Reproductive biology of the deep-sea pennatulacean

*Anthoptilum murrayi* (Cnidaria, Octocorallia)

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ABSTRACT: *Anthoptilum murrayi* has been reported from the North Atlantic, northern Mid-Atlantic Ridge, Indian Ocean and in waters around New Zealand and Australia. Recently, this species was also recorded in deep waters off Brazil, southwestern Atlantic. It was from this region (13° to 22° S) that specimens were collected, in 1300 to 1799 m, to determine the reproductive biology of *A. murrayi* using histological methods. The colony polyparium was divided into 3 zones (distal, medial and basal) to evaluate differences in gamete development between zones; dissected polyps were examined from the 3 zones to estimate fecundity. The species appears to display a continuous and long breeding activity rather than any seasonal reproductive pattern. Most oocytes were in the earliest stages of development and basal polyps presented the highest frequency of small eggs. The large mature oocytes (up to 1200 µm) indicate that *A. murrayi* produces lecithotrophic larvae. Females had 0 to 90 oocytes per polyp and 25 713 to 35 918 oocytes per colony. Male colonies of similar size to the female samples were shown to have 6 to 76 cysts per polyp and 14 014 to 27 019 cysts per colony. *A. murrayi* is a sessile gonochoric species with a 1:1 sex ratio and is most likely a broadcast spawner. The species has high fecundity, large eggs that could represent larger targets for sperm, primitive spermatophores, as well as a large number of polyps per colony. These factors, along with a patchy distribution, would enhance the chance of fertilization for *A. murrayi* and may guarantee a successful reproductive strategy for this species.

KEY WORDS: Pennatulacea · Gametogenesis · Fecundity · Deep-sea
For general reproductive patterns in deep-sea corals, data exist primarily for groups such as scleractinians (see review in Waller 2005), stylasterids (Brooke & Stone 2007) and octocorals (review in Simpson 2009). In terms of the sexual status and mode of development, most shallow-water scleractinians are hermaphroditic and spawn gametes for external fertilization (Harrison & Stone 2007) and octocorals (review in Simpson 2009). Despite the ecological importance of cold-water octocorals, there are few published studies on their reproductive processes (Cordes et al. 2001, Orejas et al. 2002, 2007), although some studies have included various aspects on the reproduction of pennatulaceans (Chia & Crawford 1973, Eckelbarger et al. 1998, Tremblay et al. 2004, Soong 2005, Edwards & Moore 2008, 2009). However, only 2 studies have focused on deep-water sea pens (Kophobelemnon stelliferum, Rice et al. 1992, and Umbellula lindahi, Tyler et al. 1995).

Gonochorism is the dominant pattern found for octocorals, with only a few alcyonacean taxa being hermaphroditic, e.g. Sinularia exilis (Benayahu 1997) and some species of Heteroxenia and Xenia (Benayahu 1991, 1997). The frequency of brooding versus broadcast spawning in octocorals varies with taxonomic order (Simpson 2009). All pennatulaceans studied to date are broadcast spawners.

Anthozoa, especially scleractinians and octocorals, are the most dominant macrobenthic group in some deep-sea areas (Bimêndrove and Espírito Santo States). Colonies between 13° and 21° S in 2000 during the Living Resources in the Exclusive Economic Zone (REVIZEE) Score Central Project (see Lavrado & Ignácio 2006), and in 2003 off Rio de Janeiro State (Caminos Basin, 21° to 22° S) during the Campos Basin Deep-Sea Environmental Project/PETROBRAS (Ocean Prof I and II campaigns) (see Falcão et al. 2006). Specimens were up to 7 cm in height (Fig. 1) and were collected at depths between 1051 and 1799 m using otter trawls. A relatively large number of specimens were collected during sampling, but only a few of these colonies (n = 24) were undamaged and still bore intact polyps suitable for study. The 24 colonies were collected in February (4 specimens), June (9), July (4) and August (7).

Gametogenesis was examined using histological methods. All colonies were fixed and preserved in 70% alcohol, as they had been primarily collected for taxonomic studies. The use of alcohol-preserved specimens for histology was far from ideal and posed many limitations during the various histological procedures. Tissues were fragile from alcohol preservation and the process of extending the sections prior to application to...
the slides was difficult, with several sections lost during reagent transfer. Polyps were dissected dehydrated by graded alcohol series and embedded in paraffin wax using standard methodology (Pantin 1948). It was ascertained that thinner sections were more adequate to obtain better histological results and as such sections of 4 to 6 µm were stained using Mallory’s triple stain for histological observations. Gametogenesis was classified according to histological characteristics and sizes of both the oocytes and spermatid cysts. Stages of development are arbitrary as they reflect a continuous process (Wourms 1987). Gamete development classification followed Pires et al. (1999), where Stage I represented the beginning of development, Stage II an intermediate step (vitellogenic process in oogenesis) and Stage III mature oocytes and/or spermatid cysts. Measurements of the different developmental stages of oocytes and spermatid cysts were made using an eyepiece micrometer in an Olympus BH2 microscope. The longest axis of the oocytes and spermatid cysts were measured. Only perfect oocytes with nuclei were measured, to avoid remeasurement of the same cells.

The polyparium of male and female colonies (from June 2000) was divided into 3 zones (distal, medial and basal) to evaluate differences in gamete development between zones. Polyps from each zone were examined using histology to access the synchrony of developmental stages among different colony zones. Polyps from different zones were also examined through dissections to determine polyp fecundity (= number of oocytes or spermatid cysts per polyp). The same colonies were used in both cases.

Gametes of 4 polyps from each zone, as well as the total number of polyps in each colony, were counted in 4 male and 4 female colonies, all collected in June 2000 (total = 48 male and 48 female polyps). One of the male colonies was used solely for fecundity counts. Counts were made using a Zeiss SV6 stereomicroscope and all oocytes and spermatid cysts seen at 25 to 32x magnification were counted. Colony fecundity was estimated as the average number of gametes in polyps from different zones of colonies multiplied by the average number of polyps of a given colony. Colony lengths were also measured.

Sex ratio was tested using a chi-square comparing the observed and expected frequencies in a 1:1 ratio (null hypothesis). Such an expected ratio was designated as the sum of males (M) and females (F) observed divided by 2 (n each sex = M/2 + F/2).

Differences in fecundity were tested using nested ANOVA, with polyp position nested in colony. Prior to testing, homocedasticity of variance (Levene’s test) and normality (Kolmogorov-Smirnov test) were tested. No significant departures from ANOVA premises were found, except for number of cysts (F_11,36 = 2.59, p = 0.016). Significant differences (p < 0.05) were further examined with post hoc tests (Tukey’s HSD). All tests were performed using Statistica 6.0 (StatSoft).

RESULTS

All examined colonies (females: 28 to 58 cm; males: 24 to 64 cm long) presented gametes and were gonochoric. It was not possible to distinguish the sex of the fixed colonies without histological preparations. Sex ratio did not significantly differ from 1:1 (11 males and 13 females examined through histology, χ² = 0.167, df = 1, p = 0.683).

Gametes appeared only in the autozooids. Mature sexual cells were visible with the naked eye (Fig. 2). A layer of mesoglea surrounded the sexual cells and stained an intense blue. The mesoglea thickened in the oocytes as they developed.
Oogenesis was classified into 3 stages (Fig. 3A–C). Stage I represented primary development, Stage II an intermediate step (most of the vitellogenesis phase) and Stage III characterized mature sexual cells. Early Stage I cells stained bluish purple. Pre-vitellogenic Stage I cells usually presented a more homogenous cytoplasm, and some cells stained light rose. The nucleus was most often positioned centrally. The smallest Stage I cell observed was 10 µm (longest axis) and the largest was 350 µm, with a mean (± SD) value of 115.32 ± 53.21 µm. Stage II oocytes stained dark rose. More lipid vesicles were present and had begun to coalesce. Stage II oocytes showed a migration of the nucleus to the cell border and they ranged in size from approximately 220 to 540 µm, with a mean value of 368.28 ± 83.89 µm. Stage III oocytes stained from dark rose to red. The cytoplasm became full of distinct colorless lipid vesicles and the nucleus had moved to the periphery of the cell. Stage III oocytes ranged in size from approximately 490 to 1200 µm, with a mean value of 901.07 ± 180.07 µm.

Spermatogenesis was also divided in 3 development stages (Fig. 3D–F). Stage I spermatic cysts ranged from 30 to 300 µm, with a mean value of 94.67 ± 54.17 µm. In Stage II, cysts had a lumen present and occasionally some tails of spermatozoa could be seen. Stage II cysts ranged from 50 to 650 µm, with a mean value of 368.28 ± 83.89 µm. Stage III oocytes stained from dark rose to red. The cytoplasm became full of distinct colorless lipid vesicles and the nucleus had moved to the periphery of the cell. Stage III oocytes ranged in size from approximately 490 to 1200 µm, with a mean value of 901.07 ± 180.07 µm.

Male and female cells were associated with the follicle layers (differentiated and flattened gastrodermal cells) during all stages of gametogenesis. These follicle layers were inconspicuous in some cells, but usually their thickness increased as the growth of sexual cells advanced. Follicle layers were approximately 20 µm thick in sperm cysts and 70 µm thick around Stage III oocytes (Fig. 3C).

*Anthoptilum murrayi* is more likely to present continuous breeding activity than seasonal cycles, since gametes in different stages of development were observed from single seasons (Fig. 3B). The lack of continuous sampling meant that an estimate of the duration of the reproductive cycle could not be obtained.

Polyps from different areas of the colony were shown to bear gametes. In females, most of the oocytes were in the earliest stages of development and polyps from the basal area presented the highest frequency of very small cells (Fig. 4).

High frequencies of small oocytes (up to 200 µm) and low frequencies of large oocytes were observed for all sampling periods (Fig. 5). All spermatic cysts observed in specimens collected in August were larger than 200 µm. Samples from August also showed the highest frequencies of the largest size classes of cysts and the occurrence of the largest cysts observed for all samples (between 700 and 740 µm) (Fig. 5).

There were large numbers of gametes in both male and female colonies. Examined specimens from June 2000 had 414 to 645 polyps in female colonies (up to 747 mm in length) and 429 to 655 polyps in male colonies (up to 650 mm in length). Female colonies presented 0 to 90 (47.6 ± 12.4) oocytes per polyp and an estimated 25713 to 35918 (31465 ± 5080) oocytes per colony. Oocytes per polyp differed from polyp to polyp (ANOVA, $F = 3.69$, df = 8, $p = 0.003$). Tukey’s HSD showed these differences always occurred between the basal polyps and the middle or distal polyps (8 out
of 32 such pairs). The number of oocytes per colony also differed significantly from colony to colony ($F = 7.33, \text{df} = 3, \ p < 0.0006$). Male colonies of similar sizes presented 6 to 76 (36.6 ± 3.6) cysts per polyp and an estimated 14 014 to 27 019 (19 871 ± 5793) cysts per colony. The number of cysts differed significantly among the different polyp positions on the colony ($F = 9.00, \text{df} = 8, \ p = 0.000001$), but did not differ significantly among colonies ($F = 1.17, \text{df} = 3, \ p = 0.33$). Basal polyps presented significantly less cysts than middle and distal polyps (Tukey’s HSD, 19 out of 32 such comparisons).

*Anthoptilum murrayi* is most likely a broadcast spawner, as no embryonic or planula stages were seen in the gastrovascular cavities of the polyps. In addition, large mature oocytes and intact cysts were commonly seen above the pharynx, well up in the hollow tentacles. Fig. 6 shows a longitudinal section of a male polyp.
where mature cysts are seen in the tentacles and at the base of the polyp. It seems that intact cysts passed by the side of the pharynx and reached the tentacles.

**DISCUSSION**

Although the lack of a continuous sampling design imposed limitations on the present study, the available samples were sufficient to detect main features of reproductive biology for this species. *Anthoptilum murrayi* followed the same general pattern of gono-choric sexual development as seen in other pennatulaceans and some octocorals (Table 1). The few available data show that sea pens have a sex ratio of 1:1 (Edwards & Moore 2008, 2009), and this was observed in *A. murrayi*.

The largest size of oocytes of *Anthoptilum murrayi* (1200 µm) was approximately 60% larger than the largest pennatulacean eggs recorded to date. The maximum oocyte size recorded for both *Kophobelemnon stelliferum* and *Umbellula lindahlii* is 800 µm (Rice et al. 1992, Tyler et al. 1995), and *Pennatula aculeata* had a maximum oocyte size of 880 µm (Eckelbarger et al. 1998). Very large oocytes (up to 1200 µm) have also been recorded in other cold-water octocorals such as *Dasystenella acanthina* (Orejas et al. 2007), as well as in solitary deep-sea scleractinians belonging to the genus *Flabellum* (Waller et al. 2008). Increased oocyte size is correlated with some corals with an extended oogenic cycles, but not all corals with long cycles produce large oocytes (Harrison & Wallace 1990). This size of oocyte also indicates that *A. murrayi* produces lecithotrophic larvae. In octocorals, larger oocytes are often associated with species which have these non-feeding larvae (Edwards & Moore 2009) and most planula observed appear to be lecithotrophic (Simpson 2009). Lecithotrophy is the pattern seen among pennatulaceans (Eckelbarger et al. 1998) and is also observed in deep-sea broadcast spawning scleractinians (Waller 2005).

Environmental factors influence coral sexual processes, synchronizing the reproductive cycles (affected by temperature, day length and salinity) and the timing of spawning within a species, which is affected by tidal patterns and lunar rhythms (Harrison & Wallace 1990). However, the great majority of deep-sea species reproduce aperiodically or continuously, not requiring periodic environmental cues to regulate their gameto-genic cycles (Young 2003). *Anthoptilum murrayi* differs from most pennatulaceans, presenting a possible continuous and long breeding activity rather than seasonal cycles. Continuous or quasi-continuous reproductive cycles are not the general pattern in pennatulaceans, but they have also been observed in...
Fig. 5. *Anthoptilum murrayi*. Relative frequency of oocyte and spermatic cyst size classes in colonies collected in February, June, July and August. Key notation: month (no. colonies/total no. gametes examined/minimum–maximum no. gametes examined in a single colony). Error bars are ±SE.
Kophobelemnon stelliferum and Pennatula aculeata (Rice et al. 1992, Eckelbarger et al. 1998; Table 1).

The presence of oocytes in different stages of development in the same polyp can indicate extended and continuous reproductive cycles, and also occurs in other deep-sea octocorals such as Antarctic primnoids (Brito et al. 1997, Orejas et al. 2002, 2007). Brito et al. (1997) identified a similar pattern for Thouarella variabilis, and showed a 2 yr cycle of oogenesis or continuous gametogenesis. Thouarella sp. and Dasystinella acanthina also have a long reproductive cycle with overlapping oocyte generations, each of which lasts more than 1 yr (Orejas et al. 2007). A gametogenic cycle of 18 mo, and possibly 2 yr, was also observed in Aimigmaptilon antarcticum, and has been seen in other deep-water anthozoans (Orejas et al. 2002). However, a different pattern was seen for 2 other deep-sea primnoid species (Fanyella rossii and F. spinosa) in which reproductive cycles are annual. Cordes et al. (2001) also observed continuous reproduction in the deep-sea brooding alcyonacean Anthomastus ritteri, inferred by the occurrence of gonads in all stages of development in the examined samples, as well as from the random temporal pattern of planulation in the laboratory. While often not possible to obtain, additional monthly samples, or even those from smaller sampling period intervals, would be necessary to produce a more robust estimate of oogenesis duration.

Polyps from different areas of the Anthoptilum murrayi colony bear gametes. In females, most of the oocytes were in the earliest stages of development and polyps from the basal area presented the highest fre-

<table>
<thead>
<tr>
<th>Species</th>
<th>Locality</th>
<th>Depth (m)</th>
<th>Sexual pattern</th>
<th>Spawning Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ptilosarcus guineyi</td>
<td>Alki Point, Seattle, USA</td>
<td>Shallow</td>
<td>Gonochoric</td>
<td>Late March</td>
</tr>
<tr>
<td>Kophobelemnon stelliferum</td>
<td>Porcupine Seabight, SW Ireland</td>
<td>350–1600</td>
<td>Gonochoric</td>
<td>No signs of seasonal reproduction</td>
</tr>
<tr>
<td>Umbellula lindahlii</td>
<td>Porcupine Seabight, SW Ireland</td>
<td>650–3850</td>
<td>Gonochoric</td>
<td>?</td>
</tr>
<tr>
<td>Pennatula aculeata</td>
<td>Gulf of Maine, USA</td>
<td>113–231</td>
<td>Gonochoric</td>
<td>Suggests continuous spawning</td>
</tr>
<tr>
<td>Renilla koellikeri</td>
<td>Southern California, USA</td>
<td>Subtidal</td>
<td>Gonochoric</td>
<td>May–July</td>
</tr>
<tr>
<td>Virgularia juncea</td>
<td>Chilton Bay, Taiwan</td>
<td>0.5–1.0</td>
<td>Gonochoric</td>
<td>August/ September?</td>
</tr>
<tr>
<td>Pennatula phosphorea</td>
<td>W Scotland</td>
<td>18.2–19.9</td>
<td>Gonochoric</td>
<td>July and/or August</td>
</tr>
<tr>
<td>Funiculina quadrangularis</td>
<td>W Scotland</td>
<td>18.9–24.3</td>
<td>Gonochoric</td>
<td>October–January</td>
</tr>
<tr>
<td>Anthoptilum murrayi</td>
<td>SW Atlantic, Brazil</td>
<td>1300–1799</td>
<td>Gonochoric</td>
<td>Continuous</td>
</tr>
</tbody>
</table>

Table 1. Sexual pattern and spawning timing of Pennatulacea (data arranged in chronological order of source)
frequency of very small cells (Fig. 4). The large number of early stage gametes has been reported in other octocorals, including the Pennatulacea (see Edwards & Moore 2008). According to Brazeau & Lasker (1989), the persistence of small oocytes throughout the year could indicate that oocytes require more than a single season to mature.

The duration of Stage III oocyte development of Anthoptilum murrayi is unknown as cells of this stage have a wide size range. As such, the presence of large, mature eggs does not necessarily indicate a spawning event is imminent. However, usually cyst development is faster and the spawning event is close when the tails of spermatozoa are present (Brazeau & Lasker 1989). The final stages of coral spermogenesis can proceed very rapidly (Harrison & Wallace 1990). Most (58%) cysts examined in samples of A. murrayi collected in August were Stage III, suggesting that the spawning event was close to this time, compared with 5% observed in June and none in February. There may be other periods of sperm release during the year, but data in the present study do not show this. A long reproductive cycle, with a probable seasonal spawning period, was also suggested to occur in Dasystinella acanthina and Thouarella sp. (Orejas et al. 2007).

Differences in fecundity among female colonies of Anthoptilum murrayi should be interpreted with caution, as the differences among the polyps suggested it would be necessary to sample more polyps for a more robust analysis. Nevertheless, although fecundity was roughly estimated, the results showed that the mean fecundity of female colonies was high (31 465 ± 5080 oocytes per colony). This figure may be an underestimate, since very small oocytes may have been overlooked in the dissected samples. Total fecundity for A. murrayi is high and comparable with fecundity estimates in other pennatulacean species, which have ranged from approximately 30 000 to 200 000 oocytes per colony (Edwards & Moore 2009). Frequency of large oocytes was low in all examined samples (Fig. 5), suggesting that only a small percentage of oocytes mature at a time. Relatively high total fecundity, but with a small proportion of developing oocytes attaining maximum size at a time, have been recorded in the sea pens Umbellula lindahlai and Funiculina quadragularis (Tyler et al. 1995, Edwards & Moore 2009).

The absence of embryos or planulae inside the gastric cavities of all examined specimens suggested a broadcast spawning mode of reproduction in Anthoptilum murrayi. To date there are no records of pennatulacean species that are adapted for brooding. It seems that male colonies of A. murrayi release intact cysts into the water, since they were seen above the pharynx level of the colonies close to the tentacle tips. Eckelbarger et al. (1998) described a similar occurrence for Pennatula aculeata and suggested that intact cysts could function as primitive spermatoophores, potentially reducing sperm dilution. High fecundity, large egg size (up to 1200 µm in histological preparations) that could represent larger targets for sperm (Levitan 1996), primitive spermatoophores (sensu Eckelbarger et al. 1998) and large numbers of polyps per colony (up to 655 in examined A. murrayi) are all strategies that could enhance the chance of fertilization (Levitan 1996). There is also evidence of large, aggregated populations of A. murrayi (authors’ unpubl. data), as has been observed with other sea pen species (Greathead et al. 2007). This would also increase the chance of fertilization in sessile gonochoric species with a 1:1 sex ratio. All these features may guarantee a successful reproductive strategy in A. murrayi.

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