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ORIGINAL ARTICLE

Brooding in *Psolus patagonicus* (Echinodermata: Holothuroidea) from Argentina, SW Atlantic Ocean

Juliana Giménez · Pablo E. Penchaszadeh

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Abstract The mode, season, and time of brooding, egg diameter, egg number per brood, and the characteristics of newly released juveniles of *Psolus patagonicus* were investigated off Mar del Plata, Buenos Aires, Argentina, between October 1999 and February 2001. Individuals were attached to the Patagonian scallop, *Zygochlamys patagonica.* Spawning occurs between February and March. The mean egg diameter, 887 ± 26 μm, is the highest reported for the family Psolidae. Eggs are brooded under the mother's sole until they develop into crawling juveniles within 7 months. The largest embryos reached a length of 1,941 ± 228 μm in September. During the brooding period (February–September) the number of brooded embryos decreased while their size increased. Our study confirms brooding behaviour in female *P. patagonicus*.

Keywords *Psolus patagonicus* · Brooding · Psolidae · Argentine continental shelf

Introduction

The sea cucumber *Psolus patagonicus* is abundant in the southwestern Atlantic Ocean. It has been reported from different substrata in a variety of habitats from southern Patagonia, including intertidal rocky shores and fronds and holdfasts of *Macrocystis pyrifera* (Bernasconi 1941; Hernández 1981). Moreover, it is found as epizoic on the shells of live scallops of economic importance (*Zygochlamys patagonica*) on the continental shelf off Buenos Aires province, Argentina (Bremec and Lasta 2002). Like many other benthic invertebrates in the region, *P. patagonicus* is distributed in shallow waters at high latitudes (Patagonian and subantarctic area), and in deeper, colder waters at low latitudes (off the Rio de la Plata estuary).

Brooding behaviour in holothurians has been observed in at least 41 species (Smiley et al. 1991). McEuen and Chia (1991) provided information on the reproductive pattern of 15 species of the family Psolidae. Eleven of these are brooding species and the remaining three have pelagic larvae.

Species belonging to family Psolidae exhibit a great diversity of brooding modes. Broods occur internally in the coelom, in dorsal depressions, folds of the sole, chambers underneath dorsal plates, interradial pouches, interradial pouches surrounding the tentacular crown, and under the sole (McEuen and Chia 1991). Juveniles of sessile species develop successfully in micro-habitats suitable for brooders (Gutt 1991).

Bernasconi (1941) reported *P. patagonicus* is a brooding species because she found a preserved adult and two juveniles together in the same flask. Brooding by *P. patagonicus* subsequently has been mentioned in the literature (Hernández 1981; McEuen and Chia 1991), but its characteristics have not been described. The present study confirms *P. patagonicus* as a brooding species and provides information on the mode, season, and time of brooding, egg diameter, egg number per brood, and the characteristics of newly released juveniles.

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J. Giménez (✉) · P. E. Penchaszadeh
Depto. de Biodiversidad y Biología Experimental,
Facultad de Ciencias Exactas y Naturales,
Universidad de Buenos Aires, Ciudad Universitaria,
Pab. II, C1428EHA Buenos Aires, Argentina
e-mail: jgimenez@bg.fcen.uba.ar

J. Giménez · P. E. Penchaszadeh
CONICET, Museo Argentino de Ciencias Naturales,
Av. Angel Gallardo 470, C1405DJR Buenos Aires, Argentina

Methods

Samples were collected off Mar del Plata (39°24′78″S and 55°56′70″W; Fig. 1), during 14 research cruises aboard the FRV Capitán Cánepa of the Instituto Nacional de Investigación y Desarrollo Pesquero (INIDEP) between October 1999 and February 2001. Specimens of the scallop *Z. patagonica* were collected by bottom trawling using a research dredge (2.5 m mouth opening, 10 mm mesh size at the cod end), at depths between 100 and 110 m. The bottom water temperature was measured with a CTD SBE 911. Immediately after collection, scallops with epizoic *P. patagonicus* (Fig. 2) were preserved in 5% formalin-seawater solution for 24 h, and then stored in 70% ethanol.

In the laboratory, *P. patagonicus* were removed from the scallops and measured with callipers. Nine hundred and-fourteen individuals were collected. Fifty-seven were brooding young under the sole.

Ten eggs and ten embryos from each brood were randomly selected each month to measure egg diameter and embryo length. The eggs and embryos in the broods were counted and photographed using a Zeiss Stemi 2000 C stereomicroscope. To avoid undercounting, the brooding specimens were carefully removed from the substratum, taking care not to leave any egg or embryo behind.

The gonad of *P. patagonicus* consists of numerous tubules joined together at their base forming a tuft attached to the dorsal mesentery. Sexes were identified by histological observations of the gonads. These were dissected, fixed in 5% seawater formalin for 24 h, stored in 70% ethanol, dehydrated in a graded alcohol series, embedded in paraffin, stained with haematoxylin–eosin (H/E) and examined under light microscope.

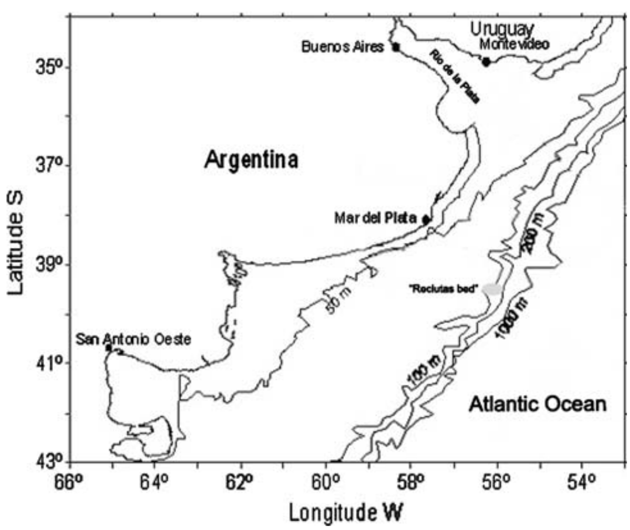

Fig. 1 Reclutas bed (39°S–55°W), sampling area of the scallop *Zygochlamys patagonicus*

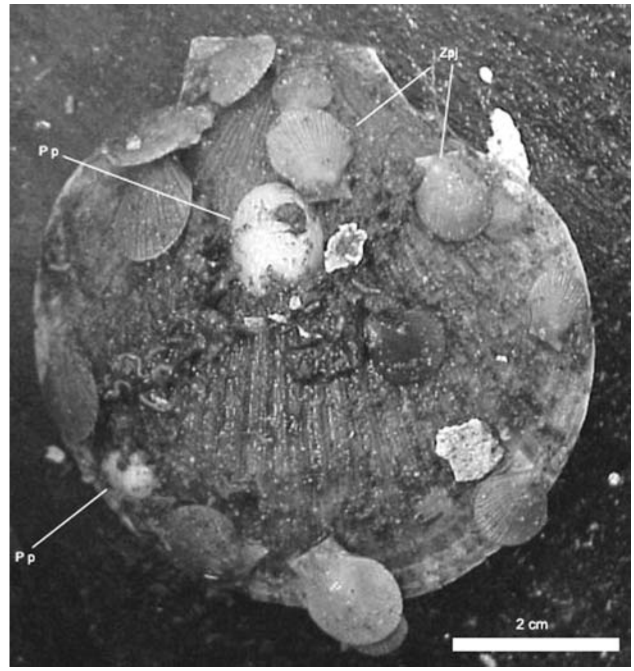

Fig. 2 *Psolus patagonicus* epizoic on the Patagonian scallop *Zygochlamys patagonica*. Two individuals of *P. patagonicus* (*Pp*) and some *Z. patagonica* juveniles (*Zpj*) are attached to a live scallop

Results

Psolus patagonicus had a single spawning season from February to March (Fig. 3a). The bottom temperature ranged between 6 and 7°C. An increase in temperature values was observed from April to June. Eggs and embryos were found under the female's sole (Fig. 3b). Histological sections of male and female gonads (Fig. 4a, b) confirmed that all brooding individuals were females.

Figure 5 shows the size-frequency distribution of the population of *P. patagonicus.* Body length ranged from 2.2 ± 0.1 mm for October 1999 and 2000 to 23.1 mm for November 2000. Brooding females were between 17 and 23 mm in length. The following brood development stages were recognised: stage 1 unsegmented egg (Fig. 6a) in February and March 2000; stage 2 segmented egg (Fig. 6b) in May 2000; stage 3 *doliolaria* larva (Fig. 6c) in June 2000; stage 4 *pentactula* larva (Fig. 6d) in July 2000; stage 5 juvenile (with clearly visible ossicles and tube feet (Fig. 6e) in August 2000; and stage 6 late brooded juvenile (Fig. 6f) in September 2000.

The brooding period lasts 7 months, from February to September (Fig. 7). Developmental stages were synchronous within and among females. Eggs were spherical in shape in February–March (at the beginning of the brooding season) and underwent cleavage at the beginning of May. The *doliolaria* larva was observed in June, the *pentactula*

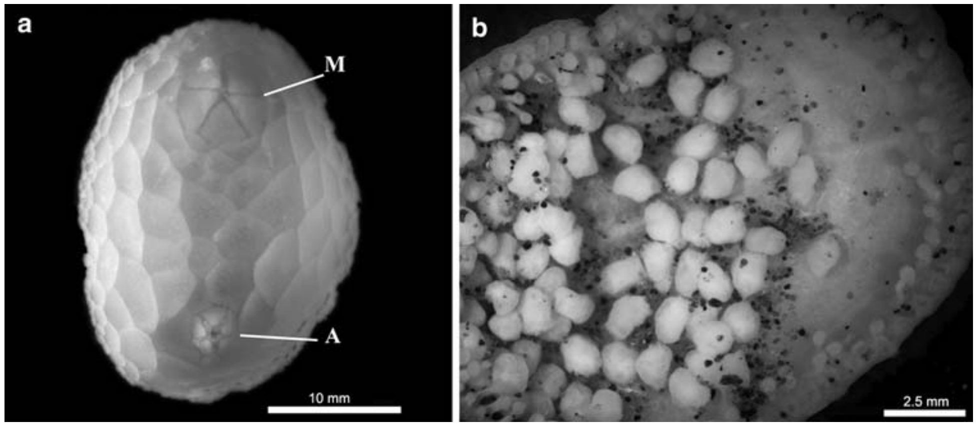

Fig. 3 *Psolus patagonicus*. **a** Dorsal view: mouth (*M*) and anus (*A*). **b** Ventral view: embryos under the sole

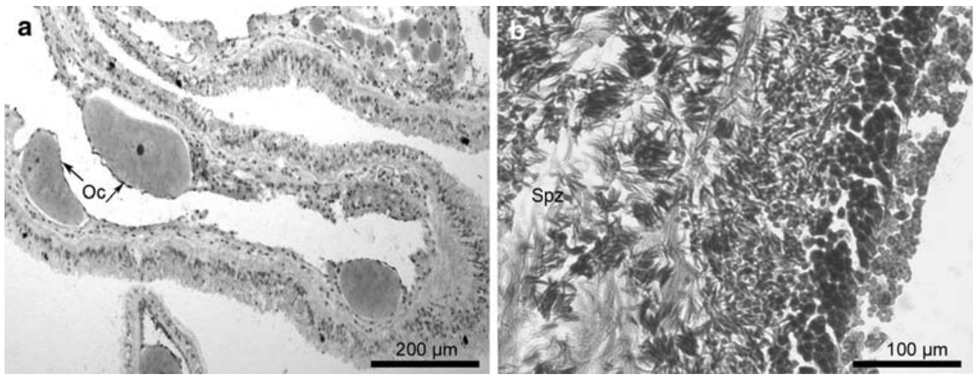

Fig. 4 *Psolus patagonicus*. **a** Light micrographs of histological sections of ovaries; note the presence of oocytes (*Oc*). **b** Light micrographs of histological sections of testes, with different stages of spermatogenesis, including some spermatozoa (*Spz*)

larva in July, early juveniles in August and late juveniles in September. The initial number of 135 ± embryos in February decreased to 70 ± embryos in September, at the end of the brooding period (Fig. 7). The free-living juveniles, found attached to the scallops in October, had a mean length of 2.2 ± 0.1 mm (Fig. 5).

Discussion

Comparison of brooding patterns between psolids

McEuen and Chia (1991) reported brooding and pelagic lecithotrophic larvae as the only modes of development in the family Psolidae. The authors described four brooding sites: on the ventral side (6 species), on the dorsum (2 species), surrounding the tentacular crown (2 species), and internally (1 species).

After spawning, the diameter of the unsegmented egg of *P. patagonicus* (887 μm) is larger than those of other psolid species (between 330 and 650 μm) (McEuen and Chia 1991). Among psolids, *Psolus antarcticus* most closely resembles *P. patagonicus* in terms of mode of brooding (Ludwig 1897, 1898). In both species, the young are brooded under the sole of the female until the formation of the coat of ossicles and the flat sole. No data are available on the uncleaved egg diameter or the mode of development for *P. antarcticus*, but large eggs and the occurrence of the doliolaria stage are common features in holothurians (McEuen and Chia 1991).

Reproductive timing and environmental conditions

There is a spatio-temporal overlap between the late brooding season of *P. patagonicus* (October, beginning of spring) and one of the two spawning seasons of *Z. patagonica*. The first spawning season is from late summer to early autumn and the second during spring (September–December) (Waloszek and Waloszek 1986). In the study area, the austral spring bloom of phytoplankton (September–October) in the upper layer of the water column is followed by the phytoplankton sinking after the development of the seasonal thermocline (October–November). This process ultimately increases food availability for benthic organisms (Schejter et al. 2002).

Psolus patagonicus, like other deep benthic animals, is subjected to small temperature fluctuations (<1°C) and complete darkness. Under this environmental condition, the sea cucumber, which is a suspension feeder, feeds on dead, dying, or decaying phytoplankton from the photic zone

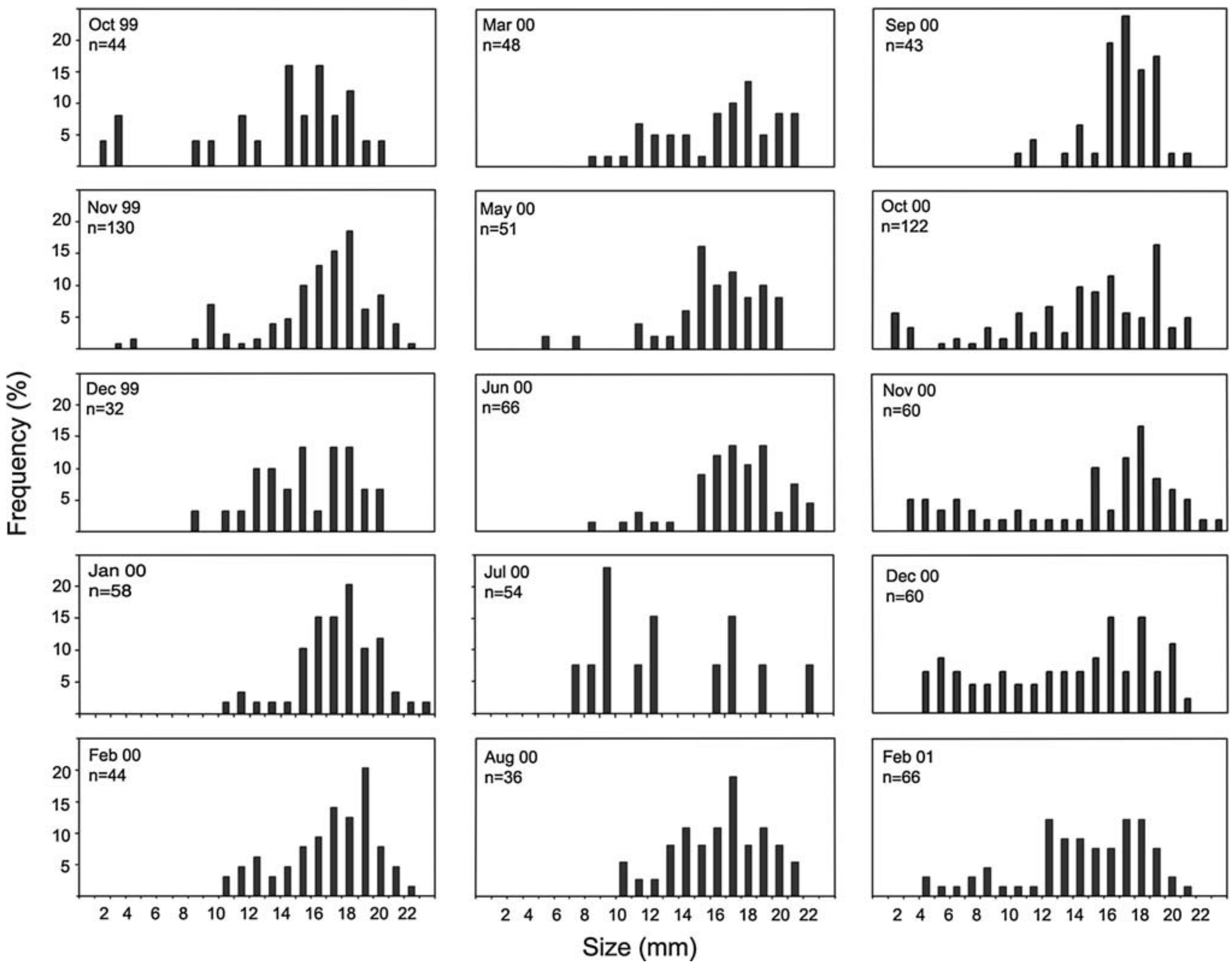

Fig. 5 Size frequency histogram (%) of *Psolus patagonicus* removed from scallop shells

(Schejter et al. 2002). On this basis, it is reasonable to assume its reproductive cycle and brooding period are related to the seasonal abundance of phytoplankton. Environmental factors such as food availability synchronize reproductive events (Himmelman 1975; Tyler 1988; Gage and Tyler 1991; Smiley et al. 1991; Himmelman et al. 2008).

Implications for brooding

Psolus patagonicus is a small sea cucumber, up to 23 mm in length. A positive relationship between small body size and brooding has been reported for marine invertebrates in general and echinoderms in particular (Chia 1974; Emson and Wilkie 1980; Strathmann and Strathmann 1982; Jablonski and Lutz 1983; Mladenov and Burke 1994; Lawrence and Herrera 2000).

It has been suggested that the small body size of brooding and fissiparous ophiuroids reflects evolutionary adaptation to patchy microhabitats (Hendler and Littman 1986; Hendler and Peck 1988). In psolids, however, the relationship between body size and type of development is not so clear. For example, a pelagic larva is part of the life cycle of large broadcast spawning species such as *P. chitonoides* (75 mm in length; Young and Chia 1982), *P. fabricii* (100 mm in length), and *P. phantapus* (150 mm in length) (Deichmann 1930), but also of the small *Psolidium bullatum* (26 mm in length) (McEuen and Chia 1991). In *P. patagonicus* the number of brooded embryos decreased at the same time as their size started to increase. This suggests space under the sole of brooding females becomes limited. More species need to be investigated to confirm this. Moreover, the fact that *P. antarcticus* (55 mm in length) (Ludwig 1897) shares similar reproductive features with the smaller *P. patagonicus* suggests the importance of studying the phylogenetic relationships among psolid species.

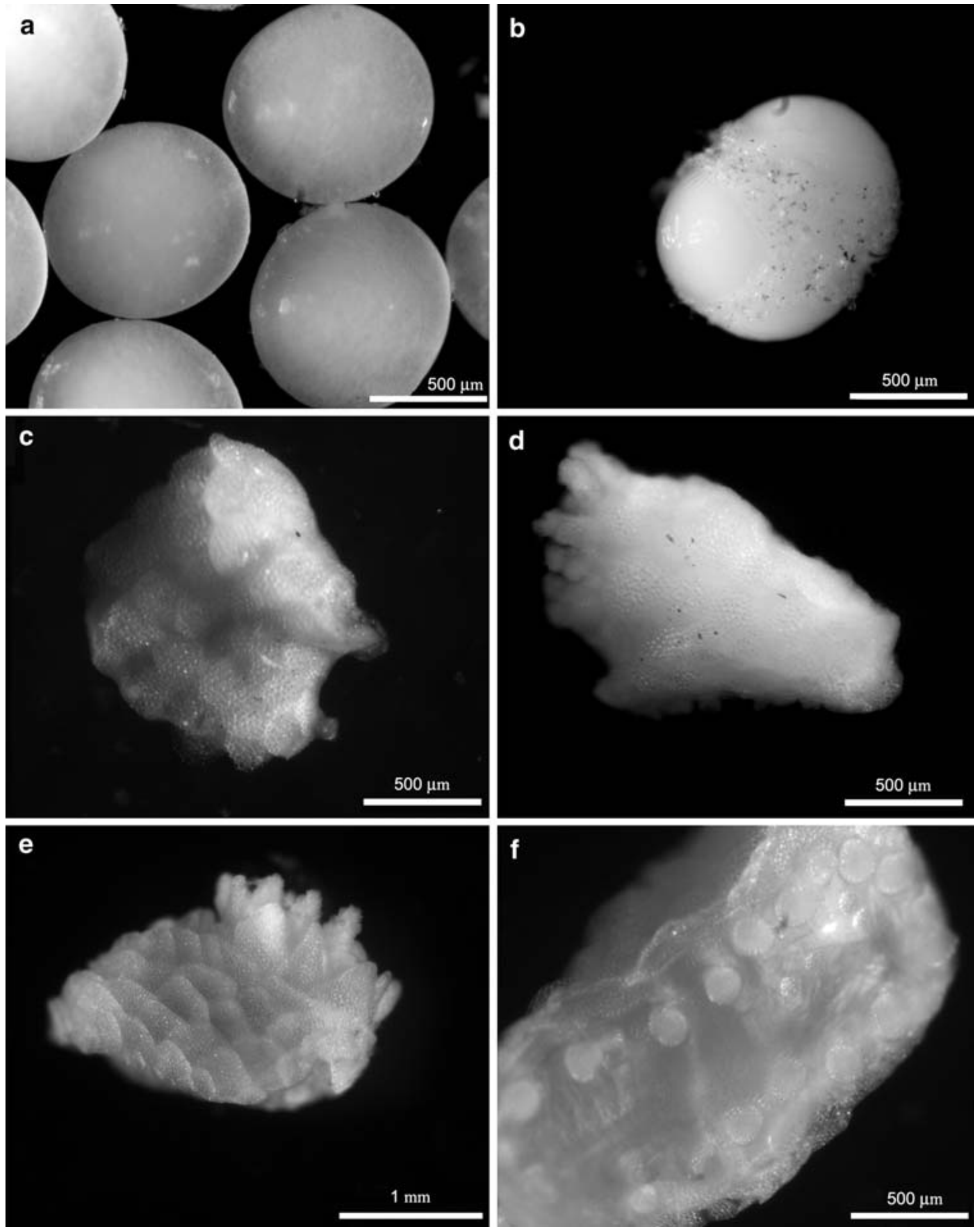

Fig. 6 Developmental stages of *Psolus patagonicus* during the brooding period. **a** Unsegmented egg, **b** segmented egg, **c** doliolaria larvae, **d** pentactula larvae, **e** young juveniles, **f** late juveniles

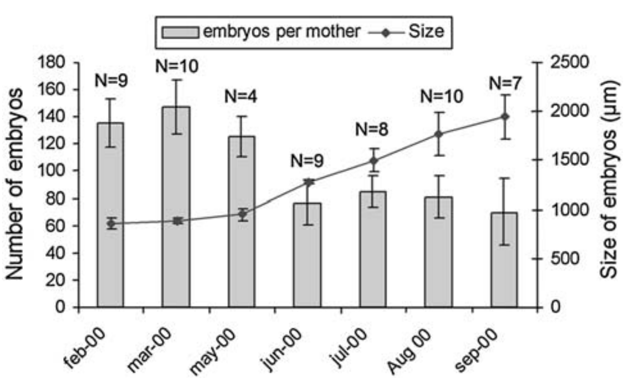

Fig. 7 Number and size of offspring during the brooding period. *Grey bar* number of embryos/eggs per brooding female. *Black line* size of the embryos/eggs. *N* number of brooding females

The number of juveniles in broods at the end of the brooding period was much higher than that of free-living juveniles. This may result from different reasons, e.g. increased survival of broods by mother's protection or inability of free-living juveniles to remain attached to the scallops during bottom trawling. One advantage of brooding is protection of the offspring from predation. But a disadvantage is that it decreases long-distance dispersal which might increase the risk of local extinctions.

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