

REPRODUCTIVE STATUS OF *OPISTHOTEUTHIS* SP. OVER AN ANNUAL CYCLE

H. I. DALY*, P. R. BOYLE* and M. A. COLLINS*

The reproductive biology of deepwater cephalopods has received relatively little attention. The cirrate octopus *Opisthoteuthis* sp. is a regular bycatch in deep benthic trawls from 800 to 1 200 m deep west of Scotland. Specimens were sampled throughout the seasonal cycle. Mature females occurred over a broad size range (600 – <3 000 g wet mass), suggesting considerable growth after maturity, with eggs released singly over a long period of time. There was no significant difference in the gonadosomatic index between spring, autumn and winter samples ($p > 0.05$), suggesting that there is no seasonal influence on the reproductive status of the population. The ovary of mature females contains eggs ranging in size from <1 mm to a maximum of 12 mm, and each egg is attached in the ovary by a short stalk. Mature females with mature eggs in the distal oviduct, after encapsulation by the single oviducal gland and ready for release, were recorded throughout the annual cycle, further suggesting that timing of spawning is not related to season.

Cephalopods of the shelf and coastal zone generally exhibit very short life cycles; a period of rapid growth is followed by a single spawning season. Recent work has shown that there is scope for considerable reproductive flexibility within that single season (Boyle *et al.* 1995), perhaps even ranging towards some definitions of iteroparity (Rodaniche 1984). Research has concentrated on the accessible neritic species, where light and nutritional factors may have a role in the onset of maturation (Rowe and Mangold 1975, O'Dor *et al.* 1977, Mangold 1987).

The deep sea at around 1 000 m deep is characterized by low light levels and constant low temperature, and it is a relatively stable environment compared with the fluctuations experienced in shallow coastal water. Orton (1920) was the first to hypothesize that species in such a deep environment would experience little effect of the seasonal cycle and may reproduce all year round, and this has been shown to be the case for species of many groups (Tyler 1988). However, there is also evidence for an annual cycle in detritus deposition to the deep sea (Lampitt 1985), and it has been shown that some deep-sea invertebrates may reproduce seasonally (e.g. Tyler *et al.* 1990, Bishop and Shalla 1994, Witte 1996). The reproductive strategy of deep-water cephalopods remains largely unknown and, given the relative absence of diurnal and seasonal cues, their spawning strategy and the timing of reproduction are of considerable interest, concerning conformity (or otherwise) to the cephalopod paradigm.

Opisthoteuthis is a widely distributed bathyenthic cirrate octopus, typically occurring at depths in excess of 800 m. A species of *Opisthoteuthis* is currently a

bycatch of deep-water trawling off the west coast of Scotland and Ireland by French vessels targeting grenadiers *Coryphaenoides rupestris* and black scabbardfish *Aphanopus carbo*. There is no market demand for cirrate octopods and they are normally discarded. However, opportunistic use of this bycatch has allowed samples to be obtained from all seasons, allowing a comparison of reproductive status over a complete annual cycle.

The specimens used in this study conform to the recently described *O. vossi* (Sánchez and Guerra 1989). However, Boyle *et al.* (1998) reported from a review of historic material by R. Villanueva (Instituto de Ciencias del Mar, Barcelona, pers. comm.) that the valid name for this species is probably *Opisthoteuthis grimaldii* (Joubin 1903).

MATERIAL AND METHODS

Samples of *Opisthoteuthis* from May 1995 until October 1996 were obtained from the port of Lochinver on the west coast of Scotland, where French trawlers land their catches. Trawls were fished on the bottom from 800 to 1 300 m and samples were grouped into season intervals (Table I).

Specimens were held on ice until landed at Lochinver, then sorted and bagged individually before being deep-frozen (-20°C) on return to Aberdeen. Loss of mass was considerable after freezing and each specimen was weighed in its bag after defrosting, so including any water which had leaked from them (*WTI*). Their sex was then determined and the complete reproductive

* Department of Zoology, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, Scotland. Email: h.i.daly@abdn.ac.uk

Table I: Summary of *Opisthoteuthis* sp. samples procured

Season and date landed	Number of specimens		
	Males	Females	Total
Spring			
24/03/96	15	6	21
12/04/96	11	4	15
Summer			
08/05/95–12/05/95	8	12	20
28/05/95–12/06/95	2	1	3
18/06/95–20/06/95	28	24	52
Autumn			
10/09/96–16/09/96	34	11	45
03/10/96	0	3	3
Winter			
31/12/95–04/01/96	13	30	43
29/01/96	44	9	53
Total sample	155	100	255

system dissected out, weighed and examined. The total body mass (*WTI*) was recorded without water loss, but because the complete gonad had to be dissected out prior to weighing, there may have been some water loss. A trial with 200 animals showed that a mean of 14.6% of *WTI* was lost as water after defrosting. It could not be determined if the water loss was equal from all tissues. All specimens received the same treatment to allow seasonal comparisons.

Females were divided into two categories, pre-breeding and breeding, using the presence of a mature encapsulated egg in the distal portion of the oviduct ready for release as the criterion for defining a breeding female. The genital complex, comprising the gonad, oviducal gland and ducts, was dissected whole and weighed to the nearest 0.1 g (*GCM*). The gonadosomatic index (*GSI*) was calculated using the *GCM* and the body mass (*WTI*) minus the *GCM*. Each ovary was opened before preservation in 10% phosphate-buffered formalin to allow eggs to separate for quantitative analysis. The total number of eggs in categories of >2 and <2 mm were counted, using a binocular microscope, in 32 females over a wide range of body size (9 from spring, 10 from autumn and 13 from winter).

RESULTS

The relationship between body mass and genital complex mass for all males and females in the whole sample (Fig. 1) is linear ($r^2 = 0.714$ for females and 0.839 for males). There is a striking difference in apparent investment in reproductive tissue between the

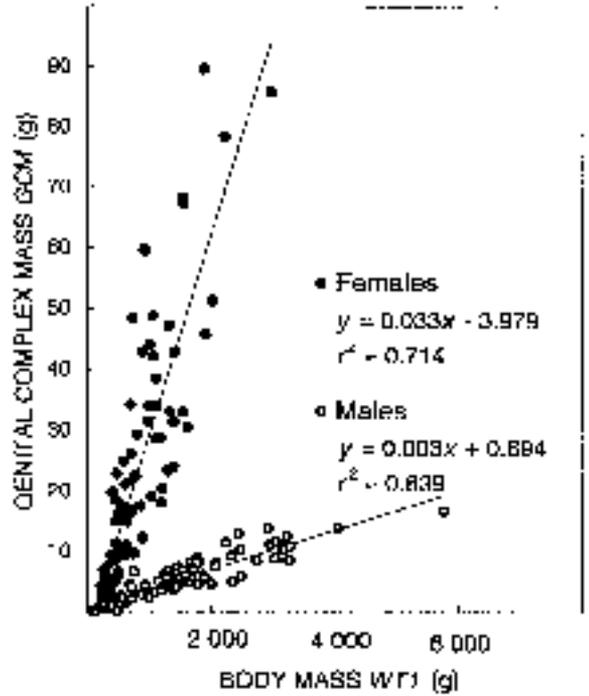


Fig. 1: Relationship between body mass and genital complex mass for *Opisthoteuthis* sp. males and females during all seasons

sexes, with relative gonad size approximately 10 times greater in females than in males of equivalent body size (slope = 0.03 for females and 0.003 for males). No males or females from any season were identified as “spent”.

The means and ranges of body mass and gonadosomatic indices for females from each season are shown in Table II. The *GSI* range and mean for the summer sample is underestimated because the ovaries were fixed in formalin before weighing, resulting in some shrinkage. There were no frozen ovaries available to allow a correction for the effect of fixation to be calculated, and consequently the summer sample was not included in further analysis. Although the *GSI*s for spring, autumn and winter may also be slightly underestimated, those seasons were given the same treatment and are comparable. The overall mean *GSI* for spring, autumn and winter was 2.88. Analysis of variance revealed no significant difference between the mean *GSI* in the spring, autumn and winter samples ($F = 2.32, p > 0.05$).

Breeding females were identified by the presence of a mature encapsulated egg in the distal oviduct. The

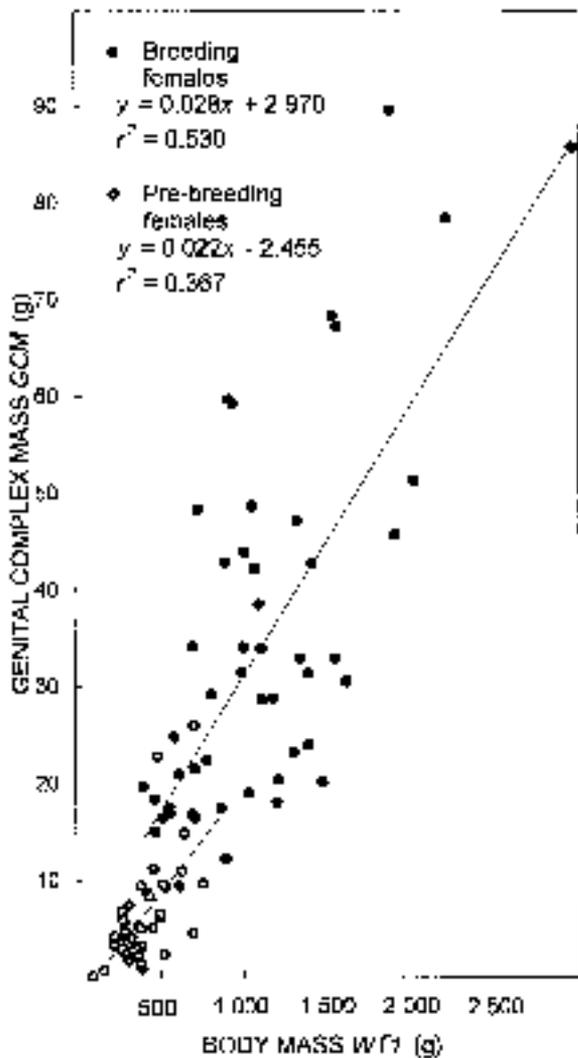


Fig. 2: Relationship between body mass and genital complex mass for breeding and pre-breeding *Opisthoteuthis* sp. females during all seasons

relationships between *WTI* and *GCM* in pre-breeding and breeding females (Fig. 2) show some overlap between the two groups. Owing to the method by which the reproductive state of females was determined, it is possible that some of the overlap between the breeding and pre-breeding groups can be explained by the erroneous identification of a breeding female between spawning events as pre-breeding. As all females >800 g body mass were breeding, any possible error in identification only applies to small females

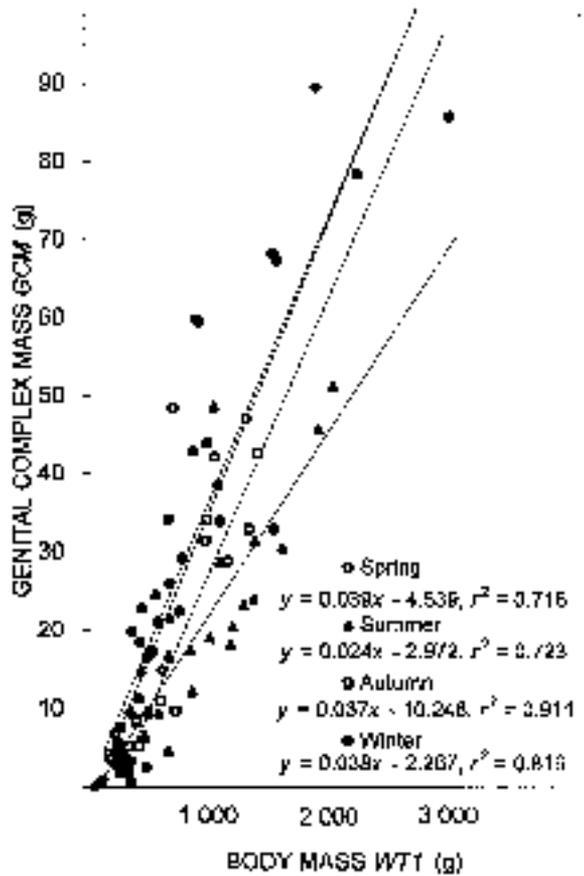


Fig. 3: Relationship between body mass and genital complex mass for *Opisthoteuthis* sp. females in spring, summer, autumn and winter

which had recently commenced spawning. Maturity is attained at a body mass of 500–800 g, i.e. the size at maturity is not defined precisely. The genital complex constituted a mean of 2.62% of the total body mass in mature females, with a range of 1.5–7%. Breeding females were observed during all four seasons and covered a broad size range, from around 500 g to almost 3 000 g.

The relationship between body mass and genital complex mass for females sorted by season is shown in Figure 3. The summer sample has a slightly shallower slope (0.024) than the other seasons, although this may be an artefact of the fixation of the specimens in this group. The reproductive systems in the summer sample were processed before the other samples and were preserved in formalin before they could be weighed, resulting in some shrinkage. The other three

Table II: Summary of means and ranges of body mass (*WTI*) and gonadosomatic index (*GSI*) for some of the *Opisthoteuthis* sp. females sampled in each season

Season	Mean <i>WTI</i>	<i>WTI</i> range	Mean <i>GSI</i>	<i>GSI</i> range
Spring (<i>n</i> = 10)	660	230–1 322	2.89	1.43–7.09
Summer (<i>n</i> = 28)	932	99–2 017	2.33	0.21–4.83
Autumn (<i>n</i> = 11)	742	362–1 412	1.97	0.84–3.53
Winter (<i>n</i> = 33)	769	169–2 959	2.99	0.48–6.97

seasonal samples, in which the genital complex was weighed immediately after thawing, have similar slopes in their relationships (0.036, 0.037 and 0.038 for spring, autumn and winter respectively), showing that the relationships between body mass and genital complex mass did not vary significantly by season. Analysis of covariance revealed no significant difference between the spring, autumn and winter relationships shown in Figure 3 ($F = 0.60, p > 0.05$).

Ovarian eggs reached a maximum length of 12 mm,

and in mature females, a full size range of eggs (<1 mm to mature 12 mm eggs) was always present in the distal oviduct. The eggs in the ovary were arranged on short strings around finger-like extensions of the epithelium of the internal ovary wall. The most mature eggs were usually found at the tip of each extension. Breeding females typically had a total of 1 000–2 000 eggs in the ovary (Table III), predominantly eggs <2 mm long.

Total numbers of ovarian eggs were expressed per gramme body mass to remove the effect of body size; the results are shown in Table III. The mean number of eggs per gramme body mass is 2.02 for spring, 1.78 for autumn and 1.51 for winter. Analysis of variance again revealed no significant differences among the three seasons ($F = 1.10, p > 0.05$).

DISCUSSION

The seasonal cycle of reproduction has been described for many incirrate octopods (Boyle and Knobloch

Table III: Body mass (*WTI*), maturity, genital complex mass, *GCM*, *GSI*, number of eggs and number of eggs per gramme body mass in a size range of *Opisthoteuthis* sp. females caught in spring, autumn and winter. There were no females available for egg counts from the summer-caught animals. Mature animals were identified by the presence of an encapsulated egg in the distal oviduct

Season	<i>WTI</i> (g)	Maturity	<i>GCM</i> (g)	<i>GSI</i>	Number of eggs	Number of eggs per gramme body mass
Spring	230	Immature	4.28	1.90	323	1.40
	233	Immature	3.44	1.50	244	1.05
	273	Immature	6.92	2.60	396	1.45
	376	Immature	5.30	1.43	1 125	2.99
	537	Mature	9.40	1.78	1 462	2.72
	650	Immature	14.90	2.35	2 097	3.23
	730	Mature	48.34	7.09	2 100	2.88
	1 069	Mature	42.20	4.11	1 839	1.72
	1 182	Mature	28.80	2.50	897	0.76
Autumn	362	Immature	3.02	0.84	901	2.49
	384	Immature	5.10	1.35	716	1.86
	391	Immature	3.31	0.85	1 020	2.61
	633	Immature	11.02	1.77	1 143	1.81
	640	Mature	25.14	4.09	1 429	2.23
	753	Immature	9.71	1.31	1 387	1.84
	991	Mature	31.44	3.28	1 064	1.07
	998	Mature	34.05	3.53	1 673	1.68
	1 220	Mature	40.56	3.44	1 638	1.34
	1 412	Mature	42.70	3.12	1 173	0.83
Winter	169	Immature	0.80	0.48	137	0.81
	280	Immature	6.01	2.19	754	2.69
	525	Immature	2.49	0.48	270	0.51
	559	Mature	17.56	3.24	1 347	2.41
	706	Immature	25.92	3.81	982	1.39
	808	Mature	29.20	3.75	1 505	1.86
	1 094	Mature	38.50	3.65	2 183	1.99
	1 533	Mature	68.30	4.66	1 717	1.12
	1 878	Mature	89.57	5.01	2 763	1.47
	2 959	Mature	85.78	2.99	2 459	0.83

1983, Forsythe and Hanlon 1988, Cortez *et al.* 1995), but studies of cirrate reproduction strongly suggest that cirrates breed continuously, i.e. with no seasonal preference (Mangold 1987, Villanueva 1992). The broad range in body size of mature female *Opisthoteuthis* sp. indicates considerable somatic growth while the females are breeding, a situation rarely seen among incirrate cephalopods, where females generally die shortly after spawning. Although incirrate cephalopods have a reputation for rapid growth (Forsythe and Van Heukelem 1987), many species in the deep sea have been shown to have slow rates of growth (Gage *et al.* 1986, Koslow 1996). As growth is closely related to temperature, deep-sea cirrates probably also grow slowly, so it can be hypothesized that they may have a lifespan of several years.

The *GSI* in *Opisthoteuthis* sp. is very low in comparison with that of other octopods, with an overall mean of 2.88% and a maximum of 7%. The *GSI* of incirrate octopods such as *Eledone cirrhosa* may reach almost 19% in the field (Boyle and Knobloch 1983) and <36% under aquarium conditions (Boyle and Knobloch 1984). The comparatively small *GSI* in *Opisthoteuthis* sp. provides further evidence for an alternative reproductive strategy, with the investment in reproduction spread over a lengthy period and eggs maturing a few at a time. However, the very gelatinous nature of cirrate octopod bodies will mean that reproductive investment may be undervalued in these terms. Only expression of dry gonad mass against dry mass of animal (or in energy terms) would allow their total investment in reproduction to be compared to that of incirrates.

The anatomy of the cirrate female reproductive system also differs from that of incirrates, with only the left oviduct and oviducal gland functional (Villanueva 1992). Mature cirrate eggs have toughened external coats which are secreted around them as they pass through the single oviducal gland. The broad size frequency of oocytes, from <1 mm in the ovary to 12 mm encapsulated eggs in the distal oviduct, is consistent with an interpretation of continuous production of eggs throughout the adult life. The eggs are released singly and are likely to give rise to completely benthic hatchlings (Boletzky 1987).

A noteworthy feature of *Opisthoteuthis* sp. reproduction is the formation of thin membranous follicles around maturing eggs. When the egg is mature the follicle splits open and releases it into the lumen of the ovary. From there it moves along the proximal oviduct, and through the oviducal gland. The "empty" follicles remain attached in the ovary and become shrivelled after the egg is released. Other data (Boyle and Daly in prep.) show that the number of follicles increases linearly with body size, which lends credence to a suggestion that follicles are not reabsorbed and that follicle number represents an index of the egg-laying

record over the female's lifetime. The egg follicles of *Opisthoteuthis* sp. and their value in estimating actual fecundity will be described in greater detail in a subsequent paper.

In contrast with incirrate cephalopods, the deep-sea cirrate *Opisthoteuthis* sp. appears to have adopted a non-seasonal reproductive strategy, with mature breeding females found throughout the year, and no significant differences between relative reproductive development or relative egg numbers between seasons.

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