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Reproductive Patterns in Six Species of *Lepidochitona* (Mollusca: Polyplacophora) from the Pacific Coast of North America

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Abstract. Reproductive patterns are documented and compared in six morphologically similar members of the chiton genus *Lepidochitona* from the west coast of North America (Oregonian and Californian Provinces). Three of the six species studied brood embryos: *L. thomasi* (Pilsbry, 1898), *L. caverna* Eernisse, 1986, and *L. fernaldi* Eernisse, 1986. The offspring of brooders are able to crawl away. In contrast, *L. dentiens* (Gould, 1846), *L. hartwegii* (Carpenter, 1855), and the less common *L. berryana* Eernisse, 1986 are free spawners whose offspring are obligate dispersers. The dispersal consequences of brooding or not brooding are exemplified by *Lepidochitona,* without major complications due to differences in larval size or larval feeding ability. Developmentally, brooders and free spawners in *Lepidochitona* differ primarily in stage (*i.e.,* age) at which larvae hatch from their egg capsules. Larval size and morphology differences are present but not as extreme as in other taxa.

As in many other taxa there is a link between brooding and particular life history traits, especially small adult size and self-fertilization. Size comparisons match the expectation that, as adults, brooders are generally as small or smaller than free spawners. The two smallest of the three brooders, *L. caverna* and *L. fernaldi,* are also simultaneous hermaphrodites, based on examination of gonads. These are the only known hermaphroditic chiton species, and are apparently fully capable of self-fertilizing multiple broods based on evidence from animals isolated for up to nine months in the laboratory. The third brooder, *L. thomasi,* is more typical of chiton species including those that brood; it has separate sexes and does not produce viable broods in isolation. Based on comparisons among chitons and among other groups that normally have separate sexes, hermaphroditism is argued to be a consequence of brooding, rather than the reverse. A mechanism is suggested that would link hermaphroditism, but not small adult size, to the consequences of crawl-away offspring. Small adult size could alternatively be attributed to the morphological constraints imposed by brooding.

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Introduction

A marine embryo, free spawned by a benthic animal into the plankton, is unlikely to return and settle where it was initially released. In contrast, some benthic marine animals brood embryos, often protecting them until they emerge as crawl-away offspring. Brooders, or egg-capsule layers, that have crawl-away offspring (called "brooders" hereafter) often share particular life history traits relative to free spawners, or even relative to animals that brood but then release larvae before they are competent to settle (*e.g.,* barnacles). The traits associated with brooders typically include small adult size and large egg size, corresponding to the release of relatively few large offspring that depend on yolk, rather than on planktonic feeding (Menge, 1975). Brooding has also been linked to hermaphroditism. At least in taxa whose majority of species are gonochoric (*i.e.,* have separate sexes), the exceptional hermaphroditic species invariably have crawl-away offspring and also tend to self-fertilize regularly (Strathmann *et al.,* 1984), unlike the vast majority of marine hermaphroditic animals (*e.g.,* barnacles, nudibranchs, tunicates) that have effective blocks to self-fertilization.

Defining the contrast between brooders and free spawners in terms of the likelihood an offspring will remain near its parent emphasizes the hypothesized differences such an opportunity might present. It would be incorrect, however, to attribute all life history traits

shared by brooders to their unique opportunity to crawl away. It is important to also recognize that certain traits may be influenced by the morphological and behavioral constraints of brooding itself. Moreover, differences between free spawning and brooding species can alternatively be explained by selection for planktonic feeding ability at the larval stage, or as a result of historical or ecological constraints on morphology. Yet there may be particular traits correlated to brooding that can be attributed specifically to the expected consequences of permitting one's offspring to remain nearby.

Brooding is distributed among ten quite distinct chiton genera (Pearse, 1979; this study). Given this presumed parallel evolution of brooding, comparisons among chitons might reveal shared patterns of brooding with other life history traits. Brooding is especially common in the genus *Lepidochitona.* No fewer than 6 of the 20 or so members of this genus are known to brood, including the subject of Kowalevsky's (1883) well-known embryological study, the Meditteranean species *Chiton polii* Philippi [=*L. corrugata* (Reeve)]. Also long known are two brooders studied by Heath (1899) as *Nuttallina thomasi* Pilsbry [=*L. thomasi* (Pilsbry)] and *Trachydermon raymondi* Pilsbry [=*L. caverna* Eernisse]. *L. caverna* is also noteworthy as the first reported hermaphroditic chiton species (Heath, 1907). Another brooder, *L. fernaldi* Eernisse, 1986, is distinguished herein as a second case of hermaphroditism in chitons. Adding to the list of brooders, Kaas and Strack (1986) have recently reported brooding in the West African species, *L. caboverdensis* Kaas and Strack, and H. L. Strack (in litt., 1986) has discovered brooding in the Canary Islands endemic species, *L. stroemfelti* (Bergenhayn).

In this study I document reproductive patterns in six morphologically similar species of *Lepidochitona* from populations along the west coast of the United States, including three of the brooders mentioned above: *L. thomasi, L. caverna,* and *L. fernaldi.* For all six species I emphasize: (*i*) evidence for free spawning or brooding; (*ii*) comparisons of early development; (*iii*) likelihood that offspring can crawl away; (*iv*) intra- and interspecific comparisons of size; and (*v*) evidence for gonochorism (*i.e.,* separate sexes) or hermaphroditism; and (*vi*) the possibility that individuals might produce viable embryos (*i.e.,* by self-fertilization or parthenogenesis) when isolated from other individuals. Using comparisons among *Lepidochitona,* I then explore hypotheses linking particular life history traits to brooding. These hypotheses may be especially relevant to patterns observed in other, normally gonochoric, marine invertebrate taxa.

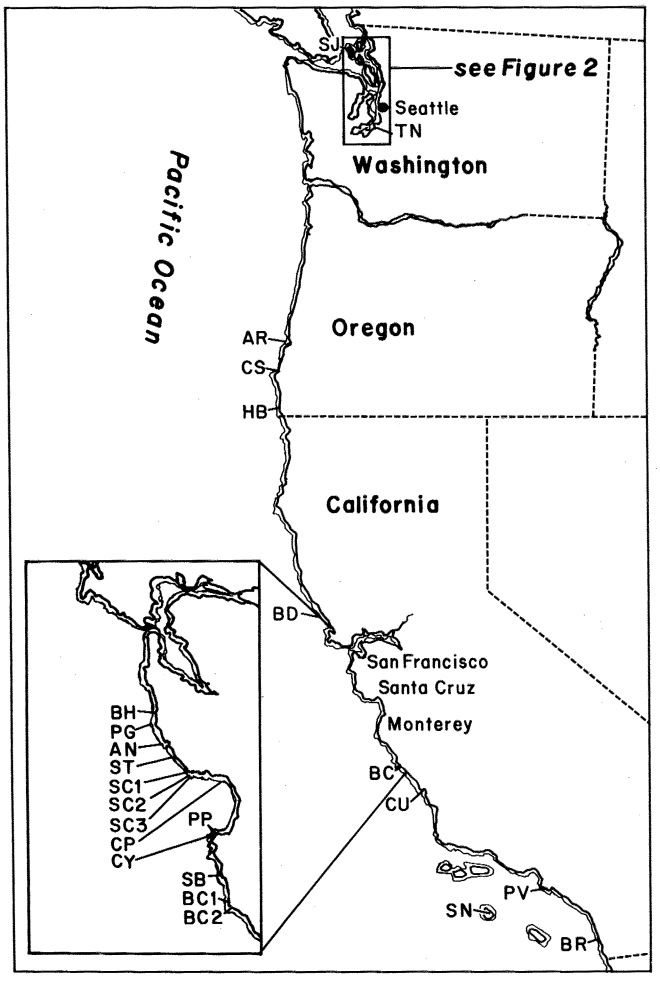

Figure 1. Primary study sites, abbreviated as follows (listed approximately north to south): In San Juan Co. (Washington): SJ = San Juan Is., west side (see Fig. 2). In Pierce Co.: TN = Tacoma Narrows. In Sonoma Co. (California): BD = Bodega Head. In San Mateo Co.: BH = Bean Hollow (Arrojo de los Frijoles); PG = Pigeon Pt.; AN = Año Nuevo Pt. In Santa Cruz Co.: ST = Scott Creek; SC = West Cliff Drive in Santa Cruz including SC1 = Auburn Ave.; SC2 = Stockton Ave.; SC3 = Pt. Santa Cruz; CP = Soquel Pt., Capitola. In Monterey Co.: PP = Pt. Pinos; CY = Cypress Pt.; SB = Soberanes Pt.; BC = Landels-Hill Big Creek Reserve. In Ventura Co.: SN = San Nicolas Is. In Los Angeles Co.: PV = Palos Verdes. In San Diego Co.: BR = Bird Rock, La Jolla.

Materials and Methods

Between January, 1980, and October, 1985, chitons were collected from a variety of locations in California and Washington (Figs. 1, 2, and Table I). Most animals were kept alive for observation at Long Marine Laboratory, Santa Cruz, California, or at Friday Harbor Laboratories, Friday Harbor, Washington. Animals transported from the field were supported by a small air pump and bubbler, cool temperatures, and frequent water changes. Aeration was especially helpful because chitons crawl out of oxygen-depleted water.

Over-zealous collecting was avoided, especially at localities where chitons occupied tightly packed barnacle hummocks, because destructive sampling was then required to obtain chitons. The pointed end of a Diamond-Deb™ fingernail file was an excellent collecting tool, su-

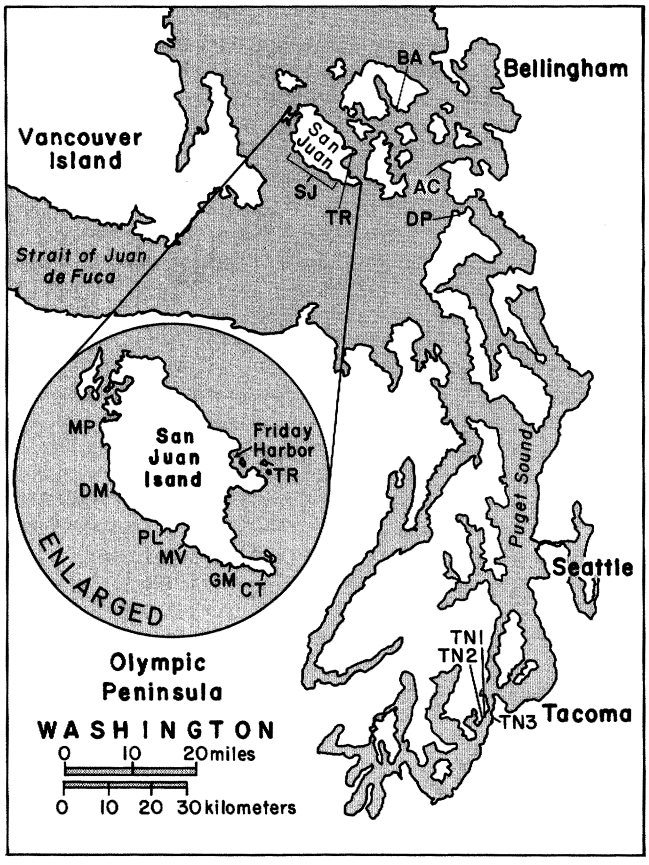

Figure 2. Study sites in San Juan Islands and Puget Sound, abbreviated as follows: SJ = San Juan Is., west side, including MP = Mitchell Pt.; DM = Deadman Bay; PL = Pile Pt.; MV = Mar Vista Resort; GM = Grandmother's Cove; CT = Cattle Pt. Also in San Juan Co. are: TR = Turn Rock; BA = Barnacle Rock. In Skagit Co.: AC = Shannon Pt., Anacortes. In Island Co.: DP = Deception Pass. In Pierce Co.: TN = Tacoma Narrows, including TN1, 2 near bridge on west side and TN3 at Salmon Beach, east side.

perior to conventional collecting blades or spatulas for small chitons. For a record of the percentage of individuals brooding (Fig. 3), all specimens of each species taken were routinely examined for brooded embryos in the pallial groove on each side of the foot. Chiton length from the most anterior to the most posterior margin of the girdle was measured in the lab by first allowing the animal to extend itself naturally on a flat surface. Length is an adequate descriptor of size for relative comparisons within and between species, but is somewhat complicated by the characteristic width and profile of each species and a shape change; individuals generally become wider and higher in profile with increasing size (Eernisse, 1984).

Size frequencies and descriptive statistics were calculated for each species from one or more population(s) (Figs. 4, 5). *L. caverna, L. fernaldi,* and *L. thomasi* were often brooding when collected, so the size of the brooding individuals is presented relative to the size frequency of all individuals measured (Figs. 5b, d, f). The collection of individuals for measurement is undoubtedly biased towards mature-sized specimens, because juveniles are inconspicuous and adults were taken preferentially.

Free-spawning species were kept in the splash tanks or, for short periods, in closed plastic containers filled nearly full with filtered seawater. This water was checked daily for evidence of spawned gametes and replaced daily with clean seawater. Observations were most detailed for *L. dentiens;* 220 adults were observed in the lab on various occasions for at least a few days after collection.

Additional specimens of *L. dentiens,* collected on six occasions from three locations in central California (BH, SC, and Mission Pt., Carmel), were prepared for histological analysis. Oocyte size frequencies were calculated for 16 females. For histological analyses of *L. dentiens* and other species, animals were relaxed in 7.3 percent aqueous $MgCl_2$, then fixed in Bouin's solution, with subsequent changes at least until decalcification was complete. Parafin sections (7–9 μm thick) were stained with Delafield's hematoxylin and counterstained with eosiny and orange-g. Six sagittal sections regularly spaced from the pallial groove to the center of the animal were prepared from each specimen. Oocyte size frequencies were then calculated by measuring only those oocytes sectioned through the nucleus (Pearse and Giese, 1966).

To examine the relationship of testis and ovary in hermaphrodites, the above methods proved inadequate. Instead, the gonads from some additional animals were dissected intact into 1.9% glutaraldehyde in 0.2 *M* Millonig's phosphate buffer (pH 7.6) at room temperature for one hour. The tissue was then rinsed in 2.5% sodium bicarbonate buffer (pH 7.2) for fifteen minutes and transferred to 2% osmium tetraoxide in 1.25% sodium bicarbonate buffer (pH 7.2) for an additional hour, dehydrated in an ethanol series. Three changes of propylene oxide were made before embedding in Medcast resin (Pelco). Sections (0.5-μm thick) were stained with Rich-

Table I

Summary of primary study sites and geographic range in six Lepidochitona *spp.*[1]

Species	Primary study sites (Figs. 1–2)	Known range limits (N to S)
L. dentiens	SJ, BD, BH, PG AN, ST, PP, BC	Alaska–so. California
L. hartwegii	PP, CY, SN, PV, BR	so. Oregon–Baja California
L. berryana	AN, CP	AN–PV
L. thomasi	BC	PP–Mill Creek (near BC)
L. caverna	SC1–3, PP, CY, SB	SC–Dolan Creek (near BC)
L. fernaldi	All Fig. 2 sites	Barclay Sound, B.C.–so. Oregon

[1] See Figures 1 and 2 for symbol key.

ardson's stain (Richardson *et al.,* 1960) and photographed with Nomarski optics.

The brooding species were collected or observed in the field in much greater detail than the free-spawning species. *L. caverna* was sampled for brooding in the field between January, 1980, and June, 1981, and again in March, 1985. *L. thomasi* was sampled between June, 1980 and August, 1982, and in March and October, 1985. *L. fernaldi* was sampled in January and September, 1981, once in August, 1982, and many times during spring, 1983, and fall, 1984, to summer, 1985.

Several methods of maintaining live animals were tried. A system of continuously splashing seawater provided the best health and survival. Large PVC or plexiglass tanks were employed either outdoors with shading, or indoors in a room with good exposure to sunlight. With too little light the chitons starve, and with too much light the substratum is overgrown by algae. To provide continuous splash, seawater lines were connected to a horizontal grid of one-half inch PVC pipes spaced approximately 20 cm apart and drilled on the bottom surface of each pipe were holes, 0.156 cm (5/32″) in diameter, approximately 2 cm apart. This grid was suspended about 20 cm above containers inhabited by chitons.

To isolate one or more chiton(s) from all other chitons, the chiton(s) was allowed to attach to a single rock (or brick) and then placed, surrounded by sand, in two-liter plastic containers approximately two-thirds full of sand. Holes in the plastic containers just above sand level permitted water outflow. This system provided low-maintenance rearing conditions for the adult chiton(s) and any brooded offspring. If no isolation was desired, chitons were allowed to mingle on a continuous hard substratum. When an animal was detached to be checked for brooding activity, there was greatly improved reattachment to hard surfaces if sand and other debris on the foot or in the pallial region were gently brushed away using wood fibers from a broken dry applicator stick. Broods were removed from the pallial region in the same manner.

Broods collected in the field or lab were usually kept in static filtered seawater cultures in small beakers with daily changes of filtered seawater. The progress of many cultures was recorded daily, with an approximate estimate of the percentage of eggs/embryos developing normally, and a recording of the appearance of the most useful developmental features. These included: early cleavage (2–64 cell stage), gastrulation and persistence of the blastopore opening, appearance of beating prototrochal cilia, appearance of eyespots, hatching, and metamorphosis. Although other potentially useful features exist (*e.g.,* foot, valve rudiments, girdle spicules), they were not generally required to determine embryonic stage of development. Some broods were collected during early cleavage and were followed through hatching and metamorphosis. The developmental schedules generated from these broods were used to assign approximate ages of appearance to the above developmental features. With this approach, it was possible to estimate to within approximately one day when any brood was spawned, even those collected at a late stage.

Postmetamorphic juveniles adhering to culture containers were introduced to outdoor splash tanks, preferably positioned away from direct spray exposure. Successful rearing to adulthood by this system required a dependable source of splash, moderately strong light source, seawater with a low level of silt, and removal of filamentous plants that tended to overgrow surfaces, by hand or by addition of grazers (*i.e., Littorina* spp. or the chiton, *Nuttallina californica*). An even higher survival rate for juveniles was attained when adult brooders kept in splash tanks were not disturbed, or if cultured larvae were allowed to metamorphose on the dorsal surface of their parent or on a small piece of barnacle plate and then introduced to a splash environment.

The early development of both brooders and free spawners was compared using light and scanning electron microscopy. All egg diameters were measured from light microscope preparations and from photomicrographs of spawned gametes. Light photomicroscopy with flash illumination as described in Eernisse (1984) was also useful for measuring active larvae. Eggs photographed with SEM were dissected from animals with mature gonads. For SEM, eggs and larvae were rinsed several times in millipore-filtered seawater, pipeted into capsules covered at each end with fine mesh nylon netting to permit fluid exchange, and immersed in 1% osmic acid in seawater buffered with 0.1 *M* sodium cacodylate. This buffer partially avoided dissolving calcareous spicules on the larvae or juveniles during fixation. After fixation, the specimens were prepared for SEM using standard procedures and scanned at 10 or 15 kV on a Nanolab 7™ SEM.

Results

The reproductive observations reported here reveal that species differ in their mode of spawning—either free spawning or brooding—and that among the species that brood there is a difference in mode of fertilization, they are either cross- or self-fertilizing. The evidence for distinctions in reproductive mode, size, and early developmental patterns is presented below, with slightly greater emphasis given to species that brood.

Evidence for free spawning in four species

Free spawning was observed in the lab for *L. dentiens, L. hartwegii,* and *L. berryana.* For *L. dentiens* from central California (BH in Fig. 1), 37 individuals (out of 220 total observed) free spawned on 11 separate occasions

from January, 1980, to May, 1981. All 11 spawnings occurred between the months of February and May 1980 and 1981. The histological analysis of 16 females collected at BH during peak spawning periods gave little indication that oocyte size reaches a maximum at any particular sample time; mature and immature eggs were present throughout February to May. The mean oocyte size of these six samples (n = 882 oocytes total) ranged from 38 to 56 μm in diameter, and only about 1.5% of the oocytes counted were large (>100 μm). The oocyte sizes in each of the 6 samples had similar ranges, usually about 8 to 168 μm in diameter (minimum range per individual = 8–152 μm). These spawning and preliminary oocyte size data, and several subsequent observations of spawning in Washington (SJ) populations, suggest that *L. dentiens* is reproductively active at least through winter and spring in both California and Washington.

Individuals of *L. hartwegii* (from PP) were observed free spawning on three separate occasions; in February, March (epidemic spawning), and October. Spawning in *L. berryana* (from SQ) was observed three times; twice in May and once in June. In the first May spawning, three *L. dentiens* males from BH were in close proximity to a *L. berryana* female who subsequently spawned. Care was taken to avoid excessive sperm and possible polyspermy by placing spawning males in another container. At the same time, eight additional individuals (4 males, 4 females) of *L. dentiens* from SJ began spawning in a separate container. Although there was ample *L. dentiens'* sperm available in both cases, only the *L. dentiens* eggs were fertilized, suggesting a block to hybridization between *L. dentiens* and *L. berryana.*

Though free spawning was not observed in the field, the laboratory observations and the absence of brooding was taken as indirect evidence that *L. dentiens, L. hartwegii,* and *L. berryana* were free spawners under field conditions. None of over 1000 specimens I have examined in more than five years were brooding. Collections were frequent enough that it is highly unlikely that brooding of brief duration was missed. A fourth species, *L. keepiana,* has been collected extensively in southern California by others but has never been reported as brooding. I have examined about 200 individuals at Palos Verdes, California (January, 1983) and over 100 individuals at Cayucos, California (April, 1984 and March, 1986). None were brooding.

Brooding in three species

Figures 3a–c depict the seasonal patterns of brooding in *L. fernaldi, L. thomasi,* and *L. caverna.* All three species brood for much or all of the year. Data from several years have been combined because the patterns appeared essentially the same from year to year.

L. fernaldi (Fig. 3a) and *L. thomasi* (Fig. 3b) have lengthy but somewhat restricted seasons, particularly with brooding activity peaking during winter and spring and little or no brooding in the field observed during August to October for either species. During this latter period, less than 1% of all *L. fernaldi* adults observed were brooding (1 of 287 at SJ; 2 of 157 at TN). Likewise, less than 1% of *L. thomasi* adults observed were brooding (0 of 108 at BC; 1 of 112 at other sites on Big Sur coastline). Only occasional brooders of either species were noted in the lab during August to October.

In contrast to *L. fernaldi* and *L. thomasi, L. caverna* may at least potentially brood throughout the year, a much longer reproductive season than previously re-

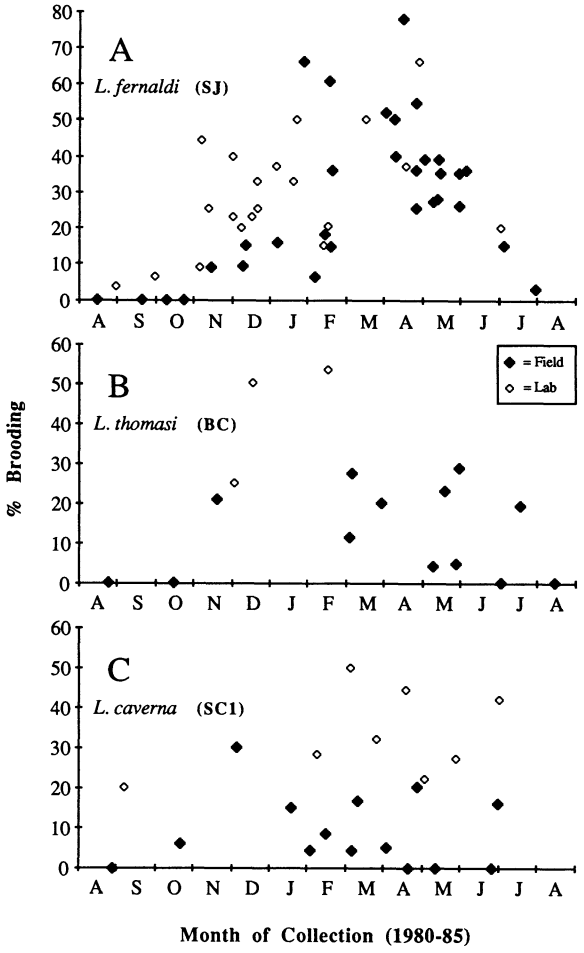

Figure 3. Seasonal brooding activity in *Lepidochitona fernaldi, L. thomasi* and *L. caverna,* between 1980–85. Dark symbols indicate field observations, open symbols indicate lab observations of recently collected animals. Statistics on field collections are given below as: n_c; n_b; n_t; $\bar{x}_b \pm s(n_{min} - n_{max})$; $\bar{x}_t \pm s(n_{min} - n_{max})$, where n_c = # of collections; n_b = total # of brooders; n_t = total # of adults observed; $\bar{x}_b \pm s(n_{min} - n_{max})$ = mean # of brooders per collection ± S.D. (range); $\bar{x}_t \pm s(n_{min} - n_{max})$ = mean # of adults observed per collection ± S.D. (range). *L. fernaldi:* 29; 163; 939; 5.6 ± 3.3 (0–12); 32.4 ± 33.7 (9–196). *L. thomasi:* 13; 79; 419; 6.6 ± 8.7 (0–31); 34.9 ± 27.2 (13–114). *L. caverna:* 14; 36; 457; 2.8 ± 2.7 (0–7); 33.7 ± 12.6 (10–50). Collection sites (see Fig. 1) are SJ, BC, and SC-1 for Figures 3a, b and c, respectively.

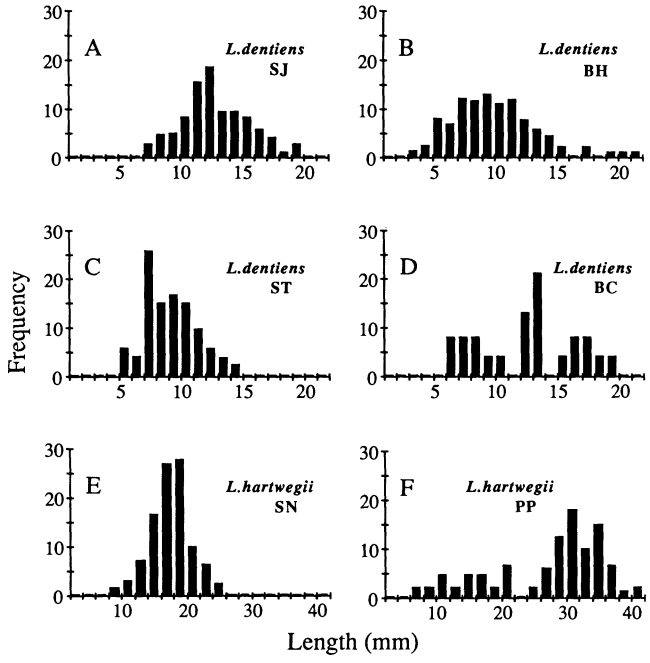

Figure 4. Size frequencies in *Lepidochitona dentiens* and *L. hartwegii* as length in mm, assigned to the nearest whole mm size class (see text). Sample statistics (given as n; $\bar{x} \pm$ S.D.; x_{max}) are as follows: (4a) 145; 12.5 ± 3.2; 27.3; (4b) 304; 9.4 ± 3.3; 22.0; (4c) 55; 8.7 ± 2.1; 14.0; (4d) 23; 12.2 ± 4.0; 18.5; (4e) 105; 16.1 ± 3.0; 23.6; (4f) 50; 25.8 ± 8.6; 39.7. Site abbreviations explained in Figure 1 legend.

ported by Heath (1907) (as *T. raymondi*). For unknown reasons in samples spread throughout the year, a high percentage of recently collected animals began brooding when brought into the lab, even during periods when no animals were observed brooding in the field (Fig. 3c—open symbols).

Size

The *Lepidochitona* considered here consist of five species with small adults and one, *L. hartwegii,* with medium-size adults (Figs. 4, 5). Among the brooding species, *L. thomasi* is largest but is still small compared to other chiton species. In the populations sampled, *L. thomasi* is characterized by a significantly greater mean length than *L. caverna* and *L. fernaldi* (Student *t*-test; $P < 0.001$ in both cases), and also has a significantly larger minimum brooding size ($P < 0.001$ in both cases). Smaller maximum adult size in *L. caverna* and *L. fernaldi* thus corresponds to smaller size at first reproduction. For each of the brooding populations, there is no apparent change in frequency of brooding with increasing size.

The size characteristics of a species vary significantly among populations, including comparisons of *L. hartwegii* (Fig. 4e, f), *L. dentiens* (Figs. 4a–d), and *L. caverna* (Figs. 5c, d) ($P < 0.001$ in each case). These size differences between populations were consistent throughout the year. Observed size characteristics of a population should therefore not be equated with those for a species throughout its geographic range.

Comparisons of early development

The eggs of *L. thomasi* and *L. fernaldi* are largest of the six species (260–280 μm diameter). Those of *L. caverna* and *L. berryana* are intermediate (220–240 μm diameter). Those of *L. dentiens* and *L. hartwegii* are smallest (200–220 μm diameter). Similarly, the larvae and recently metamorphosed juveniles become progressively smaller. These results match expectations that brooders normally have larger egg/larval size than closely-related free spawners, but the difference is not nearly as pronounced as in the case of most previous brooders and free spawners contrasted (see Discussion).

The eggs also differ in the ornate sculpturing of the transparent, extracellular egg hulls, illustrated here as imaged with SEM (Figs. 6a–f). With the exception of *L. thomasi* and *L. fernaldi,* whose egg hull sculpturing appears virtually identical (Figs. 6e, f), each species can be

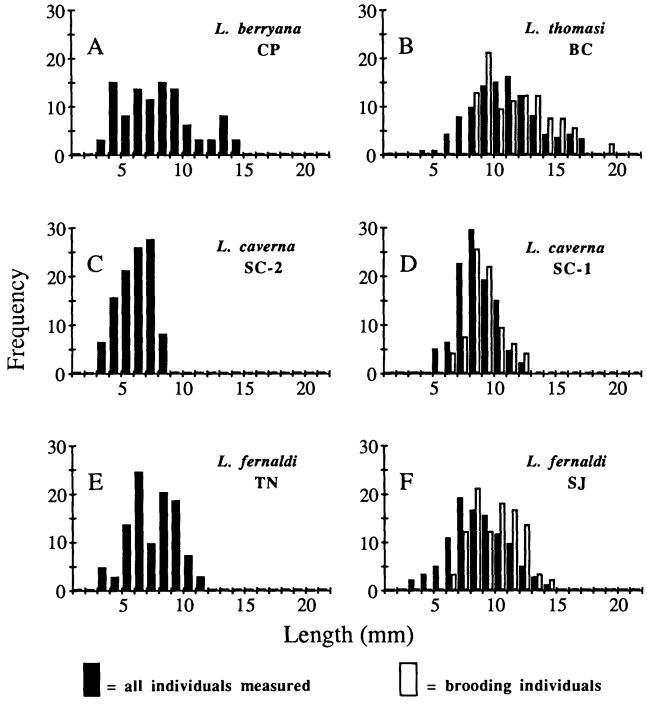

Figure 5. Size frequencies in *Lepidochitona berryana, L. thomasi, L. caverna,* and *L. fernaldi* as length in mm, assigned to the nearest whole mm size class (see text). Sample statistics (given as in Fig. 4 legend) are as follows: (5a) 38; 7.6 ± 2.9; 14.1; (5b) all individuals: 341; 10.4 ± 2.7; 18.5; brooders only: 57; 11.3 ± 3.1; 18.5; (5c) 68; 5.7 ± 1.3; 8.4; (5d) all individuals: 147; 8.2 ± 1.4; 12.0; brooders only: 42; 8.6 ± 1.4; 12.0; (5e) 45; 7.1 ± 1.8; 10.9; (5f) all individuals: 355; 8.3 ± 2.4; 17.0; brooders only: 89; 8.7 ± 2.2; 15.2. Site abbreviations explained in Figure 1 legend.

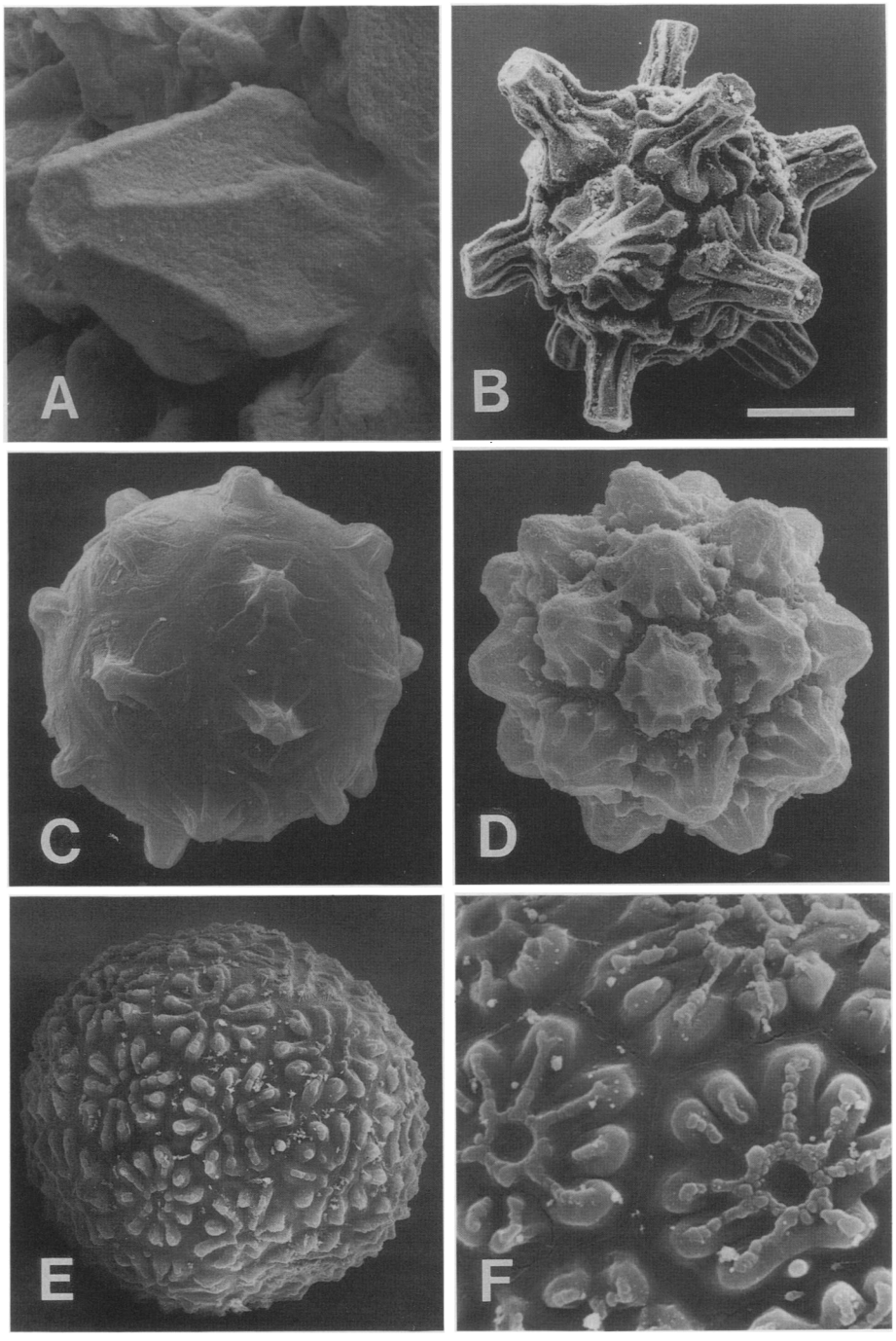

Figure 6. Eggs of six species of *Lepidochitona* as imaged with SEM. All eggs were dissected from animals with mature gonads but, except for a slightly smaller egg diameter, appear very similar to eggs that are spawned. The eggs correspond to (a) close-up of *L. hartwegii* egg hull; (b) *L. berryana;* (c) *L. caverna;* (d) *L. dentiens;* (e) *L. thomasi;* and (f) close-up of *L. fernaldi* egg hull surface. Scale bar: (a, f) = 20 μm, (b) = 63 μm, (c) = 48 μm, (d) = 54 μm, and (e) = 51 μm.

distinguished by its characteristic pattern of sculpturing. The free spawners (Figs. 6a, b, d) also have much more elongate cone-shaped hulls than the brooders (Figs. 6c, e, f).

In all three free-spawning species, the larvae at the time of hatching are very similar and can be represented by the SEM image of a two-day-old *L. hartwegii* trochophore in Figure 7a. At this stage, the larvae swam continuously and lacked a foot for attachment. In cultures with a high (>90%) fertilization success, very little variation

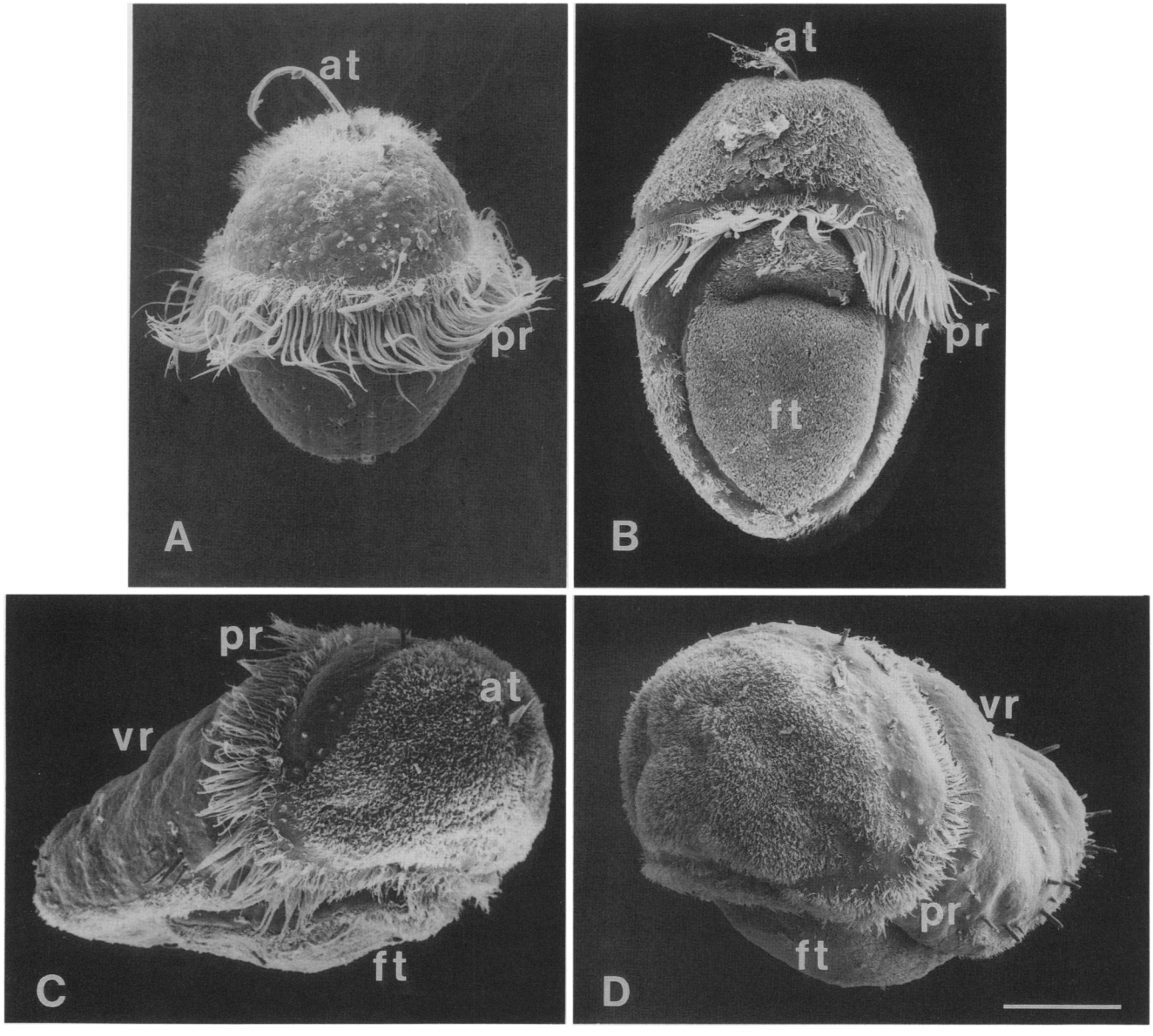

Figure 7. Larvae near stage of hatching as imaged with SEM in (a) *Lepidochitona hartwegii* (approx. 2 days old, fixed approximately 12 hours after synchronous hatching of a culture from one free-spawning female); (b) *L. caverna* (approx. 7 days old); *L. thomasi* (approx. 9 days old); and (d) *L. fernaldi* (approx. 9 days old). Explanation of symbols: pr = prototroch; at = apical tuft; ft = foot; vr = valve rudiments. Scale bar: (a–d) = 50 μm.

in time of hatching was observed in any of the species. In several cultures observed in the most detail, about 80 to 90% of the larvae hatched within a few hours of each other. A less-precise estimate is possible for all other cultures which were monitored only daily. For these, I conclude that more than 95% of the embryos hatched between 24 and 48 hours (13–16°C), if they hatched at all.

Larvae from free-spawning species swam continuously in culture for several days, until the foot and eyespots developed. These larvae began exploratory creeping behavior at about 5–6 days (at 13–16°C). Some larvae metamorphosed at about 8–10 days. However, most larvae did not metamorphose, and there was substantial variation in size of juveniles that did metamorphose. Most hatched larvae either deteriorated or remained unmetamorphosed for weeks after other larvae in the same culture had completed metamorphosis. I did not investigate stimuli to metamorphosis in these species.

In contrast, the embryos of all three brooding species hatched as larvae (*i.e.*, still with a prototroch) but at an advanced stage ready to metamorphose. Unlike recently hatched free-spawned larvae, brooded larvae already

have a well-developed foot by the time they hatch (Fig. 7b–d), and thus are capable of creeping and consequently can at least potentially remain in the vicinity of their parent(s) and siblings. Brooded larvae hatch about seven to eleven days after fertilization at 14–16°C, and thus hatch much later than do embryos of free spawners. The eyespots (a convenient unambiguous indicator of larval stage) appear about four to five days after fertilization—well after all the free-spawned larvae have hatched and well before brooded larvae hatch.

Hatching stage was influenced by the removal from the brooding parent. Broods removed from an adult at a very late stage of development often began hatching immediately, with most embryos hatching within one or two hours. Such emerging larvae could be one to two days more developed at hatching than larvae shown in Figures 7b–d. These larvae could complete metamorphosis within a day of hatching. These observations suggest that artificial culturing of broods stimulates a precocious hatching of embryos. Thus, embryos brooded naturally are even more likely to avoid pelagic dispersal upon hatching.

Larval morphology and development noticeably differs among brooders. Cultured embryos of *L. caverna* are slightly smaller and hatch earliest, roughly seven to nine days after fertilization at 14–16°C (Fig. 7b). At this stage, the larvae normally swim for one to two days in static cultures before settling and metamorphosing (defined as loss of prototroch; Pearse, 1979). Swimming, however, may be stimulated by artificial culturing, since juveniles soon appear on or near undisturbed brooders isolated in the lab (see below). Cultured embryos from both *L. thomasi* and *L. fernaldi* hatch slightly older and larger than those of *L. caverna,* about nine to eleven days after fertilization at 14–16°C (Figs. 7c, d). *L. fernaldi* embryos develop only slightly slower when cultured at the 8–12°C they normally experience when submerged at high tide (hatching by 12 days at 10°C). Again, metamorphosis generally occurs within two days of hatching in static cultures. In both *L. fernaldi* and especially *L. thomasi,* brooded larvae are sluggish when they hatch, preferring crawling to swimming. *L. fernaldi* larvae differ from *L. thomasi* (and *L. caverna*) larvae in consistently lacking an apical tuft, and possessing shorter prototrochal cilia (Fig. 7d).

Egg clusters of brooders also differ among species. The color of the egg cluster varies according to stage of development. Coloration proceeds from greenish to golden in *L. caverna,* from dark brownish green to tan in *L. thomasi,* and from light brown to golden brown in *L. fernaldi.* In *L. caverna,* the eggs are held loosely. In both *L. thomasi* and *L. fernaldi* the eggs are held together with mucus and two (or one) rod-like egg clusters can be removed intact from the brooder. Adults of these latter species are much more mobile than adults of *L. caverna* during brooding; the tightly clustered broods may permit increased mobility and feeding by adults.

In contrast to the free spawned larvae, larvae from brooders metamorphosed reliably in the lab, even in culture vessels filled only with filtered seawater. Survival was usually high (>95%) and morphological changes immediately following metamorphosis were apparent, especially a marked change in body outline as elongate larvae become oval-shaped juveniles. Girdle spicules proliferated and calcification of the valves was initiated or at least greatly accelerated. In containers with a film of microorganisms, but without overgrowth of filamentous diatoms, newly metamorphosed juveniles were observed to feed actively, leaving a trail of marks from radular scraping as they crawled (Eernisse and Kerth, 1988). Growth was generally rapid, indicating that these juveniles were developing normally.

Evidence for hermaphroditism and self-fertilization in L. caverna

Heath (1907) briefly described a simultaneous hermaphroditic condition in *L. caverna* (as *T. raymondi*). I also found that typical *L. caverna* are hermaphroditic and, as noted by Heath, have a large majority of their gonad occupied by ovaries. Figure 8 shows the intact gonad of one of two adult animals, raised in the lab, that were dissected and fixed in September, 1986. The testicular lobules in this animal are restricted to the peripheral portions of the gonad, and several contain mature sperm. This gonad is consistent with the observed gonad of more extensive field samples of *L. caverna* from 1980 to 1986. Sperm (and eggs) were regularly observed in wet dissections of live adults, and had a shape and size similar to other chiton sperm, although they were never abundant. Histological samples of 110 animals, from collections of about ten animals taken every six weeks between February, 1980 and June, 1981, showed most animals had mature gonads, regardless of the season, primarily filled with eggs. Of 94 animals sampled with detectable gonads, 89 possessed eggs. Only five males lacking eggs were found. Sixteen specimens could not be sexed either because they were reproductively immature, or because the sections were inadequate. Small amounts of sperm or testes were seen in many of these 89 "females," similar to those in Figure 8, but were not found in others, probably because of their sparseness or because of incomplete infiltration of fixative and embedding medium as indicated by open spaces in these whole mount sections.

To test the potential ability of *L. caverna* to self-fertilize broods, I repeated, on a more limited scale, the isolation experiments of Pearse and Lindberg (1980), also as in their studies (reported as *C. dentiens*) at Long Marine Laboratory with animals collected from a nearby, Santa Cruz, sea cave (the type locality). Five individuals each

produced cohorts of offspring more than one month after isolation, including some juveniles that survived to adult sizes.

Evidence for hermaphroditism and self-fertilization in L. fernaldi

When I first discovered *L. fernaldi* populations in Washington, I identified them as northern populations of the morphologically similar gonochoric species, *L. thomasi,* known previously from only a few localities in central California (Eernisse, 1986). An experiment initially designed to test the hypothesis that individuals from two geographically isolated populations could be crossbred in the lab, instead became a comparison of the capacity of *L. fernaldi* and *L. thomasi* to breed without conspecific mates.

Eight pairs, each consisting of one BC *L. thomasi* known to be a female and one randomly selected SJ *L. fernaldi,* were isolated on 8 February 1981; each pair was independently supplied with splashing seawater. Neither species is known from the vicinity of Santa Cruz, so it is highly unlikely that sperm could have entered through the Long Marine Lab seawater system. Nearly all the *L. fernaldi* individuals began brooding, and broods were fertilized; larvae hatched and metamorphosed normally. This suggested that the *L. fernaldi* brooders were hermaphrodites capable of self-fertilizing their broods. In contrast, some of the known *L. thomasi* females began brooding, but the broods failed to develop (see below).

New experiments were then performed with 32 *L. fernaldi* individuals isolated completely from all other individuals, and periodically sampled for broods. As before, animals produced a large percentage of fertilized eggs. Of 25 individuals surviving in isolation for more than 60 days, 16 produced at least one brood and nine produced multiple broods. Altogether, I collected 31 broods, including 20 that were more than 90% fertilized. Of the remaining 11, two were less than 25% fertilized and two more appeared fertilized but succumbed to bacterial infections. The large majority of embryos hatched and metamorphosed successfully in culture.

Of the individuals spawning multiple broods, one spawned five separate broods, each at least partially fertilized. I collected broods from this individual, isolated from 25 October 1981 to 13 June 1982 (231 days), on days 41, 60, 76, 88, and 195. Coincidentally, the first four broods were discovered at approximately the same advanced stage of development, so this animal was spawning broods at approximately two week intervals. Three individuals brooded three times each; again each brood was at least partially fertilized with six of the nine broods more than 95% fertilized. One of these nine broods (>50% fertilized) was collected 274 days (approx. nine months) after the animal was isolated. For all seven individuals that produced multiple broods, I detected no significant difference (Mann-Whitney U Statistic) between the mean percent fertilization of the first ($\bar{x}$ = 78.1 %; S.D. = 33.9) and last ($\bar{x}$ = 80.0; S.D. = 36.6) brood.

Approximately 25 live *L. fernaldi* were, at various times, dissected without finding any evidence of spermatophores. These dissections, combined with later histological analyses of five animals, suggest that normally only about one out of every 10 individuals produces large testes and no obvious ovaries, while most if not all other individuals possess mostly ovaries and very small amounts of testes. The *L. fernaldi* gonads appear similar to those previously described for *L. caverna* (Heath, 1907; this study). Simultaneous hermaphroditism and reproduction by isolated individuals suggests strongly that *L. fernaldi* is capable of self-fertilization, although it does not completely rule out the possibility of parthenogenesis (see Discussion).

Evidence for strict gonochorism in L. thomasi

Histological sex determination provided evidence that *L. thomasi* is normally gonochoric, since strict males were relatively common. Three separate collections were made at BC during a period of known brooding activity. Sex was determined from sections. Of 27 individuals sectioned, 9 were males with large testes, 5 were females without any trace of sperm, and 13 could not be sexed either because their gonads were immature or because the sections were inadequate. It is likely that this male-biased sex ratio resulted from a small sample size, although some male-biased inequalities have been observed for chitons (Pearse, 1979; Sakker, 1986). An additional, unquantified impression that males are at least as common as females comes from the experience of dissecting over 150 frozen or live *L. thomasi* for electrophoretic studies, including animals from seven localities on the Big Sur coastline collected over several years.

Based on morphological and electrophoretic affinities relative to other members of the genus, I previously argued that *L. fernaldi* and *L. thomasi* are closely related (Eernisse, 1984, 1986). The great similarity in egg hull sculpturing patterns presented here (Fig. 6) further corroborates this conclusion. Yet these species are, without doubt, morphologically and electrophoretically distinct from each other. Moreover, I show here that *L. fernaldi* is hermaphroditic, whereas *L. thomasi* individuals are either female or male, and females do not produce fertilized broods in isolation. Twenty-seven isolated individuals of *L. thomasi* were sampled for brooding periodically while under isolation. Although the observations were at least as frequent as for *L. fernaldi,* only eight broods were collected and none of these broods developed normally.

To be certain that this failure was due to lack of fertilization I isolated eight *L. thomasi* females known to have

Table II

Summary of reproductive patterns in six Lepidochitona *spp.*

Species	Spawning mode	Inferred fertilization mode	Mean egg size	Mean age at hatching	Max. adult length
L. dentiens	free spawner	cross	210 μm	36 h	27.3 mm
L. hartwegii	free spawner	cross	210 μm	36 h	45.1 mm
L. berryana	free spawner	cross	230 μm	36 h	14.1 mm
L. thomasi	brooder	cross	270 μm	8 days	18.5 mm
L. caverna	brooder	self	230 μm	10 days	13.9 mm
L. fernaldi	brooder	self	270 μm	12 days	17.0 mm

previously brooded, and checked daily for freshly spawned broods. During one month I collected broods from three individuals. Immediately after each collection, I dissected a male *L. thomasi,* and used its sperm to inseminate a portion of the collected eggs. Another portion of eggs was kept without sperm as a control. Each of the three times this experiment was performed at least some of the eggs with sperm added became fertilized (20, 60, and 100%), but none of the eggs without added sperm were fertilized. This demonstrates that spawned eggs are potentially viable, but do not become fertilized because of lack of viable sperm.

Discussion

The results of life history comparisons among six northeastern Pacific species of *Lepidochitona* are summarized in Table II. Similarities among the *Lepidochitona* considered here include the size and rates of development of embryos. Eggs of brooders are roughly the same or slightly larger than eggs of free spawners, and the size of newly metamorphosed juveniles of brooders and free spawners is correspondingly similar. Differences between free spawners and brooders include stage (*i.e.,* age) at which embryos hatch. Stage at hatching appears to be a relatively inflexible character within a particular species. I have presented evidence that this relatively minor developmental difference between free spawners and brooders has a functionally important consequence: brooded offspring, but not free-spawned offspring, at least have the potential to crawl away. Here I argue that a potential to remain near one's parent may explain some, but not necessarily all, of the life history traits shared by *Lepidochitona* brooders.

Most previous studies contrasting brooding and free spawning marine invertebrate species (*e.g.,* Menge, 1975) have reported dramatic differences in egg size and larval feeding between brooders and free spawners. Although the correlation of these factors with mode of spawning is an interesting subject in its own right, these differences confound interpretations concerning the consequences of crawl away dispersal that can result from brooding. Brooders typically have relatively few, large yolky eggs with lecithotropthic development, while free spawners have numerous small eggs with lengthy planktotrophic development. The comparison of brooders and free spawners in *Lepidochitona* appears less complicated by larval feeding or egg size. Specifically, planktotrophy is unknown for chitons, so the comparison of brooding and free spawning chitons is uncomplicated by a selective advantage that might be gained from larval feeding (Grant, 1983). Furthermore, although *Lepidochitona* brooders in some cases have larger eggs than free spawners, as much a two-fold volume difference, previous studies have typically reported egg diameters which translate to a five- to ten-fold egg volume difference (*e.g.,* Menge, 1975). Thus, the larval size, morphology, and feeding ability differ to a lesser degree in *Lepidochitona* than in most previous comparisons of brooders and free spawners.

The differing potential for emerging larvae to crawl away in *Lepidochitona* is repeated within chitons as a whole. *L. dentiens, L. hartwegii,* and *L. berryana* are like other free-spawning chitons (Pearse, 1979; Strathmann and Eernisse, 1987) whose pelagic embryos invariably hatch out of their egg capsules soon after their prototrochal cilia become active. In a literature search of over 50 species of chitons reported to spawn eggs freely or in loose strands of mucus, I could find no exceptions to this pattern. Free spawned embryos hatch several days before a foot has developed, so that even if embryos were retained until hatching, they would presumably have difficulty remaining attached to a substrate. The active swimming observed in laboratory cultures of free spawners also suggests they are normally pelagic.

In contrast, *L. caverna, L. fernaldi,* and *L. thomasi* are like other chiton brooders, retaining embryos in the paired pallial grooves to an advanced stage of development. Brooding is known with certainty for about 30 (out of about 800 total) chiton species as compiled by Pearse (1979) and added to by Sirenko (1973), Penprase (1979), O'Neill (1984), Burn (1984), Cochran (1986), Creese (1986), Kaas and Strack (1986), Creese and O'Neill (1987), and the present study. Additionally, there are at least a few chiton species that neither free spawn nor brood. Instead, they embed their eggs in a benthic mass of jelly-like substance, and leave this egg mass behind, often attached to rocks or algae (Heath, 1899, 1905; Risbec, 1946; Matthews, 1956). All studied chiton brooders or benthic egg mass layers hatch as larvae sufficiently advanced so that they are already endowed with a fully functioning foot for crawling. Embryos from most or all of these species hatch shortly before metamorphosis, although in some of the above cited cases, offspring may remain in the pallial groove until well after metamorphosis.

I do not claim that brooders are without variation.

Both inter- and intraspecific differences among brooders in stage of hatching suggests that there may be some variation in facultative planktonic dispersal ability of brooders. Still, the observed variation in chiton brooders is slight enough so that even the earliest hatching brooded larvae have a good opportunity to remain near parents. For example, of the three *Lepidochitona* brooders considered here, *L. caverna* is exceptional because it hatches at a "free swimming" stage when embryos are cultured apart from their parent. Similarly, other chiton brooders demonstrate flexibility in swimming behavior, depending on circumstances. For example, I have observed the same "early" hatching stage (*i.e.,* swimming, but with a well-developed foot, eyespots, shell plate rudiments, etc.) in cultured embryos of *Lepidochitona corrugata* (Reeve, 1848), collected from brooders in Yugoslavia (Eernisse, unpub.), in agreement with Kowalevsky's (1883) observations for this species (referred to as *Chiton polii* Philippi, 1836). Even in these chiton brooders that hatch at a "free swimming" stage in culture (*e.g., L. caverna* and *L. corrugata*), there appears to be a clear opportunity for offspring to crawl away.

At another level is potential variation within a species. Creese (1986) recently reported variation in both hatching stage and embryo size in brooders of what he considered to be geographically isolated populations of the same species, *Onithochiton neglectus* de Rochebrune, 1881. I searched for, but did not find, such variation in *L. fernaldi* compared from all sites in Figure 2, or in *L. caverna* from sites SC1-3, PP, CY, or SB. Egg and larval size, as well as time to hatching, appeared approximately uniform within each species. The variation Creese found is unparalleled in my own studies, but even the *O. neglectus* populations he reported as hatching earliest were sufficiently well developed to be capable of crawling away.

Likewise it is not possible to conclude that some chiton brooders never have planktonic dispersal, even for species found brooding fully metamorphosed juveniles. To my knowledge, there are no reports of adults externally brooding *unhatched,* fully metamorphosed juveniles, so I suspect that all chiton brooders may have at least some occasional ability to swim as larvae. An exception might be the possibly ovoviviparous chiton, *Calloplax vivipara* (Plate, 1899), a single animal of which was reported by Plate (1899) to be internally brooding 15 metamorphosed juveniles. In practice, it would be difficult to estimate the degree to which planktonic dispersal is important for a particular brooder.

Differences in spawning and stage at hatching may explain why brooders tend to have a patchy distribution, relative to free spawners. The free spawners that hatch early are typically found wherever suitable habitats are searched throughout their range. *Lepidochitona* free spawners are typically widespread (although not always common) and are more uniformly distributed than the species that brood (Eernisse, 1986). The brooders, whose larvae can crawl away, are found in isolated but locally dense populations. For example, several authors during the last century have expressed exasperation after failing to find *L. thomasi,* even though this brooder is quite abundant locally (Eernisse, 1986). Likewise, the only recently described and rarely collected *L. caverna* and *L. fernaldi* have similar, patchy, distributions. Quantifying the absence of brooders from localities that appear suitable is difficult, but the abundance of brooders where they do occur speaks strongly for their colonization ability. For example, a dramatic colonization event was observed after the recent opening of the Monterey Bay Aquarium, Monterey, California. In July, 1986, within a year of the creation of an artificial, high energy tidepool environment at the aquarium, I observed *L. caverna* to be present in densities estimated at 500 per square meter (Eernisse, unpub.), especially individuals less than 3 mm length. None of the many chiton free spawners of the Monterey Peninsula region were observed in even moderate densities.

Brooders also tend to have a somewhat more restricted geographic range than free spawners, although the range of some brooders is relatively great. For example, the geographic range of *L. fernaldi* extends a considerable distance (many sites in the San Juan Islands, several sites in Puget Sound, on Bordelais Island in Barclay Sound, Vancouver Island, British Columbia, and several sites in southern Oregon) (Eernisse, 1986; this study). In contrast, *L. thomasi* appears to be restricted to the Big Sur coastline and vicinity, a total known distribution stretching only approximately 30 km. Of 137 intertidal invertebrate species surveyed at the Landels-Hill Big Creek Reserve near Big Sur, which had a known geographic range, *L. thomasi* was the invertebrate species with the most restricted distribution (Lindberg, 1984). The rather extensive range of *L. fernaldi* may either indicate that facultative planktonic dispersal has been relatively effective in some cases or that juveniles or adults have rafted successfully, perhaps on algae, as Simpson (1977) first proposed for the widespread subantarctic brooder, *Hemiathrum setulosum* Carpenter, in Dall, 1876. *Lepidochitona* could conceivably be dispersed by rafting; I often observed them in the field on holdfasts and in laboratory cultures on algae such as *Ulva* spp.

Two of three brooding species in *Lepidochitona* are hermaphroditic. *L. thomasi,* and the free spawners, have separate sexes. The first and only previous report of hermaphroditic chitons by Heath (1907) is often cited, but is repeatedly attributed to the wrong species, usually *Trachydermon raymondi* Pilsbry [=*Lepidochitona dentiens* (Gould)] (Eernisse, 1986). Heath (1907) called attention to the simultaneous hermaphroditic condition of small brooding chitons he collected from high tidepools at Pa-

cific Grove, California, commenting briefly on how early and in what form the gonad appears. Figure 8 adds new detail to Heath's description of the hermaphroditic gonad of *L. caverna.* Like Heath, I observed testicular lobules and sperm in the peripheral regions of the gonad, with the great majority of total gonad space occupied by ovaries.

As in predominantly selfing plants which, as a rule, have dispropotionately small amounts of pollen, a small amount of testis relative to ovary in a hermaphroditic animal is usually indicitive of self-fertilization (Charnov, 1982). This possibility is supported by my observations of successful brooding in isolated *L. caverna* and *L. fernaldi,* which excluded the possibility that isolated individuals were outcrossing, provided that long-term sperm storage (up to nine months) was not occurring. For both species, the possibility of sperm storage seems remote based on the simple gonoduct arrangement possessed by chitons and the lack of copulatory structures. Storage for this length of time would seemingly require highly specialized spermatophores, although the prosobranch gastropods, *Urosalpinx cinerea* and *Eupleura caudata,* which copulate but appear to lack discrete spermatophore structures, have been reported to store sperm for periods exceeding one year (Hargis and MacKenzie, 1961). If the possibility of sperm storage is discounted, these isolation experiments provide indirect evidence that these animals are capable of self-fertilization (or parthenogenesis).

Because both hermaphroditic chiton species are brooders, but most brooding chiton species are not hermaphroditic, hermaphroditism is most likely secondary to brooding. Ghiselin (1974) made a similar argument for echinoderms. An association between brooding and hermaphroditism has been noted for a variety of marine invertebrates (reviews by Ghiselin, 1969, 1974; Charnov, 1982; Strathmann and Strathmann, 1982). Strathmann *et al.* (1984) and Eernisse (1984) suggest that when hermaphroditism is found in normally gonochoric groups, such as chitons, it is usually in species that release crawl-away larvae or juveniles. Moreover, these hermaphrodites are typically capable of frequent self-fertilization, an exceptional practice that most hermaphroditic marine invertebrates have elaborate adaptations to avoid. By linking the infrequent occurrence of self-fertilization via hermaphroditism to the potential to crawl away that results from brooding, these authors are postulating that some consequence of limited dispersal must result in conditions favorable for the spread of self-fertilization or, conversely, for the escape from dependence on cross-fertilization.

The mechanism proposed to explain the association of brooding and selfing involves a trade-off between the normally deleterious consequences of inbreeding and the theoretical advantages that departure from outbreeding should entail, all other factors being equal. Strathmann *et al.* (1984) and Eernisse (1984) suggested that brooding (or other types of development with low dispersal) may serve as a precondition to the spread of self-fertilizing hermaphrodites in a population of gonochorists. These authors assumed that occasional hermaphroditic individuals could arise in a normally gonochoric population and, additionally, not always experience a block to self-fertilization. Inbreeding depression would normally reduce the viability of selfed offspring in outcrossing populations, thus usually blocking the success of occasional hermaphrodites. However, if brooding results in prolonged inbreeding, thus exposing and eliminating deleterious alleles, then there may be little additional inbreeding depression if selfing were to occur. If a hermaphrodite were to arise in a highly inbred population and, additionally, had a reduced allocation to male reproduction (*i.e.,* by producing more ovaries than testes) and an ability to self, then it would be expected to gain a reproductive advantage, relative to the cross-fertilizing brooders in the population (Williams, 1979; Charlesworth and Charlesworth, 1981; Charnov, 1982; Bell, 1982). Comparisons of genetic diversity patterns among free spawning and brooding species of *Lepidochitona* (Eernisse, 1984; unpub. data) are at least consistent with this several part hypothesis since gonochoric brooders are less genetically diverse (*i.e.,* possibly more inbred), as detected by allozyme variation, than are free spawners. These results will be considered in subsequent studies, as will the results of paternity analyses using allozyme markers, which support self-fertilization (but do not exclude parthenogenesis) as the normal mode of reproduction in at least *L. fernaldi.*

Another trait linked to brooding may have little to do with limited dispersal. Brooding is linked to small adult body size of chitons in general (Pearse, 1979) and this study documents similar trends among West Coast *Lepidochitona.* All three brooding species are small, generally not exceeding 2 cm in length (Figs. 5b–f). By contrast, the free spawning *L. hartwegii* (Fig. 4f) can exceed four cm in length (Eernisse, 1986). The two smallest *Lepidochitona* species examined here are both brooders. Although the largest brooding species, *L. thomasi,* is as large as some of the free-spawning *Lepidochitona,* this species is certainly small compared to other chiton species in general.

Strathmann and Strathmann (1982) reviewed the association of small adults and brooding in a variety of marine invertebrates and the hypotheses that may explain this correlation. Such hypotheses are not necessarily mutually exclusive, so several factors may be responsible for the observed small adult size of brooding species of *Lepidochitona.* Distinguishing the relative importance of these factors remains to be attempted experimentally, but some hypotheses do not seem likely explanations.

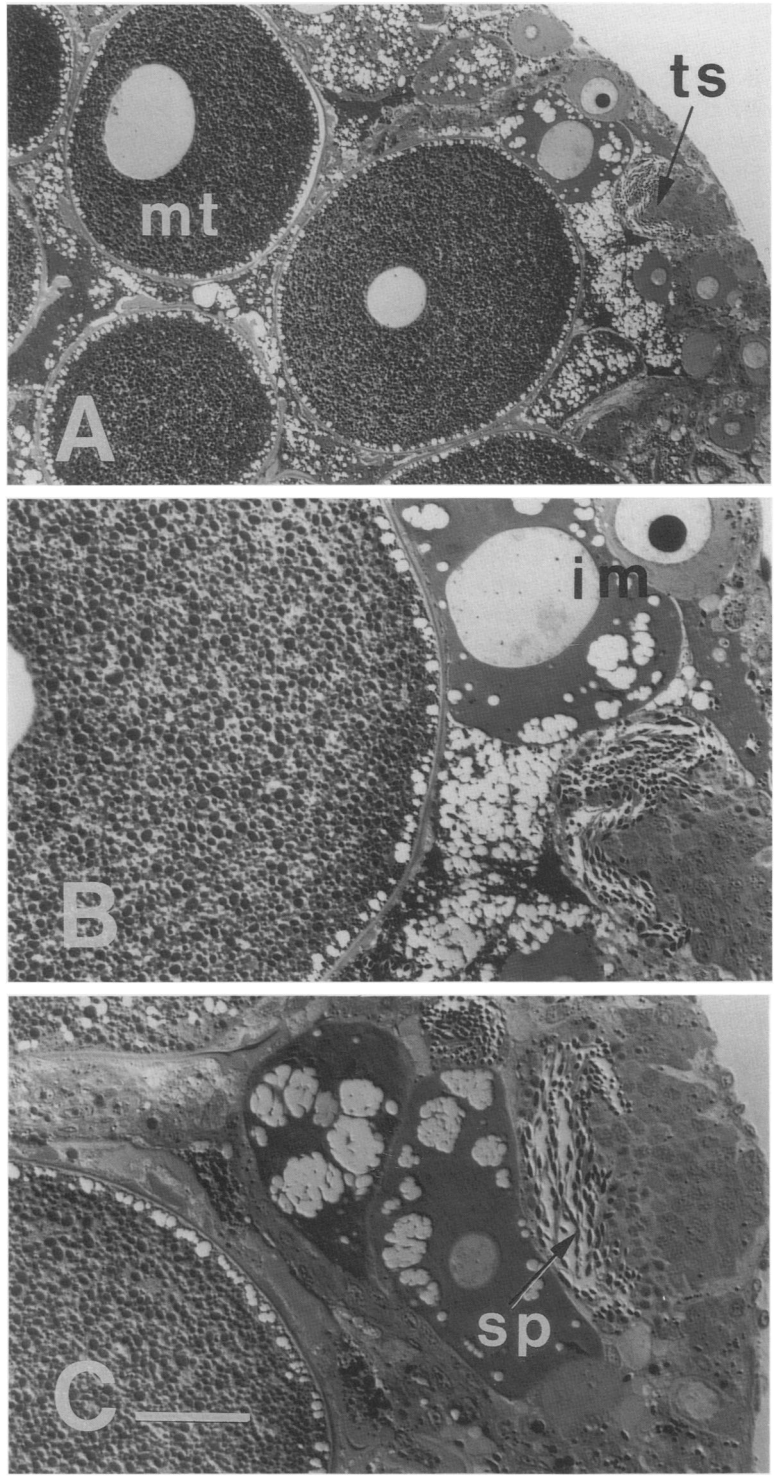

Figure 8. Sections (0.5 μm thick) of the hermaphroditic gonad of one specimen of *L. caverna*. Explanation of symbols: im = immature oocytes; mt = slightly more mature oocytes (still not full size); ts = testicular lobule; sp = mature sperm. Scale bar: (a) = 25 μm; (b, c) = 10 μm.

For example, the suggestion by Christiansen and Fenchel (1979) that the prevalence of brooding in small animals is due to a smaller size at metamorphosis does not hold for *Lepidochitona*. Size at metamorphosis of brooders is equal to, or greater than, size at metamorphosis of non-brooders.

Another hypothesis, first proposed by Ghiselin (1963) and often repeated, is that animals with small adult body

size are limited in energy and thus cannot afford a planktonic stage, which is assumed to depend on high fecundity. Strathmann and Strathmann (1982) consider this an incomplete explanation because it fails to explain why large animals should not brood. I argued above that the most obvious difference between free spawners and brooders was a developmental difference—stage at hatching—which imposed obligatory dispersal for free spawners but not brooders. It is possible that a less-obvious difference may account for the link between brooding and small adult size.

A hypothesis that can be tested experimentally or by comparison is that brood volume increases only as a function of available surface area, while body (and hence, gonad) volume increases as a function of body volume. This would lead to an allometric limitation in available brood space with increasing body size, so that large females could produce more eggs than they could brood (Strathmann and Strathmann, 1982; Strathmann *et al.,* 1984).

Brooding may also place constraints on the embryos or egg coverings themselves. As previously noted and as illustrated in Figure 6, *Lepidochitona* brooders have much more reduced egg hull sculpturing than free spawners. This general pattern holds true when the egg hulls of other chiton brooders are compared to the hulls of their free-spawning congeners (Eernisse, 1984). A hypothesis related to the allometric limitation hypothesis is that a reduction of egg hull sculpturing is under selection to permit tighter packing of eggs within the brood space. Evidence for this hypothesis would provide an indication that brood space is indeed limiting. However, an alternative hypothesis may equally well explain the "smooth" egg hulls of brooders. If the elaborate egg hulls function in some way as planktonic adaptations (*i.e.,* to facilitate suspension via chain formation or to discourage predators) and the hulls are costly to produce, then brooded embryos may be released from such a selective force. Perhaps comparisons with the egg hulls of benthic egg layers, where no brood space constraints or pelagic selective forces should apply, will help distinguish among these alternative hypotheses.

Another allometry hypothesis is that respiration or successful ventilation of a brooded egg mass is increasingly restricted with increasing body size. Such would be the case if gill surface area does not increase at the same rate as gill volume so that, as body size increases, the increase in respiratory efficiency does not keep pace with potentially greater brood output. The placement of brooded chiton embryos within the pallial groove certainly must impede normal respiratory patterns, as Heath (1905) first suggested. Heath (1905) noted but did not specify in detail an observation that brooding *L. caverna* appeared to greatly increase the respiratory powers of their mouth cavities and skin surfaces during brooding. The extent to which chitons can "breathe" through these other body surface areas may also be subject to allometric limitations, with larger animals able to use this means of respiration to a lesser extent. However, this reasoning assumes it is the adult that is respiration-limited. It may be preferable to consider the limitations imposed on the individual embryos of a brood mass. Although these allometric hypotheses are appealing, it is technically difficult to accurately measure either relative volume or respiration in large and small brooding *Lepidochitona.* Again it might be useful to consider chiton species known to lay benthic egg masses which, as discussed above, are like brooders in having late hatching embryos but, because they do not brood, have no increasing respiratory demands associated with increasing body size. The fact that the only two well-documented examples of this habit, *Stenoplax heathiana* Berry, 1946 and *Ischnochiton acomphus* Hull & Risbec, 1930, are also species with large adult size, suggests that such a relationship with body size is plausible.

Life history variation in *Lepidochitona* is considerable yet can be reduced to relatively few factors. The larvae of these morphologically similar brooders or free spawners differ primarily in their potential to remain near their parent(s). Adults differ in their patterns of distribution in the field and, in two out of three species that brood, in their hermaphroditic condition. I have emphasized the potential consequences of low dispersal parental care, while cautioning that some life history traits shared by brooders may be better explained by less obvious morphological or other constraints imposed by brooding itself.

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