, when the identity of the mother was known independently. Capsules larger than Y were produced almost exclusively by *L. plena*, while those smaller were produced almost exclusively by *L. scutulata*.

Spawning season

Both species began spawning in early April and continued through early October. Fig. 3 shows densities of eggs of *L. scutulata* in capsules collected in the plankton, and those of both species spawned in the laboratory. Capsules collected from the plankton were identified to species using capsule morphology, diameter, number of eggs, and the criteria described above. Very few capsules of *L. plena* were collected in San Juan Channel (data not shown). Because of the naturally patchy distribution of plankton (Abraham 1998) and relatively small volume of water sampled, the plankton data should not be taken as accurate measurements of total concentration of eggs in capsules across a larger area. Instead, they indicate the presence of egg capsules at certain times of the year and some of the range of variability in concentration.

Fecundity

Both species were reproductively mature by 2–3 mm shell height: one male of *L. plena* measuring 1.8
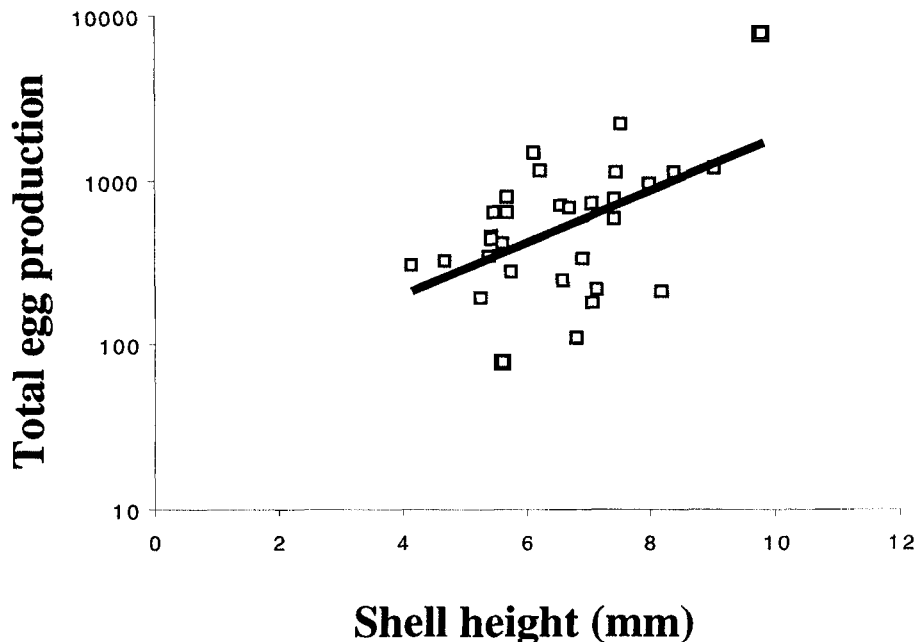
Fig. 4. Total fecundity of females of *L. scutulata* kept in the laboratory for 3 months. Egg production is significantly related to shell size (r = .508; p = .004).

mm had a fully developed penis, and several females of *L. scutulata* measuring under 3 mm produced egg capsules in the laboratory. Total fecundity of females of *L. scutulata* was significantly related to shell height (regression analysis on log-transformed data: $\ln(Y) = 3.83 + 0.37X$, where Y = total number of eggs and X = shell height in mm; r = .508; p = .004) but varied widely to almost 10,000 eggs over 3 months (Fig. 4). Eggs of *L. scutulata* measured 95.0 ($\pm$ 4.3 μm in diameter (n = 40) and were significantly larger (t-test; p < .001) than those of *L. plena* (90.2 $\pm$ 2.0 μm; n = 12).

Larval development

At 12–14°C, veligers of *L. scutulata* hatched after 9 days and those of *L. plena* hatched after 8 days. Prehatching development is summarized in Table 1. At hatching, shells of *L. scutulata* measured 140–150 μm in diameter, and those of *L. plena* measured 130–140 μm in diameter. The aperture lip formed a horn be-

Table 1. Early development of *L. scutulata* and *L. plena* at 12–14°C.

| Stage	*L. scutulata*	*L. plena*
2-cell (hours)	3	3
4-cell (hours)	5	5
Trochophore (hours)	35	35
Early veliger (days)	4	4
Well-developed veliger (days)	5	5
Hatching (days)	9	8

tween two velar notches. Sinusigeral growth lines differentiated the post-hatching shell (protoconch II) from the smooth pre-hatching shell (protoconch I) (Fig. 5A). At hatching, the shell was approximately planispiral, but translation down the axis of coiling began about one-quarter of a whorl after hatching. As the larvae grew, the right velar lobe became larger than the left, perhaps to support the shell apex. Velar cilia were 20–30 μm long. Juvenile structures developed gradually as the larvae grew. With medium or high food concentrations, a foot appeared within the first 2 weeks and continued to enlarge and become muscular and agile. Anterior tentacles with eyespots at their base appeared in the third week. After 4 weeks the animals began crawling on the substrate with their velar lobes still extended, possibly testing for appropriate settlement cues. Larvae raised at the minimal food concentration did not develop any of these juvenile structures or exhibit this crawling behavior.

Fig. 6 shows growth of larvae of *L. scutulata* at 4 different food concentrations from one brood. Of the 4 growth functions tested, the one that best fit the data was of the form

$$S = (\alpha + \beta d)^{1/3}$$

where S is shell length (μm), d is time (days), α is a coefficient related to size at hatching, and β is a growth coefficient. This function suggests that total mass, which should vary roughly as the cube of the length, increased linearly during larval development. No significant difference was found either in growth coefficients at the various food concentrations (2-way AN-
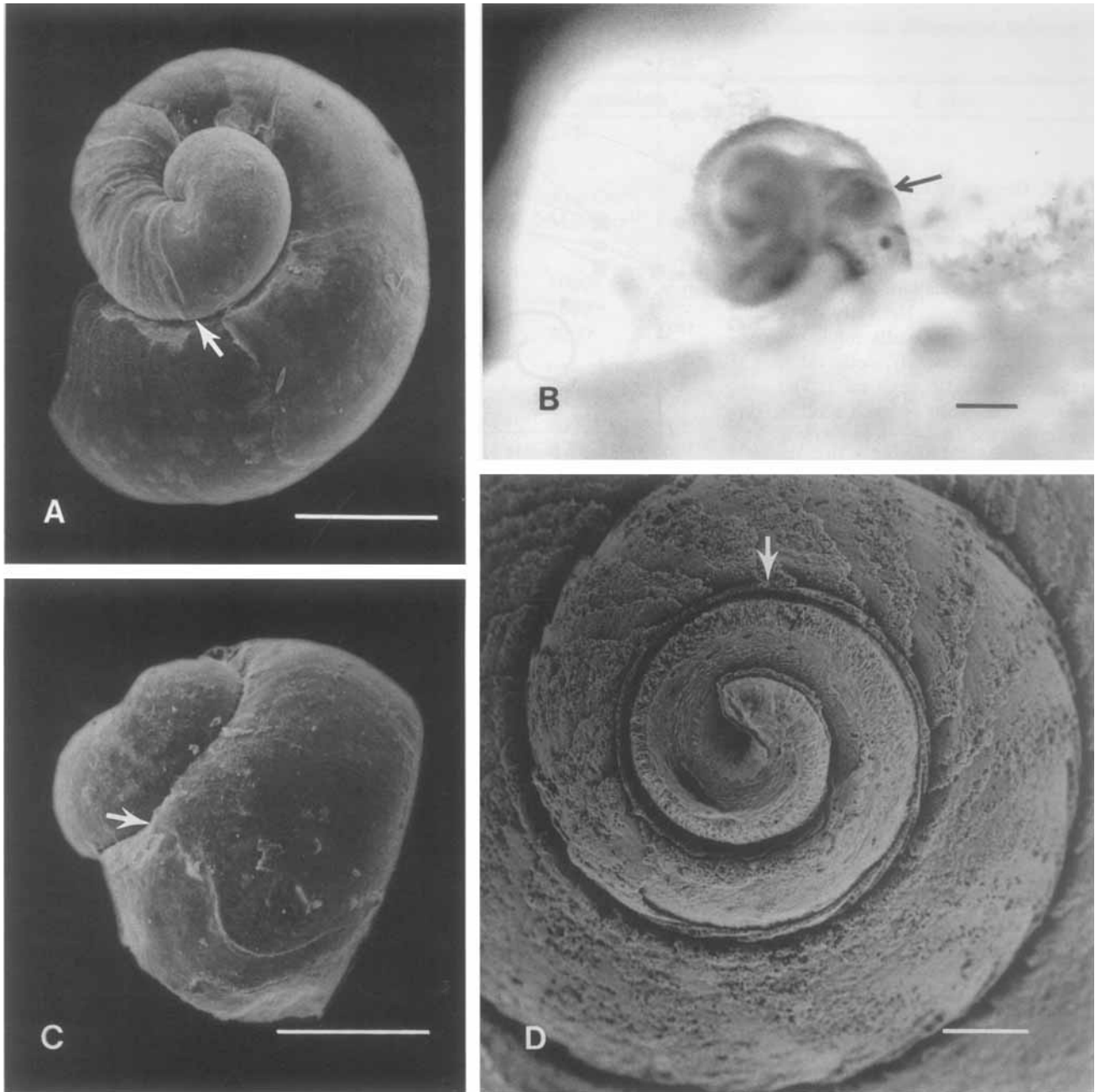
Fig. 5. A. Veliger shell of *L. scutulata* in oblique view. Arrow marks the protoconch I/protoconch II boundary (hatching). Note sinusigeral growth lines and velar notches at the aperture lip. SEM. **B.** Newly settled juvenile of *L. scutulata* on the barnacle shell on which it metamorphosed. Arrow points to the protoconch/teleoconch boundary. The juvenile right eye can also be seen. LM. **C.** Newly metamorphosed juvenile of *L. scutulata*. Arrow marks the protoconch/teleoconch boundary. SEM. **D.** Shell of *L. plena* collected in Puget Sound, shown in apical view. Arrow marks the onset of the imbricate pattern in the outer calcified layer. SEM. Scale bars, 100 μm.

OVA; .05 < p < .1) or in growth rates of the two species (2-way ANOVA; p > .1). However, both species exhibited the trend, seen in Fig. 6, of fastest growth at the medium food concentration and very little growth at the minimal food concentration.

Planktonic period

Only larvae fed at the medium or high food concentrations metamorphosed in the laboratory: 8 larvae of *L. scutulata* and one larva of *L. plena*. Larvae set-

Fig. 6. Growth of larvae of *L. scutulata* from one brood at 4 food concentrations of the microalgae *Isochrysis galbana* and *Rhodomonas lens*. Mean ± standard error; diamonds = minimal, 10^3 cells ml^{-1}; squares = low, 10^4 cells ml^{-1}; triangles = medium, 10^5 cells ml^{-1}; circles = high, 10^6 cells ml^{-1}. The measurement taken on each shell is shown in the lower left. Arrow marks the time at which larvae in the medium food concentration began crawling on the substrate.
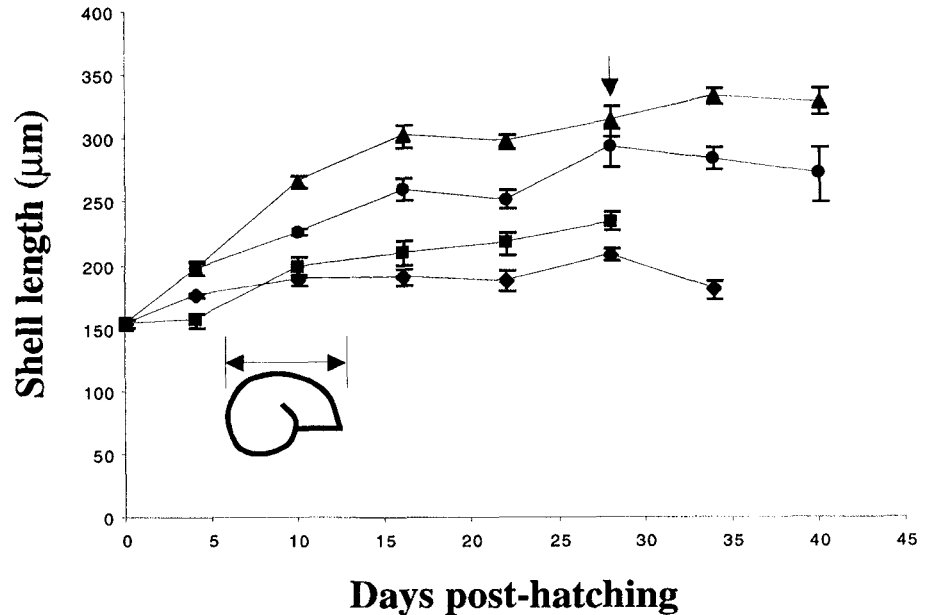
tled in the presence of barnacle shells, intertidal rocks, conspecific adults, and in the absence of any introduced cue. Total planktonic period for *L. scutulata*, including pre-hatching development, was 37–70 days, and the single larva of *L. plena* metamorphosed at 62 days. An additional 6 larvae metamorphosed in the laboratory at 48–61 days. However, these larvae hatched from type B capsules, and neither the capsules nor the mothers could be independently identified to species. Shell sizes at metamorphosis in *L. scutulata* were 320–408 μm, with a mean of 355 μm, and there was no relationship between age and size at metamorphosis (p > .5; n = 9). Fig. 5B shows a recently metamorphosed individual of *L. scutulata* that was crawling on the barnacle shell on which it had settled. The sinusigeral protoconch/teleoconch boundary is clearly visible. Fig. 5C shows a newly metamorphosed juvenile shell under SEM, and again the horn of the protoconch aperture lip is clearly visible where it is underlain by new accretionary growth. Even in lab specimens, however, this evidence of the protoconch/ teleoconch boundary in the outer shell layers is quickly worn away.

Protoconch size

Most shells of both species collected from the field were very degraded, and the degree of degradation seemed to vary consistently among collection sites. No surface sculpture, such as the boundary shown in Fig. 5C, was ever seen, but some parts of the shell microstructure remained. As in *Carinapex minutissima* (Hickman 1995a), the outer calcified layer showed a clear imbricate pattern (Fig. 5D). Toward the apex of

the shells this layer was typically worn away, but remnants of it were still visible in the sutures, where the imbricate pattern is seen in cross section. On the shell of *L. plena* shown in Fig. 5D, the imbricate pattern begins only at about 327 μm shell diameter. Shell diameter at this point was taken to be the size of the protoconch for 8 shells of *L. scutulata* and 25 of *L. plena*. Protoconch size in *L. scutulata* was 293–436 μm (mean = 339 μm), and in *L. plena* it was 256–405 μm (mean = 330 μm). These sizes were not significantly different (t-test; p > .5). Protoconch size in field-collected *L. scutulata* also did not differ significantly from lab-raised specimens (t-test; p > .5).

Discussion

Egg capsule morphology

The majority of the life history differences between the sympatric sibling species *Littorina scutulata* and *L. plena* are associated with the egg capsules (Table 2). This study corrects previous misinterpretations and establishes new diagnostic criteria for assigning capsules to species. However, capsule morphology may vary geographically. Buckland-Nicks et al. (1973) first described type B capsules from Puget Sound. Murray (1979) assigned these capsules to *L. scutulata*, but pictured a capsule with two unequal rims (type A) from his California collections. Similarly, Mastro et al. (1982) described California capsules of *L. scutulata* as having "two rims of very different diameters" (type A), and did not report finding any capsules with one rim (type B).

Number of eggs per capsule and egg size may also

Table 2. Summary of current reproductive data on *L. scutulata* and *L. plena*. Asterisks mark differences between the species.

Character	*L. scutulata*	*L. plena*
Size at maturity (mm shell height)	2–3[f]	2–3[c,f]
Spawning season		
Puget Sound	Apr–Oct[a,f]	Apr–Oct[f]
California	Mar–Oct[d]	Mar–Oct[d]
Maximum fecundity (eggs/female)		
*daily	1000[d]	1500[d]
over 2 weeks	7000[b,f]	
over 3 months	8000[f]	
*Egg capsule morphology (see Fig. 1)	type A,B[b,c]	type C[b,e]
	type A,B[f]	type B,C[f]
*Capsule outer diameter (μm)	700–1000[e]	1100[e]
	A,B: 337–976[f]	B: 730–1000[f]
		C: 841–1340[f]
Correlated with no. of eggs?	yes[f]	yes[f]
*Eggs per capsule [range (mean)]		
British Columbia	1–4 (2.9)[b]	
Puget Sound, San Juan Isl.	A,B: 1–11 (3)[f]	B: 2–11 (5)[f]
		C: 6–47 (19)[f]
Oregon		C: 4–35 (17)[b]
California	1–14 (7)[b]	C: 5–41 (23)[b]
*Egg size (μm)	105[b]	96[b]
	95[f]	90[f]
*Hatching at 12–14°C (days)	9[f]	8[f]
*Hatching at 15°C (days)	8[b]	7[b]
Size at hatching (μm)	155[b]	169[b]
	140–150[f]	130–140[f]
Total planktonic period in lab (days)	37–70[f]	62[f]
Size at metamorphosis (μm)		
laboratory [range (mean)]	320–408 (355)[f]	
field [range (mean)]	293–436 (339)[f]	256–405 (330)[f]

[a] Buckland-Nicks et al. 1973.
[b] Murray 1979.
[c] Mastro et al. 1982.
[d] Chow 1987.
[e] Reid 1996.
[f] This study.

vary geographically (Table 2). Murray (1979) found more eggs in capsules of *L. scutulata* from California compared to those from southern British Columbia. Consistent with these results, Behrens Yamada (1989) reported more eggs per capsule of *L. scutulata* from Oregon (mean of 10.7) than those from Puget Sound (means of 2.8, 3.0, and 3.1). In the current study, capsules of *L. scutulata* from Puget Sound contained 1–11 eggs (mean of 3), whereas Buckland-Nicks et al. (1973) reported 3–4 eggs per capsule from the Puget Sound area, although species identification by Behrens Yamada (1989) and Buckland-Nicks et al. (1973) is

uncertain. In the current study, I was able to identify capsules to species by combining morphology, diameter, and number of eggs. Murray's (1979) egg size measurements for California snails are larger in both species than those reported here for Puget Sound. Murray (1979) also reported lower fecundity for *L. scutulata* in California compared to Puget Sound, though this appears to be based on inaccurate recalculations of data from Buckland-Nicks et al. (1973). Given the evidence for geographic variation in these characters, future work outside of the Puget Sound area should first establish criteria for identifying egg capsules, us-

ing females that have been independently identified by tentacle coloration, shell characters, or other means (Hohenlohe & Boulding 2001).

Spawning season

This study and previous work suggest that females of *L. scutulata* and *L. plena* spawn sporadically from early spring to early fall (Table 2). Buckland-Nicks et al. (1973) found spawning on San Juan Island, Washington, from May through September with a peak in July, although both species may have been included. Behrens Yamada (1989) reported finding egg capsules, possibly of both species, around southern Vancouver Island from March to October. In northern California, Chow (1987) did distinguish the two species and found each to spawn from March to October. However, <25% of females of either species spawned in any single month, so individual females may concentrate their reproduction at different times during the spawning season.

Fecundity

Females of both species can reproduce by the time they are 3 mm in shell length (Mastro et al. 1982 and this study). Combining these sizes with the growth rate data given by Chow (1987) indicates that both species reach sexual maturity within a year. Chow (1987) reported higher peak daily fecundity in *L. plena* compared to *L. scutulata* in California (Table 2), and both the current study and that of Murray (1979) found that egg volume in *L. plena* was 17–30% smaller than in *L. scutulata*. These data suggest that *L. plena* may invest the same amount of energy in a larger number of smaller eggs than *L. scutulata*. In support of this possibility, larvae of *L. plena* in this study hatched at a smaller size, though Murray (1979) found the opposite result. Although seasonal fecundity estimates for *L. plena* are not available for comparison with those for *L. scutulata*, there appears to be a high level of intraspecific variability in fecundity.

Larval development

Murray (1979) and this study both found that larvae of *L. plena* hatch one day earlier than those of *L. scutulata*, so that pre-hatching period may be related to egg size as in species of the gastropod genus *Conus* (Perron 1981). Comparison of my results with those of Murray (1979) also suggests the expected pattern of faster development at a higher temperature (Table 2). In my study, all larvae that metamorphosed in the laboratory were fed at medium or high food concentrations. These larvae exhibited substantial growth in

size after hatching, developed juvenile structures such as tentacles and a foot, and exhibited a corresponding change in behavior. In contrast, larvae fed at the minimal food concentration showed little growth (though there was no statistically significant difference in growth coefficients among food concentrations), did not develop juvenile structures, did not show the same behavioral change, and did not survive to metamorphosis. Taken together, these results suggest that a substantial precompetent period, including both pre- and post-hatching development, is required before settlement and metamorphosis.

Planktonic period

The few individuals of *L. scutulata* that metamorphosed in the laboratory exhibited a nearly 2-fold variation in total planktonic period. Previous studies of marine molluscan larvae have likewise found a high level of intraspecific variation in planktonic period. For example, slower larval growth can extend the competent period (Pechenik 1980), while faster growth can shorten the precompetent period (Pechenik 1984; Pechenik & Lima 1984). Variation in larval growth rates may be the result of food availability (found here), genetic variation (Hilbish et al. 1993; Pechenik et al. 1996), or temperature (Zimmerman & Pechenik 1991). Geographic variation in response to settlement cues (Schubart et al. 1995) may also result in variation in planktonic period. Some species, particularly those with a wide range of settlement cues and adult habitats, may have a short and relatively constant competent period (e.g., Davis 1994). However, most species seem to fit the pattern predicted by Jackson & Strathmann (1981) that selection should favor flexibility in timing of metamorphosis and a large ratio of competent to precompetent period (Scheltema 1986b). In an extreme example, larvae of the prosobranch *Fusitriton oregonensis* survived as veligers for 4.5 years in the absence of settlement cues, and were still competent to metamorphose (M. Strathmann, pers. comm.).

Protoconch size

The data presented here suggest a model for shell growth in these species of *Littorina*: the larval shell is formed by accretionary growth of an organic matrix with relatively little calcification (Hickman 1995b). At metamorphosis, as in the prosobranch *Carinapex minutissima* (Hickman 1995a), accretionary growth begins beneath the lip of the protoconch and fills in the velar notches, replacing the sinusigeral aperture lip with a straight lip. The protoconch is calcified simultaneously, not by the mantle edge, but by the entire mantle surface. Further accretionary growth involves some

advance and retreat of the mantle, which produces the imbricate pattern seen in the outer calcified layer that begins only after metamorphosis. As the shell continues to grow at the aperture, the protoconch is filled with shell material until it is solid, as seen in Fig. 5D. Since this infilling takes place after metamorphosis and continues into the teleoconch as the snail grows, it would not retain any evidence of the protoconch/teleoconch boundary. However, in the outer calcified layer, metamorphosis is marked by the point at which the imbricate pattern begins.

Combining protoconch sizes with larval growth rates could yield an estimate of total planktonic period, though the many sources of variation discussed above should reduce confidence in such estimates. In this case, *L. scutulata* and *L. plena* did not differ significantly in either larval growth rates in the laboratory or protoconch size in field-collected adults, suggesting that there are no interspecific differences in planktonic period in nature.

Implications for gene flow

The life history data summarized here do not suggest differences in dispersal potential sufficient to explain the greater genetic subdivision of populations found in *L. plena* by Ward (1990) and Kyle & Boulding (2000). Interspecific differences in capsule morphology and diameter are unlikely to affect dispersal. All capsule types are slightly negatively buoyant and should behave similarly as passive particles in the water column, although their movement in flow has not been studied. The slightly longer pre-hatching period in *L. scutulata* would keep siblings together in an egg capsule for an additional day. On the other hand, capsules of *L. plena*, especially type C, tend to have more eggs, thus keeping larger numbers of siblings together before hatching. However, these effects are likely to be minor, since any differences in dispersal of capsules, spread of siblings, or pre-hatching development would affect no more than ~20% of the total planktonic period.

One might expect length and timing of spawning season to affect gene flow among populations by influencing the variability in ocean currents encountered by larvae, and thus the geographic range of new recruits from a given source. However, females of both *L. scutulata* and *L. plena* spawn during a broad period from spring to early fall, and their planktotrophic larvae presumably encounter a similar wide range of seasonal ocean currents.

Total planktonic period might also affect gene flow by determining dispersal potential. For example, Waples (1987) found a significant inverse correlation between planktonic period and population genetic differentiation in 10 species of marine fishes. Kohn & Perron (1994) found a significant correlation between minimum planktonic period and geographic range among species of *Conus*, suggesting a relationship between planktonic period and dispersal ability, though Scheltema (1989) found no such pattern among prosobranch gastropods. If the species examined here follow the relationship between egg size and precompetent period found in *Conus* species by Perron & Kohn (1985), *L. plena* should have a longer precompetent period. If larvae of *L. plena* on average have longer planktonic periods in nature, one would expect higher levels of gene flow and less genetic subdivision in this species, in contrast to the results of Kyle & Boulding (2000). However, neither the growth rates of larvae raised in the laboratory nor the protoconch sizes of field-collected adults suggest a consistent interspecific difference. In addition, theoretical considerations suggest that, with a minimum time of several weeks as measured here, differences in planktonic period have only a limited effect on gene flow (Hohenlohe 2000). Given the general similarity in larval characteristics of these two species and the expected high levels of intraspecific variation, any interspecific differences in planktonic period appear insufficient to influence gene flow and produce the different genetic patterns that have been observed.

The laboratory settlement data presented here indicate that both species can remain in the plankton for more than 2 months, placing them in the teleplanic, or maximum dispersal potential, category of Scheltema (1989; see also Levin & Bridges 1995). This presents the potential for dispersal across hundreds of kilometers per generation. In addition, both species spawn over a 6-month period, which should introduce high levels of variation into the direction that larvae travel from any population. These traits should produce panmictic populations, rather than the population genetic structure seen in both species, especially *L. plena*. Several other factors may have produced the current population genetic structure. These include historically isolated populations that leave a signature of genetic divergence (Hellberg 1995; Palumbi 1995), interannual variation in fecundity or recruitment success that results in stochastic gene frequency differences (Benzie & Stoddart 1992), or localized selection that severely limits recruitment of long-dispersing larvae (Burton 1986; Bertness & Gaines 1993). While each of these factors has been observed in other groups, they remain to be studied in these *Littorina* species.

ship in mathematical biology. Thanks to Danney Grae for mounting and photographing the SEM specimen shown in Fig. 5D, to Dianna Padilla for advice on raising larvae, and to Alan Kohn, Richard Strathmann, and Megumi Strathmann for general advice on this research.

References

Abraham ER 1998. The generation of plankton patchiness by turbulent stirring. Nature 391: 577–580.

Behrens Yamada S 1989. Are direct developers more locally adapted than planktonic developers? Mar. Biol. 103: 403–411.

Benzie JAH & Stoddart JA 1992. Genetic structure of outbreaking and non-outbreaking crown-of-thorns starfish (*Acanthaster planci*) populations on the Great Barrier Reef. Mar. Biol. 112: 119–130.

Bertness MD & SD Gaines 1993. Larval dispersal and local adaptation in acorn barnacles. Evolution 47: 316–320.

Buckland-Nicks J, Chia F-S, & Behrens S 1973. Oviposition and development of two intertidal snails, *Littorina sitkana* and *Littorina scutulata*. Can. J. Zool. 51: 359–365.

Burton RS 1986. Evolutionary consequences of restricted gene flow among natural populations of the copepod *Tigriopus californicus*. Bull. Mar. Sci. 39: 526–535.

Chow V 1987. Patterns of growth and energy allocation in northern California populations of *Littorina* (Gastropoda: Prosobranchia). J. Exp. Mar. Biol. Ecol. 110: 69–89.

———— 1989. Intraspecific competition in a fluctuating population of *Littorina plena* Gould (Gastropoda: Prosobranchia). J. Exp. Mar. Biol. Ecol. 130: 147–165.

Crisp DJ 1978. Genetic consequences of different reproductive strategies in marine invertebrates. In: Marine Organisms: Genetics, Ecology, and Evolution. Battaglia B & Beardmore J, eds., pp. 257–273. Plenum Press, New York.

Davis M 1994. Short-term competence in larvae of queen conch *Strombus gigas*: shifts in behavior, morphology and metamorphic response. Mar. Ecol. Prog. Ser. 104: 101–108.

Hellberg ME 1995. Stepping-stone gene flow in the solitary coral *Balanophyllia elegans*: equilibrium and nonequilibrium at different spatial scales. Mar. Biol. 123: 573–581.

Hickman CS 1995a. Asynchronous construction of the protoconch/teleoconch boundary: evidence for staged metamorphosis in a marine gastropod larva. Invertebr. Biol. 114: 295–306.

———— 1995b. Models of development and calcification of the gastropod larval shell. Am. Zool. 35: 52A.

Hilbish TJ, Winn EP, & Rawson PD 1993. Genetic variation and covariation during larval and juvenile growth in *Mercenaria mercenaria*. Mar. Biol. 115: 97–104.

Hohenlohe PA 2000. Larval dispersal, gene flow, and speciation in the marine gastropod genus *Littorina*. Ph.D. dissertation, University of Washington, Seattle. 126 pp.

Hohenlohe PA & Boulding EG 2001. A molecular assay identifies morphological characters useful for distinguishing the sibling species *Littorina scutulata* and *L. plena*. J. Shellfish Res. 20: 453–457.

Jablonski D & Lutz RA 1983. Larval ecology of marine

benthic invertebrates: paleobiological implications. Biol. Rev. 58: 21–89.

Jackson GA & Strathmann RR 1981. Larval mortality from offshore mixing as a link between precompetent and competent periods of development. Am. Nat. 118: 16–26.

Kohn AJ & Perron FE 1994. Life History and Biogeography in *Conus*. Clarendon Press, Oxford. 106 pp.

Kyle CJ & EG Boulding 2000. Comparative population genetic structure of marine gastropods (*Littorina* spp.) with and without pelagic larval dispersal. Mar. Biol. 137: 835–845.

Levin LA & Bridges TS 1995. Pattern and diversity in reproduction and development. In: Ecology of Marine Invertebrate Larvae. McEdward L, ed., pp. 1–48. CRC Press, Boca Raton, FL.

Mastro E, Chow V, & Hedgecock D 1982. *Littorina scutulata* and *Littorina plena*: sibling species status of two prosobranch gastropod species confirmed by electrophoresis. Veliger 24: 239–246.

Murray T 1979. Evidence for an additional *Littorina* species and a summary of the reproductive biology of *Littorina* from California. Veliger 21: 469–474.

Palumbi SR 1994. Genetic divergence, reproductive isolation, and marine speciation. Ann. Rev. Ecol. Syst. 25: 547–572.

———— 1995. Using genetics as an indirect estimator of larval dispersal. In: Ecology of Marine Invertebrate Larvae. McEdward L, ed., pp. 369–388. CRC Press, Boca Raton, FL.

Pechenik JA 1980. Growth and energy balance during the larval lives of three prosobranch gastropods. J. Exp. Mar. Biol. Ecol. 44: 1–28.

———— 1984. The relationship between temperature, growth rate, and duration of planktonic life for larvae of the gastropod *Crepidula fornicata* (L.). J. Exp. Mar. Biol. Ecol. 74: 241–257.

Pechenik JA & Lima GM 1984. Relationship between growth, differentiation, and length of larval life for individually reared larvae of the marine gastropod *Crepidula fornicata*. Biol. Bull. 166: 537–549.

Pechenik JA, Hilbish TJ, Eyster LS, & Marshall D 1996. Relationship between larval and juvenile growth rates in two marine gastropods, *Crepidula plana* and *C. fornicata*. Mar. Biol. 125: 119–127.

Perron FE 1981. The partitioning of reproductive energy between ova and protective capsules in marine gastropods of the genus *Conus*. Am. Nat. 118: 110–118.

Perron FE & Kohn AJ 1985. Larval dispersal and geographic distribution in coral reef gastropods of the genus *Conus*. Proc. Fifth Int. Coral Reef Cong., Tahiti 4: 95–100.

Reid DG 1990. A cladistic phylogeny of the genus *Littorina* (Gastropoda): implications for evolution of reproductive strategies and for classification. Hydrobiologia 193: 1–19.

———— 1996. Systematics and Evolution of *Littorina*. The Ray Society, London. 463 pp.

Scheltema RS 1971. Larval dispersal as a means of genetic exchange between geographically separated populations of

shallow-water benthic marine gastropods. Biol. Bull. 140: 284–322.

———— 1986a. Long-distance dispersal by planktonic larvae of shoal-water benthic invertebrates among central Pacific islands. Bull. Mar. Sci. 39: 241–256.

———— 1986b. On dispersal and planktonic larvae of benthic invertebrates: an eclectic overview and summary of problems. Bull. Mar. Sci. 39: 290–322.

———— 1989. Planktonic and non-planktonic development among prosobranch gastropods and its relationship to the geographic range of species. In: Reproduction, Genetics, and Distributions of Marine Organisms. Ryland JS & Tyler PA, eds., pp. 183–188. Olsen & Olsen, Fredensborg.

Schubart CD, Basch LV, & Miyasato G 1995. Recruitment of *Balanus glandula* Darwin (Crustacea: Cirripedia) into empty barnacle tests and its ecological consequences. J. Exp. Mar. Biol. Ecol. 186: 143–181.

Thorson G 1946. Reproduction and Larval Development of Danish Marine Bottom Invertebrates, with Special Reference to the Planktonic Larvae in the Sound (Øresund). C.A. Reitzels Forlag, Copenhagen. 523 pp.

———— 1950. Reproductive and larval ecology of marine bottom invertebrates. Biol. Rev. 25: 1–45.

Waples RS 1987. A multispecies approach to the analysis of gene flow in marine shore fishes. Evolution 41: 385–400.

Ward RD 1990. Biochemical genetic variation in the genus *Littorina* (Prosobranchia: Mollusca). Hydrobiologia 193: 53–69.

Zar JH 1984. Biostatistical Analysis. Prentice Hall, Englewood Cliffs, NJ. 718 pp.

Zimmerman KM & Pechenik JA 1991. How do temperature and salinity affect relative rates of growth, morphological differentiation, and time to metamorphic competence in larvae of the marine gastropod *Crepidula plana?* Biol. Bull. 180: 372–386.