

Reproduction, development and population biology of the Caribbean ophiuroid *Ophionereis olivacea*, a protandric hermaphrodite that broods its young

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Abstract. Reproduction and development of the ophiuroid *Ophionereis olivacea* H. L. Clark, a species that broods its young, were investigated in Florida from January through December 1985 and in Belize in June 1985 and April 1986. *O. olivacea* has a maximum disc diameter of 5.2 mm and is a protandric hermaphrodite. Sex reversal occurs at disc diameters ranging between 2.2 and 4.0 mm; it has 400 μm diameter oocytes and is ovoviviparous. As expected from an obligate out-crosser, this ophiuroid has a similar allocation to male and female reproduction, in terms of gonad volume. Reproduction of the Florida population was monitored for one year. *O. olivacea* is a sequential brooder and incubates its young during the coldest months of the year. The females produce a single clutch of juveniles each year. Spawning starts in November and peaks in January and February. The ova are spawned synchronously from all the ovaries of each female into the bursae and are retained there. Fertilization is accomplished by intake of sperm into the female bursae. *O. olivacea* develops through a ciliated embryonic stage that appears to be a modified vitellaria larva lacking ciliary bands. These modified larvae swim in seawater in the bursae. Direct development in this species has resulted from heterochrony in the loss of ophiopluteus features. The number of brooding females in the population increases in November and reaches a maximum in March. Juvenile *O. olivacea* emerge from the bursae at a disc diameter of 480 μm and each arm has three segments. Recruitment was first detected in April, and juveniles were particularly abundant in June. Small size in *O. olivacea* is associated with paedomorphic heterochrony with precocious maturation of the males at a disc diameter of 1.5 mm, ~ 2 mo after taking up their free existence. Brooding as a life history mode in *O. olivacea* is examined and the adaptations of this species to its warm-water habitats are discussed.

Introduction

Brooding is a mode of reproduction that lies at one end of the spectrum of life history patterns of marine invertebrates. Species that care for their young to the juvenile stage lack a dispersive phase in their life history; and for several phyla, brooding is considered to be a derived mode of development that arose through the loss of a feeding larva (Strathmann 1978). The significance of this pattern of development and the suite of life history traits associated with it, have been the subject of considerable debate (Chia 1974, Heath 1977, 1979, Strathmann and Strathmann 1982, Emler et al. 1987, Eernisse 1988). In many invertebrate taxa, particular life history traits are linked with brooding, including diminutive size and hermaphroditism. The association of hermaphroditism with brooding is considered to be a secondary consequence of this reproductive mode, resulting from extensive inbreeding in poorly dispersed populations (Strathmann et al. 1984, Eernisse 1988). Several hypotheses have been proposed for the association of brooding with small size in marine invertebrates (Chia 1974, Heath 1977, Strathmann and Strathmann 1982). One of these, the energetic hypothesis, proposes that diminutive species do not have the energetic reserves for a high-fecundity dispersive life history (Chia 1974). The allometry hypothesis proposes that large species can not brood their young because they produce more embryos than they could care for, such as when egg production increases volumetrically with ovary size while the brood capacity increases with surface area (Heath 1977, Strathmann and Strathmann 1982, Strathmann et al. 1984). This hypothesis is based on comparisons of gonad size and brood space, and has been tested for several taxa including asteroids and bivalves (Strathmann et al. 1984, Kabat 1985, McGrath and ÓFoighil 1986).

In the Ophiuroidea, parental care typically involves incubation of the embryos in the bursae and the expected suite of life history traits is seen in many genera (Hendler 1985, 1991). The majority of brooding ophiuroids are small and hermaphroditic (Hendler 1985, 1991). Brood-

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ing ophiuroids may have viviparous or ovoviparous development. Viviparous species such as *Amphipholis squamata* invest considerable post-vitellogenic reserves into embryogenesis (Fell 1946, Walker and Lesser 1989). *A. squamata* has a minute 100 μm egg, yet its progeny vacate the bursae at a disc diameter of 800 μm (Fell 1946, Walker and Lesser 1989). Specialized sinuses in the bursal wall appear to supply nutritive material to the developing embryo (Fell 1946, Walker and Lesser 1989). In *A. squamata*, the requirement for a large egg is circumvented through direct delivery of parental nutrient reserves to the developing embryo, potentially augmented by nutrients derived from symbiotic bacteria (Fell 1946, Walker and Lesser 1989). Ovoviparous species, on the other hand, have large ova containing sufficient yolk reserves to support embryogenesis. In these ophiuroids, specialized bursal sinuses are not present (Byrne 1988, 1989). The majority of brooding ophiuroids are ovoviparous (Hendler 1991).

The reproductive anatomy of ophiuroids is associated with ten bursae, one on either side of the base of each arm. Each bursa is a sac that projects into the coelom and opens to the outside through a narrow slit. The margins of the slit are formed by the genital plates and scales to which the gonads are attached. In broadcasting ophiuroids, the bursae have a respiratory function and serve as the outlet for the gametes during spawning. Ophiuroids that care for their young retain their eggs in the bursae, which serve as brood chambers. The bursae are an anatomical feature considered to be a pre-adaptive trait for the evolution of parental care in the Ophiuroidea (Hendler 1979). The presence of the bursae would have made the shift from broadcasting to brooding a relatively simple matter of retention of the eggs in a structure ideal for protection and ventilation of embryos.

The increase in the number of brooding species at high latitudes across a range of taxa, a trend known as "Thorson's rule", has also generated interest (Thorson 1950, Chia 1970, Mileikovsky 1971, Vance 1973, Highsmith 1985, Emler et al. 1987). Based on the geographic pattern of reproduction in marine invertebrates, Thorson (1950) suggested that parental care may be an adaptation to the rigors of the polar environment where development times are long and planktonic food is of short duration. In the Ophiuroidea, Mortensen (1936) noted that about 50% of subantarctic and arctic ophiuroids are brooders. Recent work in the Caribbean, however, has shown that brooding ophiuroids are more prevalent in the tropics than previously realized (Hendler 1979, Emson et al. 1985, Hendler and Littman 1986, Hendler and Turner 1987, Byrne 1988, 1989, 1991, Hendler and Peck 1988).

Ophionereis olivacea H. L. Clark is a small Caribbean ophiuroid that broods its young in the bursae. In this investigation, the life history traits of this species are described from populations in Florida and Belize. The morphology of the embryos is examined by means of scanning electron microscopy, and particular attention is paid to the presence of larval features which are often modified in the shift to direct development (Strathmann 1978, Emler et al. 1987). Brooding as a life history mode in *O. olivacea* is discussed, and the allometry hypothesis is

tested. The adaptations of this species to its warm-water habitats are also considered.

Materials and methods

Specimens of *Ophionereis olivacea* H. L. Clark were collected at low tide in shallow water (0.5 m depth) from Conch Key, Florida, USA (25°N; 81°W), in a series of monthly samples from January through December 1985. At Conch Key, the ophiuroids inhabit a dense turf of the calcareous alga *Halimeda opuntia*. Clumps of the alga were collected at random, placed in trays, and all the brittlestars were removed. This resulted in random samples of 58 to 178 specimens (see Fig. 8). The seawater temperature at Conch Key was measured at high tide each month. *O. olivacea* were also collected from Twin Cays and the Blue Ground Range on the Belize Barrier Reef (17°N; 88°W) in June 1985 and April 1986. In Belize, specimens were collected in shallow water (0.5 to 1.0 m depth) from *H. opuntia* attached to mangrove roots and coral, and also from turfs of the algae *Dictyota cervicornis* and *Amphiroa fragillissima* in mangrove areas. The ophiuroids were narcotised in 7% MgCl_2 in distilled water, and their disc diameters were measured with an ocular micrometer. They were then dissected in order to examine the gonads, and the embryos were removed from the bursae of brooding females. The sex of each specimens was noted and the number and size of the embryos were recorded.

Reproduction of *Ophionereis olivacea* in Florida was monitored by monthly measurements of the oocytes and examination of the brood condition. Each month, all the oocytes in the ovaries of ten females were measured with an ocular micrometer. These data are represented in size-frequency polygons. To examine the population dynamics and recruitment of the Florida population, disc-diameter size-frequency distributions were constructed for each monthly sample. The disc diameters of male and female *O. olivacea* were compared by Student's *t*-test.

The allocation to male and female reproduction was determined from estimates of the total volume of the testes and ovaries in 20 mature males and 20 mature females. For the most part, the ovaries and testes were spherical and so their volumes were calculated from their diameter. Some females, however, had elongate ovaries and their shapes were approximated to a geometrical shape (ovoid or cylinder) and their dimensions (length, width, diameter) were used to calculate volume. The estimates do not take into account irregularities in the ovary such as bumps from bulging oocytes. To avoid the confounding influence of size, only specimens with 4 to 5 mm diameter discs were used for the estimate of gonad volumes. The total testis and ovary volumes were compared by Student's *t*-test.

To test the allometry hypothesis, the number of unspawned late-vitellogenic oocytes (fecundity) in the ovaries of 78 gravid females and the number of late-stage embryos (clutch size) in 62 brooding females were counted. The relationship between fecundity and female disc diameter and the relationship between clutch size and female disc diameter were examined by linear regression. In order to obtain the allometric exponent for these relationships, the data were plotted on log-log scales.

For histological examination of the gonads and bursae, entire discs of male and female *Ophionereis olivacea* were placed in Bouin's fluid. This fixative also decalcified the specimens. The tissues were dehydrated in alcohol, cleared in xylene, embedded in paraffin, and sectioned at 6 to 7 μm . Echinoderm yolk usually reacts positively to Schiff's reagent (Byrne 1988) and so the Alcian blue/periodic acid Schiff's method (AB/PAS) was used to stain the sections. Some sections were stained with haematoxylin and eosin (H/E).

The embryos were removed from the bursae for microscopy, photography, and scanning electron microscopy (SEM). The presence of a larval skeleton was investigated by viewing whole-mounted embryos in polarized light. For SEM, the embryos were fixed in 2.5% glutaraldehyde in 0.45 μm -filtered seawater for 1 h at room temperature, washed in 2.5% NaHCO_3 (pH 7.2), and post-fixed in 2% OsO_4 in 1.25% NaHCO_3 for 1 h at room temperature.

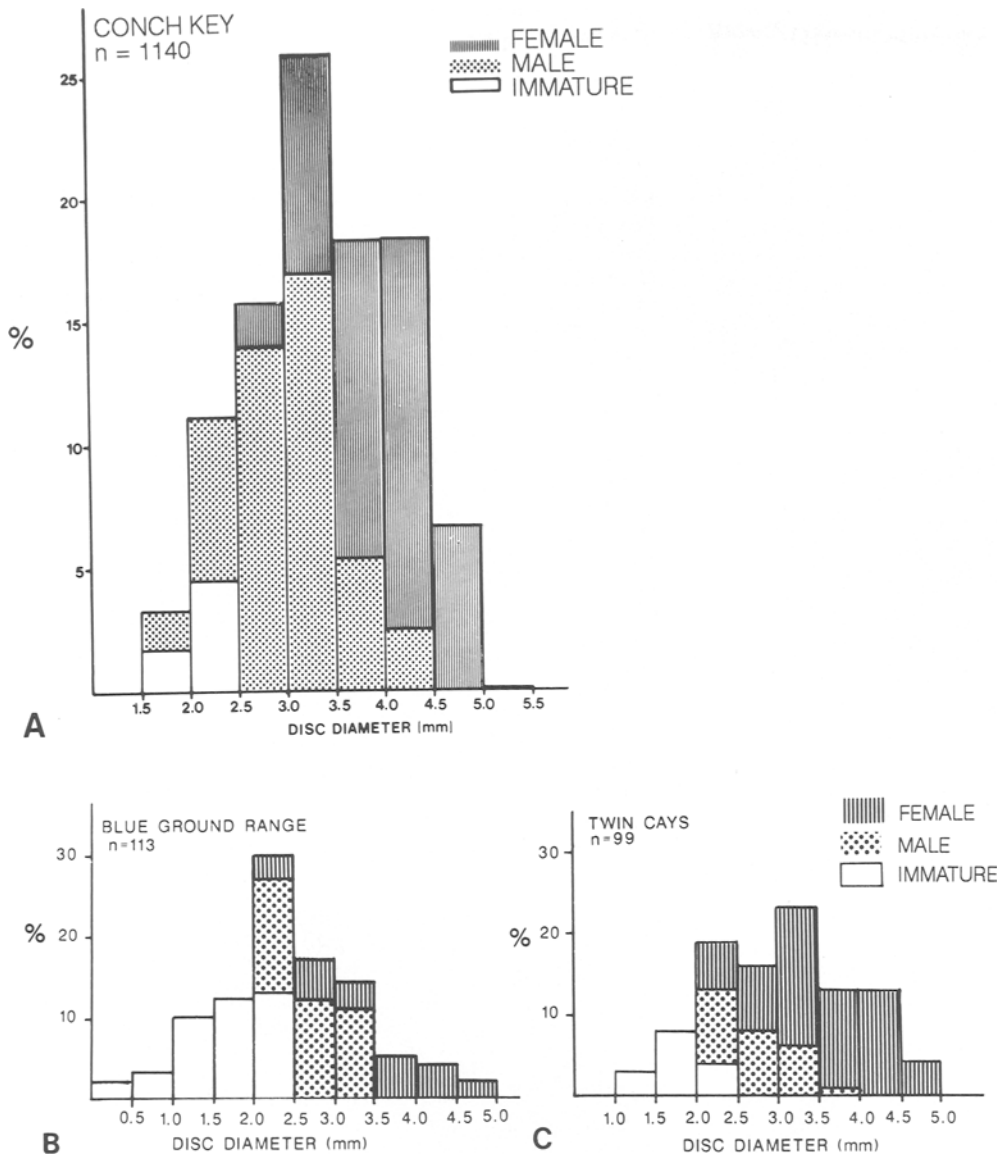


Fig. 1. *Ophiureis olivacea*. Size-frequency histograms showing relationship between disc diameter and sex in ophiuroids from Florida (A) and Belize (B, C)

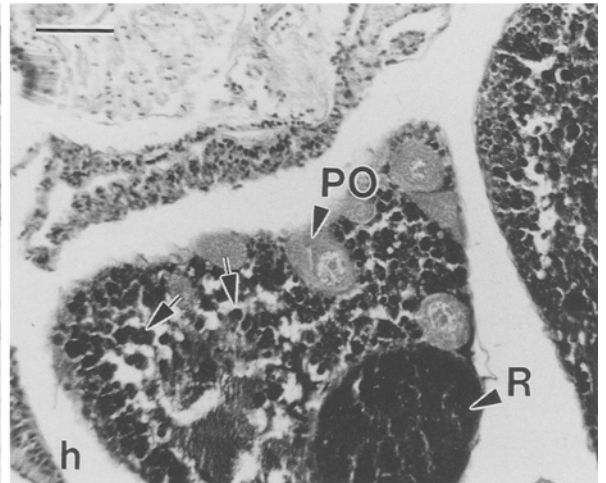
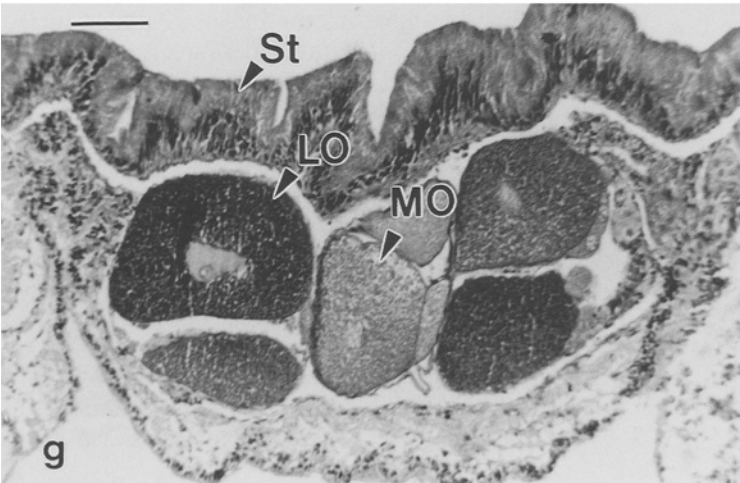
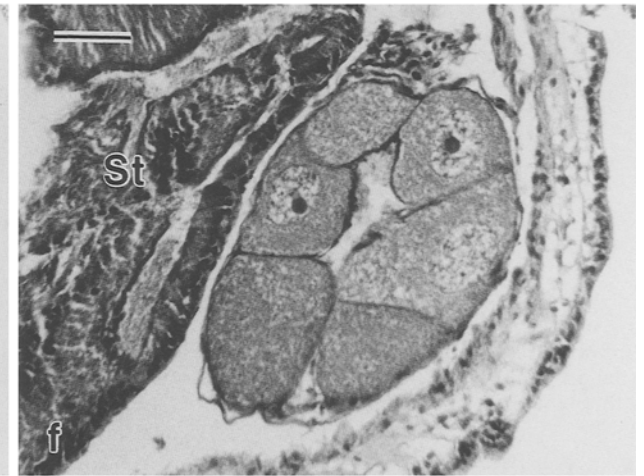
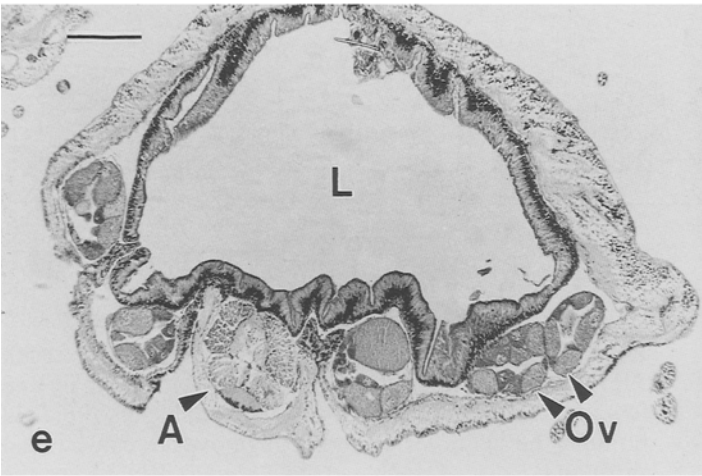
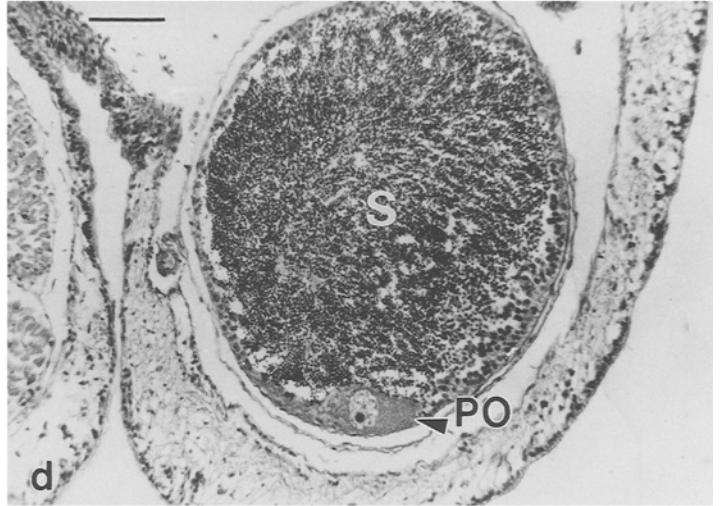
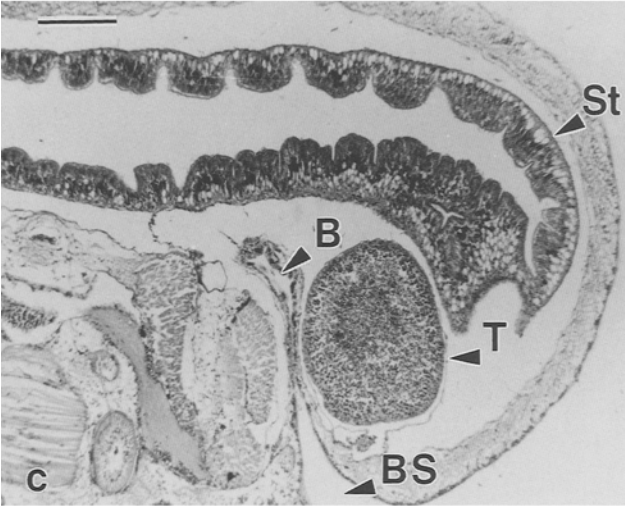
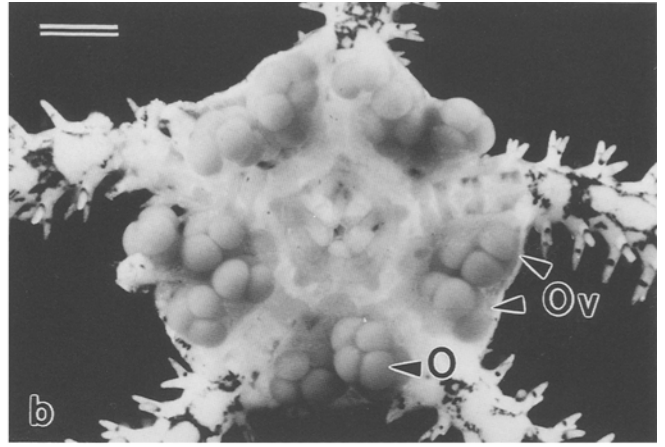
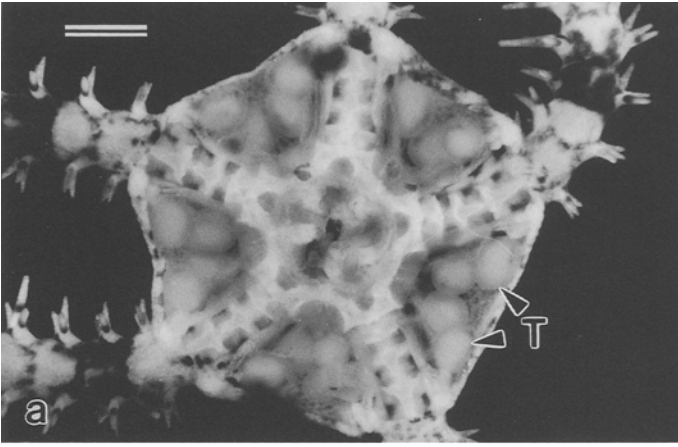
They were then washed in distilled water and dehydrated in ethanol. After dehydration, the embryos were critical-point dried and viewed with a Novascan 30 SEM.

Results

Sexuality

Ophiureis olivacea is a protandric hermaphrodite and sex reversal occurs at disc diameters between 2.2 and 4.0 mm (Fig. 1). This ophiuroid has a maximum disc diameter of 5.2 mm. Male *O. olivacea* from the Florida population had a mean disc diameter of 2.8 mm (SE=0.02, range = 1.5 to 4.5 mm, $n=477$), while females had a mean disc diameter of 3.5 mm (SE=0.03, range=2.2 to 5.2 mm, $n=479$). In Belize, the mean disc diameters of males and females were 2.6 and 3.6 mm, respectively (males: SE=0.05, range=1.6 to 3.6 mm, $n=66$; females: SE=0.07, range=2.2 to 5.0 mm, $n=82$).

Females were significantly larger than males in both Florida and Belize (Fig. 1) ($p=0.0001$). Mature male and female *O. olivacea* are readily discernable in the field because the white testes and orange ovaries can be seen through the disc. They have 2 to 3 gonads attached to the genital scales positioned on the inter-radial side of the bursal slits (Fig. 2a, c). Mature males have an average of 15 round testes (SE=0.8, range=10 to 23, $n=22$), with a mean diameter of 490 μm (SE=41, range=200 to 800 μm , $n=22$). Gravid females have an average of 17 ovaries (SE=0.8, range=11 to 27, $n=38$) that are round to elongate, with a mean diameter of 690 μm (SE=37, range=400 to 1200 μm , $n=35$) (Fig. 2b, e). Histological examination of the gonads reveals that the switch from male to female reproduction does not usually include a transitional stage of simultaneous hermaphroditism (Fig. 2c, f, g). Only one of the ophiuroids sectioned ($n=50$) had a gonad with sperm and an oocyte (Fig. 2d). The allocations to male and female reproduction in *O. olivacea* based on total gonad volume were 7.8 \times



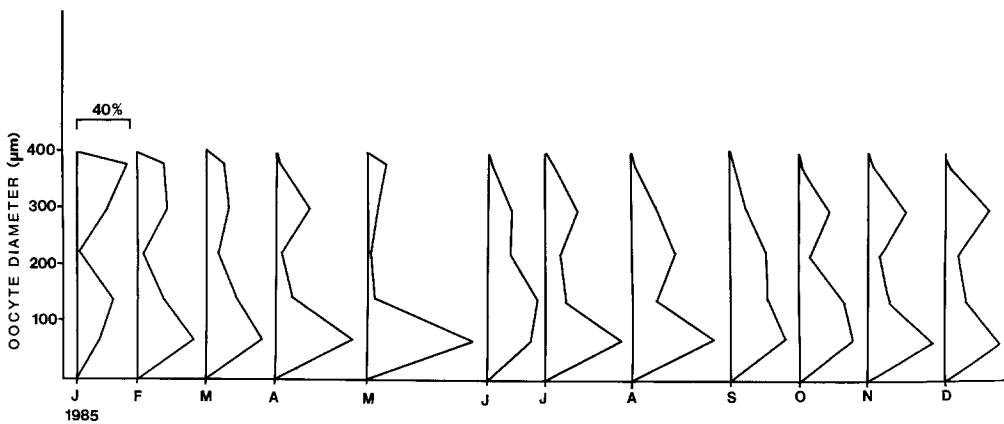


Fig. 3. *Ophionereis olivacea*. Oocyte size-frequency distributions of females from Conch Key

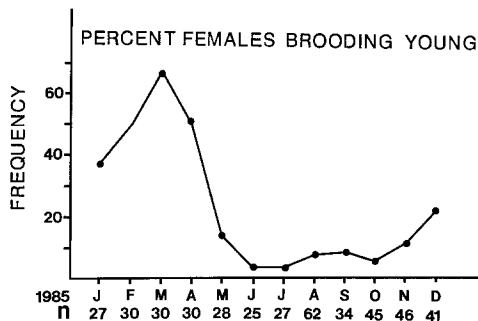


Fig. 4. *Ophionereis olivacea*. Percent of females brooding young at Conch Key in 1985; number of females in each sample is shown under each month

$108 \mu\text{m}^3$ (SE = 1.3, $n = 20$) and $12.2 \times 108 \mu\text{m}^3$ (SE = 1.5, $n = 20$), respectively. These values are not significantly different ($p > 0.05$).

Gametogenic cycle

Oocytes at different stages of development are distinguishable by their colour in life, histological staining properties, and size. The largest oocytes of *Ophionereis olivacea* have a diameter of $400 \mu\text{m}$ (Fig. 2b) and are orange in colour. Previtellogenic oocytes, up to $120 \mu\text{m}$ in diameter, are white, and in sections are basophilic and PAS-negative (Fig. 2h). The onset of vitellogenesis is evident in oocytes 120 to $150 \mu\text{m}$ in diameter that are slightly

eosinophilic and PAS-positive. Vitellogenic oocytes gradually take on an orange colour as they increase in size. These oocytes are eosinophilic and PAS+ (Fig. 2f, g). Late-vitellogenic oocytes are 360 to $400 \mu\text{m}$ in diameter and are intensely PAS+ (Fig. 2g). Cohorts of 6 to 12 eggs undergo synchronous vitellogenesis in each ovary (Fig. 2b, f).

The reproductive cycle of *Ophionereis olivacea* at Conch Key is annual (Figs. 3 and 4). In 1985, gravid females with late-vitellogenic oocytes were present during October through February. Unspawned oocytes remaining in the ovaries in March and April were relict, and were subsequently resorbed. The number of brooding females started to increase in November, indicating the onset of spawning (Fig. 4). The oocyte size-frequency data show that peak spawning occurred between January and February (Fig. 3). Spawning was a synchronous event among the ten bursae of individual females. An average of 67 eggs (SE = 0.2, range = 22 to 171, $n = 78$) were spawned directly into the bursae and retained there. Fertilization presumably involves sperm being drawn into the bursa in the ventilation current. During the peak incubation period, February through April, the ovaries of brooding females were minute and contained few or no vitellogenic oocytes. By May, relict oocytes were being resorbed and the ovaries contained the PAS+ products of oolysis (Fig. 2h). In May, the ovaries were dominated by pre-vitellogenic and early vitellogenic oocytes (Fig. 3). By June, most females had ovaries with vitellogenic oocytes for the next breeding season (Fig. 3).

Embryogenesis

Incubation of embryos by *Ophionereis olivacea* at Conch Key was seasonal, with brooding females most abundant in January through April (Fig. 4). All the embryos brooded by each female were at the same stage of development, suggesting that fertilization is simultaneous in all the bursae (Fig. 5d, e). This synchrony of development was seen in every clutch examined, from early development shortly after formation of the fertilization membrane through metamorphosis. Although broods within female *O. olivacea* undergo synchronous embryogenesis, clutches of different females may be at a different stage. As the

Fig. 2. *Ophionereis olivacea*. (a) Male with aboral surface removed to show testes (T). (b) Female with ovaries (Ov) containing fully grown oocytes (O). (c) Cross-section of disc of a male showing location of testis (T), bursa (B) and bursal slit (BS); St: stomach. (d) Ovotestis of a hermaphrodite with sperm (S) and a previtellogenic oocyte (PO). (e) Cross-section of disc of a female showing position of ovaries (Ov); A: arm, L: stomach lumen. (f) Ovary containing eosinophilic vitellogenic oocytes; St: stomach. (g) Ovary stained with AB/PAS containing PAS+ late-vitellogenic oocytes (LO); MO: mid-vitellogenic oocyte; St: stomach. (h) Spent ovary filled with PAS+ material (arrowed) derived from a relict oocyte (R); PO: previtellogenic oocyte. Scale bars: (a, b) $500 \mu\text{m}$, (c) $200 \mu\text{m}$, (d) $250 \mu\text{m}$, (e) $350 \mu\text{m}$, (f) $150 \mu\text{m}$, (g) $160 \mu\text{m}$, (h) $140 \mu\text{m}$

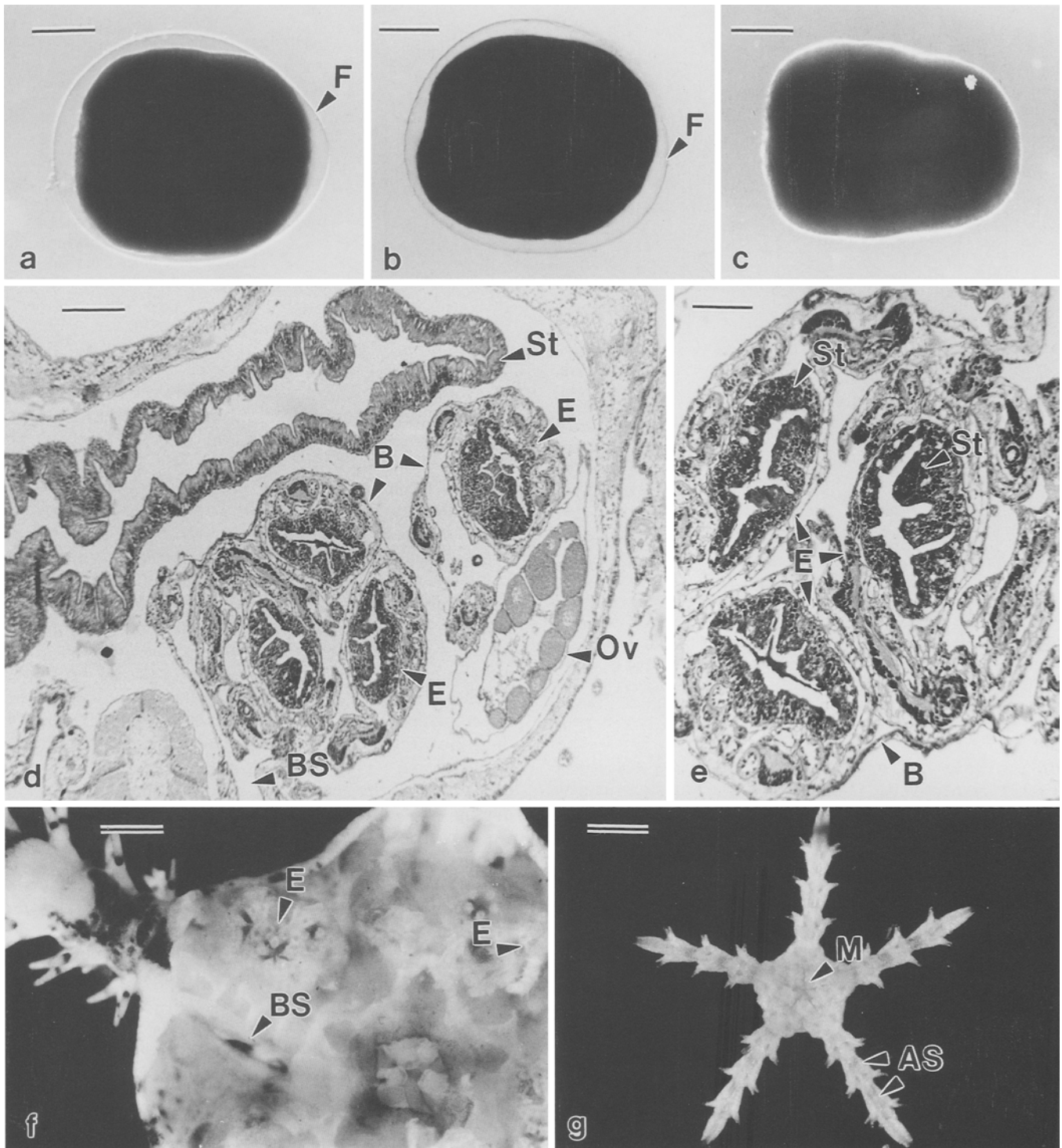


Fig. 5. *Ophionereis olivacea*. (a) Blastula, (b) gastrula, (c) hatched gastrula; F: fertilization membrane. (d) Cross-section of disc of a brooding female with embryos (E) in adjacent bursae; bursal wall (B) is distended to a thin layer of tissue; ovary (Ov) contains previtellogenic oocytes; BS: bursal slit, St: stomach. (e) Advanced embryos (E) from Fig. 5d in a bursa with thin walls (B); stomach (St)

cells of embryos are filled with PAS+ vitellogenic material. (f) Brooding female with aboral surface removed to show embryos (E) in bursae; one bursa has been removed to show position of bursal slit (BS). (g) Oral surface of juvenile that has recently left bursa; AS: arm segment; M: mouth. Scale bars: (a, b) 125 μm , (c) 90 μm , (d) 200 μm , (e) 135 μm , (f) 450 μm , (g) 320 μm

embryos develop, the walls of the bursae expanded to accommodate their growth and, by March, the bursae of most females were distended by late-stage yolky stars (Fig. 5d–f). Brooding females were easily identified in the field because of their distended discs.

The earliest embryos encountered in the bursae were blastulae and gastrulae surrounded by the fertilization membrane (Fig. 5a, b). Newly hatched gastrulae are round and then develop an elongate shape. The embryos are covered by scattered cilia (Fig. 6d). A hydropore

forms and the embryos take on a barrel-shape (Fig. 5c and 6a, b). At this stage the embryos appear to be modified vitellariae larvae with a narrow anterior end and a flattened posterior end. These modified larvae are covered by cilia, but lack ciliary bands and do not have a mouth or anus (Fig. 6a–d). Their anterior region has an extracellular glycocalyx-like covering that gives the epithelial surface a smooth appearance (Fig. 6a–c). Cilia protrude through this coat (Fig. 6c, e). The rest of the embryonic surface does not have an extracellular coat (Fig. 6d). Examination of the embryos with polarized light did not reveal the presence of larval spicules. The embryos of *Ophionereis olivacea* swim in seawater in the bursae and, on transfer to culture dishes, they move along the bottom of the dishes, rotating around their main axis. The modified vitellaria metamorphose into yolky stars, with the formation of a star-shaped adult rudiment at the posterior end followed by gradual resorption of the larval body. The adult skeleton forms as thin ossicles over the central yolk mass (Fig. 6f). Metamorphosed embryos orient with their mouths against the bursal wall and with their arms curled over their disc (Fig. 5f). The embryos of *O. olivacea* are not attached to maternal tissue at any stage of development and, on dissection of the female, roll out of the bursae.

Histological examination of the bursal wall of brooding females did not reveal the presence of bursal sinuses and there was no morphological evidence for extra-embryonic nutrition (Fig. 5d, e). Development of *Ophionereis olivacea* appears to be ovoviviparous, with the ova containing sufficient reserves to support embryogenesis. The PAS+ products of vitellogenesis are present in the gut wall of the embryos through metamorphosis (Fig. 5e). The juveniles leave the bursae at a disc diameter of 480 μm , and each arm has 3 segments (Fig. 5g). At this stage they still have orange-pigmented yolk reserves in the gut.

Most juveniles emerge from the bursae between April and May, 3 to 4 mo after peak spawning in January–February (Fig. 3). This indicates that intrabursal development takes ~ 3 to 4 mo. The number of brooding females declined between March and May and through the summer and autumn to a low level (Fig. 4). *Ophionereis olivacea* broods a mean clutch of 71 embryos (SE = 0.2, range = 40 to 165, $n = 62$) and, because brooding is seasonal, these data represent the annual number of juveniles produced per female.

Seawater temperatures recorded at Conch Key ranged from 18°C in January to 30°C in August. Spawning, fertilization and the onset of brooding in January coincided with the lowest temperature of the year, and the juveniles emerged from the bursae in April at a sea temperature of 25°C. *Ophionereis olivacea* broods its young during the coldest months of the year.

Allometry of reproduction

The relationships between fecundity, clutch size and disc diameter of female *Ophionereis olivacea* are shown in Fig. 7. The number of unspawned fully-grown oocytes in

the ovaries and the number of metamorphosed embryos soon to leave the bursa both increase with female disc diameter. The relationship between fecundity and disc diameter has an allometric exponent of 1.85, while the relationship between clutch size and disc diameter has an exponent of 1.83 (Fig. 7). Thus, fecundity and clutch size both scale approximately as a square of the disc diameter. The similarity of the two exponents indicates that the oocytes and embryos occupy similar portions of the female body. It is evident that brooding by *O. olivacea* involves a simple shift in space utilization from the ovaries to the bursae. Moreover, the ovaries are minute during the incubation period, so there is no competition for body space between the ovaries and embryos. That the relationship between juvenile production and adult disc diameter does not decrease in the larger females is evidence that female *O. olivacea* successfully brood all the embryos they produce, irrespective of adult size (Fig. 7).

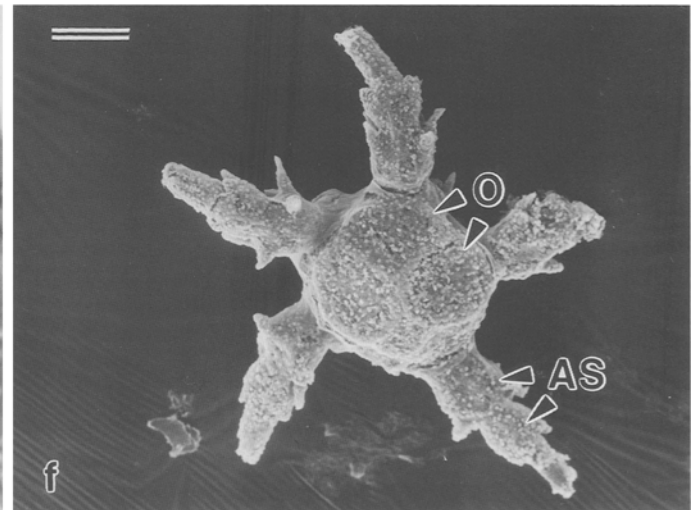
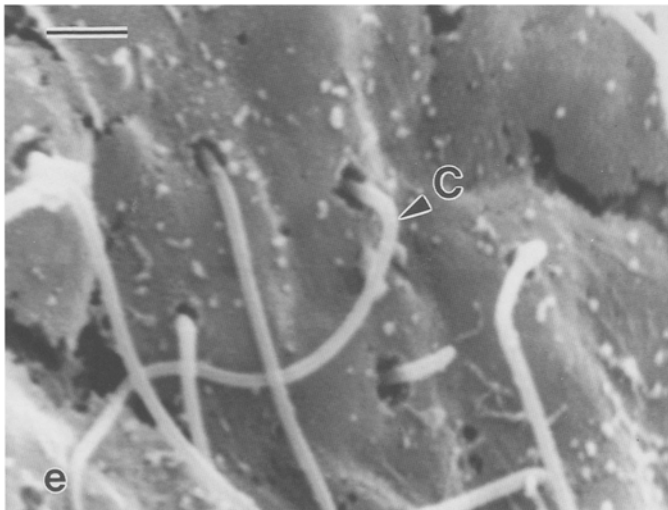
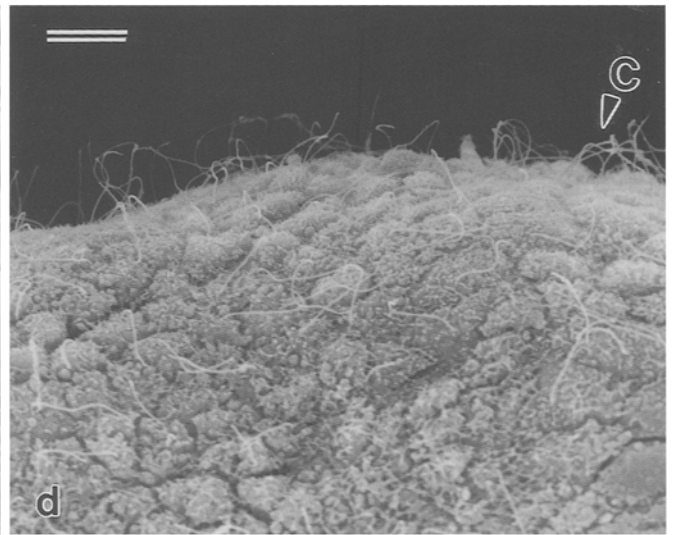
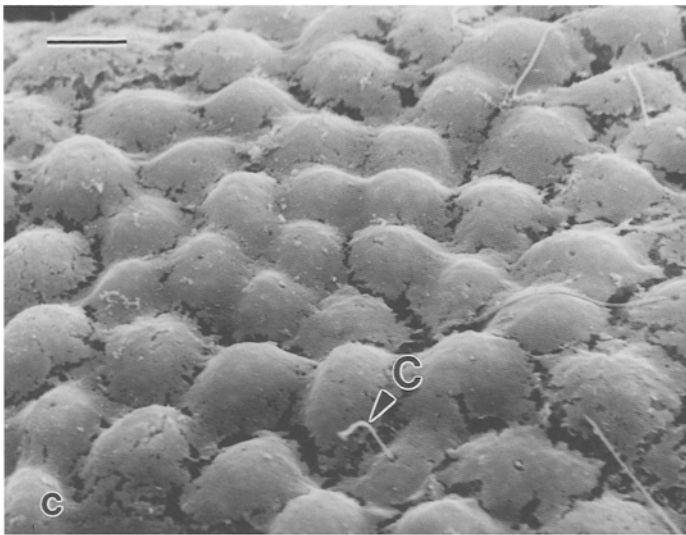
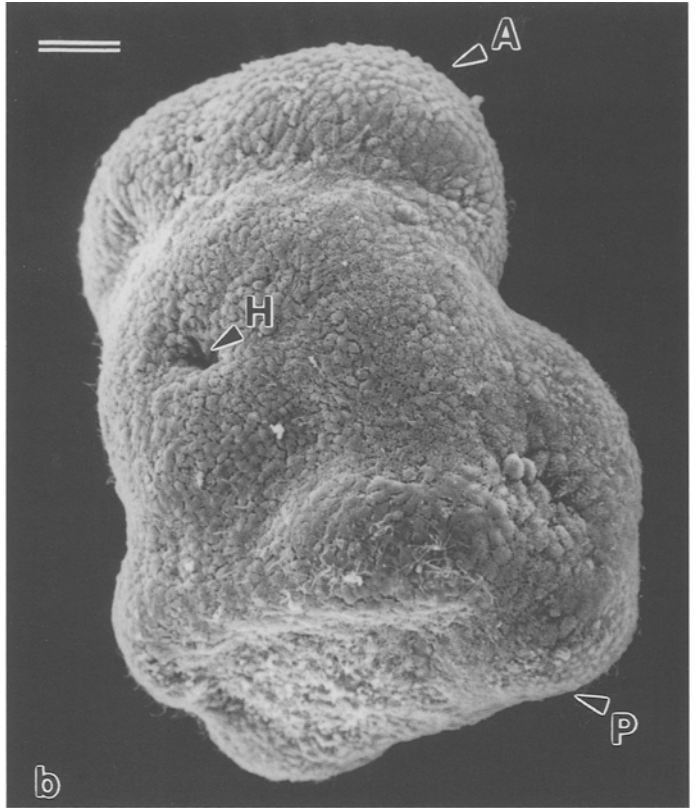
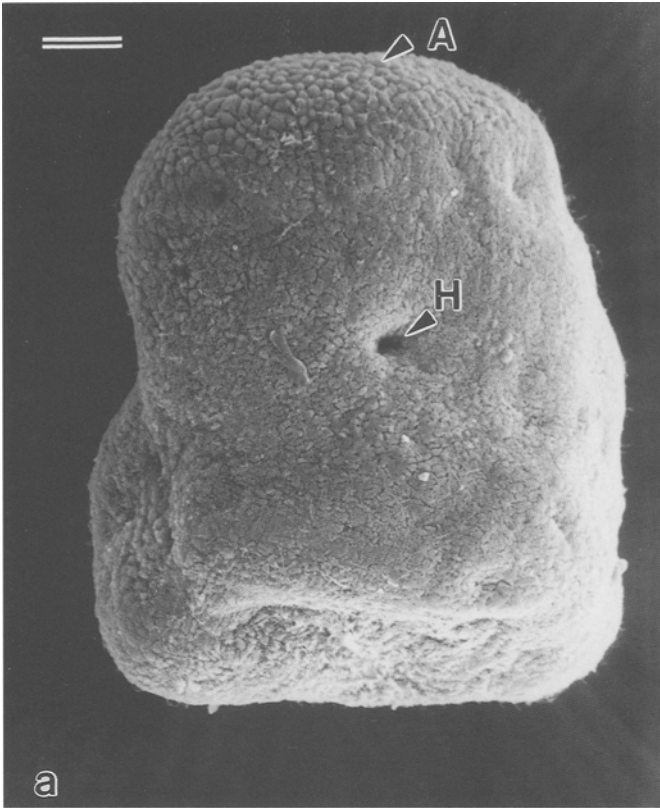
Population dynamics

The disc-diameter frequency data for the Florida population are shown in Fig. 8, which also illustrates the relationship between size and sexual maturity. From November to April when the females were brooding, the population was dominated by mature adults. Small immature *Ophionereis olivacea* present in the April sample indicate that juveniles start to leave the bursae in March. Juveniles with disc diameters of 0.5 to 1.0 mm were present in the May and June samples, the smallest of these, with 3 arm segments, had recently left the bursae (Fig. 5g). The highest pulse of recruitment was recorded in June, by which time brooding females were largely absent (Fig. 4). By June, many of the juveniles had attained disc diameters of 1.5 to 2.0 mm and developed testes, approximately 2 mo after taking up their free life (Fig. 8), providing further evidence that *O. olivacea* is protandric.

Discussion

Ophionereis olivacea is ovoviviparous with embryonic development supported largely by the products of vitellogenesis. The specialized bursal sinuses associated with viviparous development are not present in *O. olivacea*, nor are they present in another ovoviviparous brooder, *Ophirolepis paucispina* (Byrne 1988, 1989). At a disc diameter of 480 μm , the advanced embryos of *Ophionereis olivacea* are similar in size to the spawned egg. Dissolved organic molecules may also play a role in embryonic nutrition, as demonstrated for the brooded embryos of *Amphipholis squamata* (Fontaine and Chia 1968).

There are at least ten ophiuroids in the Caribbean that brood their young, several of which co-occur with *Ophionereis olivacea* (Hendler 1979, Hendler and Littman 1986, Hendler and Turner 1987, Byrne 1988, 1989, 1991); the life history traits of several species are compared in Table 1. Like *O. olivacea*, *Ophirolepis paucispina* has 400 μm -diameter ova, a size intermediate between the small 100 to 200 μm eggs typical of plank-



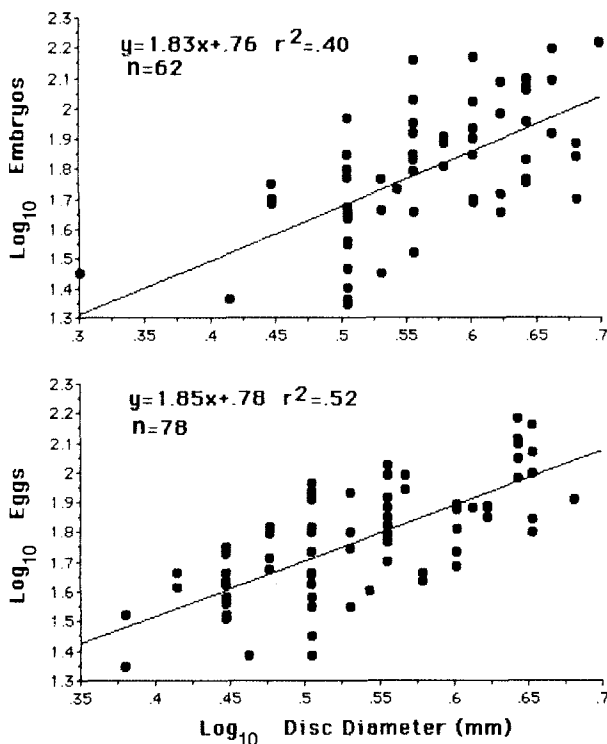


Fig. 7. *Ophionereis olivacea*. Log-log plots showing number of unspawned fully grown oocytes and number of advanced embryos per female as a function of adult disc diameter. Equation for each regression is shown; allometric exponents = 1.85 and 1.83, respectively

totrophic ophiuroids and the large 500 to 900 μm eggs typical of most ovoviviparous species (Hendler 1975, 1988, 1991, and present Table 1). This intermediate oocyte provides an exception to the hypothesis of Vance (1973), that only the extremes of planktotrophy or lecithotrophy should be selected for and that intermediate-sized eggs are evolutionary unstable. Not surprisingly, *Ophionereis olivacea* and *Ophiolepis paucispina* give rise to similar-sized progeny, with 480 μm diameter discs (Table 1). By contrast, most brooding ophiuroids give rise to large juveniles, with long arms, that leave the bursa when they have attained up to one-half the size of the parent (Mortensen 1936, Hendler 1975, 1991, and present Table 1). In the Ophiuroidea, recruitment of large progeny with enhanced mobility is considered to be the major advantage of brooding over broadcasting, because the crawl-away juveniles would be less susceptible to predation (Hendler 1975, 1991). This is not the case, however for *Ophionereis olivacea* and *Ophiolepis paucispina*, which give rise to juveniles similar in size to the newly settled

post-larvae of broadcasting ophiuroids (Hendler 1975, 1991).

Ophionereis olivacea has seasonal reproduction, and the clutch size given in Table 1 represents the annual output of juveniles by this species. This annual pattern contrasts with that of other brooders, which have prolonged reproduction, with asynchronous clutches, and release 1 to 2 juveniles at a time. As exemplified by *Amphiura stimpsonii*, *Amphipholis squamata*, and *Ophiurochaeta* sp., most ophiuroids that care for their young are characterised by reduced fecundity with production of a few eggs and small clutches (Table 1). Although it is not possible to calculate the annual fecundity of species with asynchronous reproduction, it appears that *Ophionereis olivacea* has a comparatively higher fecundity. This is undoubtedly due to differences in egg size, with *O. olivacea* producing a higher number of smaller eggs. If the annual fecundity of *Ophiolepis paucispina* could be measured it would probably approximate that of *Ophionereis olivacea*, since *Ophiolepis paucispina* has continuous reproduction and broods clutches of 12 embryos (Byrne 1988, 1989, and present Table 1).

Spawning and brooding by *Ophionereis olivacea* during the winter may be cued by exogenous factors such as water temperature and day length. The winter incubation period suggests that conditions in the summer may not be optimal for embryonic development. During the summer, sea temperatures increase to 30°C and would be higher at low tide. With reduced levels of dissolved oxygen at high temperature, it might be difficult for female *O. olivacea* to ventilate their broods adequately. The seasonal and synchronous brooding pattern of this ophiuroid may also result in the release of juveniles at an optimal period for their survival.

The similarity in the number of unspawned oocytes and the number of embryos, suggests that most if not all the eggs produced by female *Ophionereis olivacea* are fertilized (Table 1). This fertilization success is undoubtedly influenced by the aggregated distribution of this species (Byrne personal observations). Although spawning was not observed, sperm are presumably drawn into the female bursae for fertilization. Seasonal reproduction of *O. olivacea* results in the release of a large cohort of juveniles over a 1 to 2 mo period and, due to the small size of the recruits, this may be associated with high juvenile mortality. It appears, however, that *O. olivacea* has predictable recruitment, based on the abundance of this ophiuroid at Conch Key and in other *Halimeda opuntia* areas examined over several years (Byrne personal observations). The release of a large number of juveniles may be a strategy to ensure survival to maturity of a critical number of juveniles.

The invariable association of hermaphroditism and parental care in the Ophiuroidea has been recognized for some time, and the majority of brooding ophiuroids are simultaneous hermaphrodites (Mortensen 1920, 1933, Hendler 1979, 1991, and present Table 1). Brooding may be linked to the shift from gonochorism to simultaneous hermaphroditism because of the inbreeding depression resulting from limited dispersal of progeny (Strathmann et al. 1984, Eernisse 1988). According to Strathmann

Fig. 6. *Ophionereis olivacea*; SEMs of embryos. (a) Modified vitellaria larva; A: anterior region, H: hydropore. (b) More advanced embryo showing narrow anterior region (A) and flattened posterior region (P); H: hydropore. (c) Surface coat on anterior surface of embryo gives this region a smooth appearance; C: cilia. (d) Lateral surface with scattered cilia (C), lacks surface coat. (e) Cilia (C) protruding through surface coat. (f) Advanced embryo with two arm segments (AS) and thin ossicles (O) over aboral surface. Scale bars: (a, b) 40 μm , (c) 4.5 μm , (d) 9.0 μm , (e) 0.8 μm , (f) 200 μm

Table 1. Life history traits of Caribbean ophiuroids that brood their young; data from present study, from Byrne (1988, 1989), from Hendler (1988) and from own unpublished observations. Volume of ovary/testis: mean male and female allocation = total ovary or testis volume $\times 10^8 \text{ mm}^3$ (SE); No. of eggs: fecundity [= mean number of

large oocytes in ovaries (SE)]; No. of embryos: mean clutch size (SE); Size of embryos: maximum disc diameter of embryos and number of arm segments (AS); (n): number of specimens examined. P: protandric hermaphrodite; S: simultaneous hermaphrodite

Species	Max. disc diam (mm)	Sex	Max. egg diam (μm)	Volume of:		No. of eggs	No. of embryos	Size of embryos (μm)	(n)
				ovary	testis				
<i>Ophionereis olivacea</i> H. L. Clark	5.0	P	400	12.1 (1.5)	6.9 (1.0)	67.0 (3.5)	71.4 (4.5)	480 (3 AS)	(140)
<i>Ophiolepis paucispina</i> (Say)	6.2	S	400	8.4 (0.9)	1.0 (0.1)	22.0 (1.3)	12.0 (1.6)	480 (2 AS)	(192)
<i>Amphiura stimpsonii</i> Lütken	4.0	S	680	5.4 (0.6)	0.3 (0.1)	3.5 (0.5)	2.2 (0.4)	800 (9-10 AS)	(34)
<i>Amphipholis squamata</i> (Delle Chiaje)	3.2	S	100	0.03 (0.004)	0.02 (0.02)	1.6 (0.2)	2.1 (0.2)	880 (10-12 AS)	(57)
<i>Sigsbeia confiera</i> Koehler	6.4	S	760	10.3 (1.0)	0.2 (0.03)	5.0 (0.5)	11.0 (1.0)	800 (7-8 AS)	(51)
<i>Ophiurochaeta</i> sp. A ^a	4.8	S	680	3.5 (0.4)	0.1 (0.1)	2.0 (0.1)	2.2 (0.2)	1 000 (12-15 AS)	(108)

^a Designated species A in Hendler and Peck (1988)

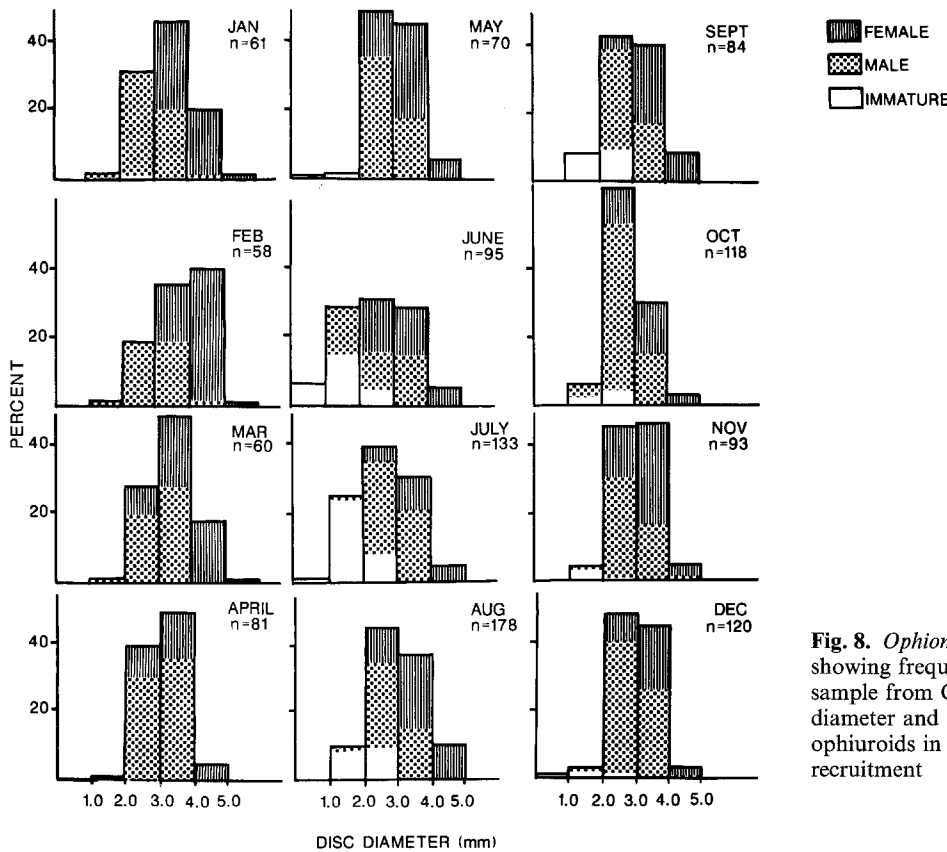


Fig. 8. *Ophionereis olivacea*. Size-frequency histograms showing frequencies of disc diameters in each monthly sample from Conch Key and relationship between disc diameter and sex. The high proportions of immature ophiuroids in June and July samples are due to recent recruitment

et al. (1984) and Eernisse (1988), the barrier to the spread of self-fertilizers would be low in gonochoric populations of brooders because of their reduced genetic diversity. Moreover, because self-fertilizers are expected to produce just enough sperm to ensure fertilization of all the ova

produced, a reduced male allocation would be selected for (Heath 1977, Charnov 1982, Strathmann et al. 1984). This hypothesis fits several of the Caribbean species which have minute testes and reduced male allocations (Table 1). Moreover, for *Ophiolepis paucispina*, *Amphiura*

stimpsonii, *Amphipholis squamata*, *Sigsbeia conifera* and *Ophiurochaeta* sp., the association of ovaries and testes with individual bursae would facilitate self-fertilization (Mortensen 1933, Fell 1946, Hendler 1991, Byrne personal observations).

In contrast to the other Caribbean species, *Ophionereis olivacea* is a protandric hermaphrodite and does not have a reduced male allocation (Table 1). The scarcity of simultaneous hermaphrodites suggests that sex reversal in *O. olivacea* is rapid and probably occurs after spawning, during the gonadal recovery period in May and June. In having separate sexes *O. olivacea* is an obligate out-crosser and, as expected from species that broadcast sperm, the allocation to male and female reproduction is similar. Although the limited dispersal of the progeny of *O. olivacea* undoubtedly results in inbreeding and decreased genetic diversity, simultaneous hermaphroditism has not been selected for in this ophiuroid.

The allometry hypothesis provides an explanation for the link between brooding and small adult size in several taxa (Strathmann et al. 1984, Kabat 1985). In *Asternia phylactica*, the asteroid used as the model for the allometry hypothesis, the capacity to produce eggs increases volumetrically with size, whereas brood space increases with surface area (Strathmann et al. 1984). In contrast to *Ophionereis olivacea*, this asteroid broods its young externally under the oral surface. There is a physical constraint on brooding by *A. phylactica*, with larger adults producing more eggs than they can hold, resulting in embryo loss and mortality (Strathmann et al. 1984). These observations support the allometry hypothesis, which proposes that brooding does not occur in large species due to spatial limitations on brood space with increasing size (Heath 1977, Strathmann and Strathmann 1982).

The number of eggs and juveniles produced by *Ophionereis olivacea* scale as a square of the disc diameter. As this ophiuroid is a sequential brooder producing an annual cohort of large oocytes, it is not surprising that utilization of body space simply transfers from the ovaries to the bursae during brooding. Although it is intuitive that egg production and brood space should have a volumetric relationship with body size, this is not indicated by the data. Female *O. olivacea* are capable of brooding all the embryos they produce and do not exhibit brood loss with increasing size, observations counter to the allometry hypothesis. For this species, the allometry hypothesis does not provide a direct explanation for the association of diminutive size with brooding. By scaling fecundity and clutch size as a square of the disc diameter, however, *O. olivacea* may be overcoming the allometric constraints of ventilation with increasing brood volume. Ventilation of the embryos depends on the ciliated surface of the bursal wall, and if the number of embryos increased volumetrically, then larger females may have greater difficulty in oxygenating their broods. The need for oxygen exchange and waste elimination by developing embryos limits the size and shape of egg masses (Strathmann and Chaffee 1984).

The energetic hypothesis (Chia 1974) proposes that small size is linked to brooding due to the energetic con-

straints, where small species can not produce enough eggs to ensure recruitment through dispersal and thus have to brood their young. Although not testable, the energetic hypothesis is consistent with the life history traits of *Ophionereis olivacea* and the other Caribbean ophiuroids that exhibit parental care. All these species have reduced fecundity and, with the exception of the viviparous ophiuroid *Amphipholis squamata*, have large eggs (Table 1). As noted by Strathmann and Strathmann (1982), however, the energetic hypothesis does not explain why brooding is not also typical of large species.

The embryos of *Ophionereis olivacea* lack ophiopluteus features and are morphologically similar to the non-feeding vitellariae of the closely related species, *O. annulata* and *O. squamulosa* (Mortensen 1921, Hendler 1982). Like the embryos of *O. olivacea*, the larvae of *O. annulata* and *O. squamulosa* are yolky and barrel-shaped, but differ in having the transverse ciliary bands typical of vitellariae. In addition, the larva of *O. annulata* has a vestigial ophiopluteus skeleton (Hendler 1982). Ophiuroid vitellariae are considered to have evolved through modification of the ophiopluteus form (Hendler 1982, Mladenov 1985). In *O. olivacea*, the transition to direct development has resulted in further modification of the ophiopluteus, with loss of the ciliary bands and larval skeleton. The presence of a vestigial ophiopluteus in the bursal development of *Amphipholis squamatus* and *Ophionotus hexactis* provides further evidence that parental care in the Ophiuroidea is a derived mode of reproduction and that brooding species have ancestors with feeding larvae (Mortensen 1921, Fell 1946, Strathmann 1978).

In the shift from broadcasting to brooding in *Ophionereis olivacea*, the evolution of a large egg would have been a pre-adaptive trait for parental care. The pathway in the evolution of brooding in *O. olivacea* probably included an intermediate ancestor that had a non-feeding planktonic larva similar to the vitellariae of *O. annulata* and *O. squamulosa*. Subsequent steps in the evolution of brooding would have included retention of the egg in the bursa for fertilization and embryogenesis. Brooding in *O. olivacea* has resulted from heterochronies in the suppression of larval features. Small size in *O. olivacea* is also associated with paedomorphic heterochrony, as demonstrated by the precocious maturation of male *O. olivacea* at a disc diameter of 1.5 mm, two months after they vacate the bursae. The evolution of small size through paedomorphosis is also evident in diminutive *Ophiolepis* spp. that brood their young (Hendler 1979).

The obvious advantage of brooding in *Ophionereis olivacea* is the retention of juveniles in a favourable adult habitat that enhances the chance of survival to reproductive maturity. *Halimeda opuntia* turf has a complex infrastructure, and this alga contains a toxic secondary metabolite that makes it unattractive to grazing fishes (Littler et al. 1983, Paul and Fenical 1983). In the Caribbean, predation by grazing fishes exerts a major influence on the distribution and behaviour of ophiuroids (Aronson 1987, Sides 1987). *H. opuntia* is considered to be a refuge-substratum where ophiuroid survival is en-

hanced by protection from predation and environmental stress (Hendler and Littman 1986).

Although brooding by polar ophiuroids is undoubtedly adaptive to conditions at high latitudes, it is evident that selection for parental care in the Ophiuroidea has also occurred in the warm-water habitats of the Caribbean. The generic diversity of the ophiuroids listed in Table 1 adds credence to the suggestion that brooding has evolved in ophiuroid clades irrespective of geography and phylogeny (Mortensen 1933, Hendler 1991). Like *Ophionereis olivacea*, the other Caribbean brooders occupy algal and reef microhabitats that would select for small size (Emson et al. 1985, Hendler and Littman 1986, Byrne 1991). Whether small size in *O. olivacea* and the other species was a pre-adaptive trait or whether it evolved in response to selection for microhabitat utilization, is not known. Occupation of their present habitats, however, depends on their diminutive size. The protection from predation provided by *Halimeda opuntia* may have influenced selection for colonization of this alga by *O. olivacea* and the other brooders.

Selection for small size in *Ophionereis olivacea* undoubtedly influenced the shift to brooding, and it appears that small size and brooding arose as co-adaptive traits that allowed successful utilization of refugia microhabitats. The evolution of diminutive size and brooding is associated with paedomorphosis, and may have been influenced by factors such as energetics and a requirement for retention of progeny in a favourable habitat. For the Ophiuroidea, the presence of brooders in the Caribbean appears to be a function of habitat rather than latitude. It would be interesting to examine turfing *Halimeda* spp. elsewhere in the tropics to investigate the relationship between this habitat and ophiuroid life histories. Such an investigation may reveal the presence of brooding species in regions where parental care is considered to be rare.

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