Reproductive characteristics of the bathyal viviparous fish *Cataetyx alleni* (Osteichthyes: Bythitidae) from the southeastern Sardinian Sea (central-western Mediterranean)

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**SUMMARY:** The reproductive biology of the bathyal viviparous fish *Cataetyx alleni* was described based on 34 specimens (17 females and 17 males) caught during experimental trawl surveys carried out between 800 and 1700 m depth in the southeastern Sardinian Sea (central-western Mediterranean). Males and females were present at similar size intervals. For the first time, the internal fertilization was demonstrated by the finding of free spermatozoa scattered in the ovarian cavity. Based on macroscopic and histological gonad analysis, mature females were found in summer, autumn and winter, suggesting a long reproductive period. Inseminated females were observed only in November and January, in correspondence with the higher maturity of the males. These results suggest a probable reproductive peak with copulation in autumn-winter. Moreover, histological examination demonstrated that the ovaries of this member of the family Bythitidae had a ‘group-synchronous’ pattern. No spermatophores in males and embryos in females were observed in the histological sections analysed.

**Keywords:** *Cataetyx alleni*, reproduction, internal fertilization, Sardinian Sea.

**INTRODUCTION**

The reproduction of the more than 20000 species of teleostean fishes includes both oviparity and viviparity (Wourms, 1991). In the oviparity adopted by most species, males and females release sperm and eggs in the aquatic environment almost at once. On the other hand, viviparity has been found in approximately 510 species (Wourms, 1991). In these species, females copulate with males and eggs are fertilized internally with sperm. Therefore, embryos at various embryonic stages are usually found in the ovaries of collected fishes.
The Bythithoidei are viviparous, a style confirmed by the presence of an intromittent organ in males (Cohen and Nielsen, 1978). The brotulas of the family Bythitidae are small benthic fishes that inhabit shallow waters of the continental slope, with circumglobal distribution in tropical and temperate waters (Leis and Rennis, 2000). Viviparity is one of the adaptations which allows brotulas to occupy a wide range of habitats.

The family contains 107 species included in 37 genera (Nelson, 2006). All bythitids are livebearers (Wourms and Bayne, 1973; Goodwin et al., 2002) and release elongate larvae with a striated gut.

The genus Cataetyx has a worldwide distribution in tropical and temperate regions (Bañón, 2001) and contains 12 known species. It is represented in the Mediterranean Sea by two species, Cataetyx alleni (Byrne, 1906) and Cataetyx laticeps Koefoed, 1927 (Nielsen, 1986), which are closely associated with depths greater than 1000 m (Stefanescu et al., 1992).

Cataetyx alleni is benthic, usually found below 600 m and not commercially important. The species is distributed in the western basin [Catalan Sea between 984 and 1851 m (Matallanas, 1983; Stefanescu et al., 1992), Balearic Islands between 700 and 1600 m (Moranta et al., 1998, 2004; Morales-Nin, 2001), Ligurian Sea between 473 and 750 m (Relini Orsi, 1971, 1974; Relini et al., 1986) and Sardinian Channel between 800 and 1420 m (Follesa et al., 2005, 2010)], and in the eastern basin only in the southern Adriatic Sea between 1074 and 1196 m (Ungaro et al., 2001). Only a few specimens of C. alleni have been collected in the eastern North Atlantic (Porcupine Seabight) between 770 and 1205 m (Merrett et al., 1991).

As the reproductive biology of C. alleni has received little attention, the aim of our work was to provide further information on the reproductive characteristics (internal fertilization, maturity and spawning period) of this species caught in southeastern Sardinian deep waters (central-western Mediterranean) at depths of between 800 and 1700 m.

MATERIALS AND METHODS

A total of 34 specimens of C. alleni were caught during seasonal experimental trawl surveys carried out between 793 and 1701 m depth on compact mud bottoms off southeastern Sardinian deep waters (central-western Mediterranean) (Fig. 1). Table 1 shows the indication of the positive hauls by month, depth range (m) and number of specimens collected.

Table 1. – Indication of the positive hauls by month, depth range (m) and number of specimens collected of Cataetyx alleni.

<table>
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<tr>
<th>Haul</th>
<th>Month</th>
<th>depth range (m)</th>
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gonadal development. Transverse sections of 3.5 μm (glycol-methacrylate method) were stained with Harris hematoxylin and counterstained with eosin. Oocyte development stages were identified according to the scale proposed by Wallace and Selman (1981), whereas the development stages of the testicular germinal cells were identified based on the spermatogenic differentiation developed by Grier (1981), both with the use of an optic microscope (Laborlux 12) at original magnification x25-400. Oocyte size and number of a defined number of microscopic fields, obtained by taking the mean and maximum diameter, were only recorded from those oocytes which had been sectioned through the nucleus with the image analysis program, tpsDig2 (Rohlf, 2005).

RESULTS

The length sizes of *C. alleni*, showed a standard length (SL) ranging between 43 and 116 mm, with a mean±SD of 81±18.9 mm. Of the total of individuals caught (n=34), macroscopically 17 were females and 17 were males. Males and females were present at similar size intervals: the length ranged between 43 and 116 mm SL (mean 82±20.2 mm) and 64 and 106 mm SL (mean 86±13.4 mm) for males and females, respectively.

Males of *C. alleni* were characterized by a copulatory shield-shaped organ found after the anal opening, showing a flattened portion at rest on the ventral side,
whose posterior margin is invaginated like a glove finger (Fig. 2a).

Females showed no detectable sexual character (Fig. 2c). The gonads are saccular (Fig. 2d) and merge to the caudal tip and the connective tissue capsule surrounding them continues in a short and unequal oviduct.

The macroscopic and microscopic stages of female maturity were as follows:

Stage I, immature: the gonad (belonging to young females that have not taken part in reproduction) consists of two thin and whitish parallel filaments. Oogonia have a characteristic large nucleolus and abundant cytoplasm (size range 10-28 μm, mean±SD 15.3±10 μm), while primary oocytes (PO) have an abundant cytoplasm and several nucleoli (size range 17-83 μm, mean±SD 44.9±20 μm) (Fig. 3a).

Stage II, developing: the ovary appears light orange in colour with eggs not visible to the naked eye. Primary oocytes and cortical alveoli oocytes (CAO) with very small lipid droplets (size range 61-142 μm, mean±SD 85.4±27.4 μm) are present. The follicle layer is thick (Fig. 3b).

Stage III, maturing: orange gonads fill almost entire abdominal cavity; eggs are visible to the naked eye. The ovary tissue shows oocytes at different stages of vitellogenesis that are surrounded by an evident follicular layer. In early vitellogenic oocytes (EVO) size range 124-288 μm, mean±SD 226.1±45.5 μm) the yolk droplets are in the periphery of the cytoplasm; the nucleus is not round and a circular zone consisting of oil droplets of various size is localized around the nucleus. Late vitellogenic oocytes (LVO), size range 294-517 μm, mean±SD 358.8±57.3 μm) show continuous yolk accumulation until it obscures the cortical alveoli with more and larger yolk droplets occupying the whole cytoplasm. Their nucleus shows rounded nucleoli (Fig. 3c). The zona radiata of EVO and LVO is homogeneous and begins to regress in thickness.

Stage IV, mature: dark orange gonads that fill the entire abdominal cavity; individual orange eggs are distinguishable wrapped in a sturdy transparent tunica albuginea (Fig. 2d). A few primary oocytes and many mature eggs in advanced late vitellogenesis (ALVO) that reach maximum dimensions (size range 376-943 μm, mean±SD 702.7±138.6 μm) are visible. Complete fusion of yolk droplets does not occur at ALVO stage (Fig. 3d). The zona radiata of ALVO is very thin (Fig. 3e). Sperm are deposited in the oviducts and ovarian lumen, where they may be stored for a long time, being nourished by cells of the luminal epithelium (Fig. 4).

Stage V, spent: the gonad appears flaccid and reddish. The tissue is collapsed (Fig. 3f) and characterized by oocytes in PO and in reabsorption.

In mature females, at least two populations of oocytes were recognized at any one time, as shown in Figure 5: a fairly synchronous population of ALVO and a more heterogeneous population of smaller oocytes.

In males, the macroscopic and microscopic stages of maturity were the following:

Stage I, immature: small and translucent testes are made up of distinct lobes. Spermatogenesis has started, but testes lack a well-defined tubular system. Only spermatogonia (SPG) are present (Fig. 6a).

Stage II, developing: whitish testes. Spermatogenesis is generalized with all spermatogenic stages (Fig. 6b).

Stage III, maturing: white gonads fill almost the entire abdominal cavity. Spermatogenic cysts at all stages of development are present with spermatids (SPD) rod-shaped and stringy (filiform) spermatozoa (SPZ) that can be observed in all tubules (Fig. 6c).

Stage IV, mature: white gonads fill the entire abdominal cavity (Fig. 2b). Spermatic activity is intense. Greatly enlarged tubules are fully filled with spermatozoa (SPZ). Sometimes nests of spermatogonia on the walls of the tubules and spermatids are present (Fig. 6d).

Stage V, spent: testes are flaccid and short. Collapsed tubules are again full of spermatozoa. The spermatogenic activity is very limited, with residual sperm (Fig. 6e).

In immature females the SL was 64 to 68 mm, while in immature males it was 43 to 67 mm. The smallest
Table 2. – Number of females (F) and males (M) of *Cataetyx alleni* at immature (I), developing (II), maturing (III), mature (IV) and spent (V) stages for each sampled month. (+) indicates fertilized females.

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mature female and male had an SL of 77 and 83 mm, respectively, while the largest ones had an SL of 114 and 103 mm, respectively.

The maturing female specimens were detected in May, June, July and November, while the mature ones were found in June, September, November and January, with fertilized individuals only in the last two months. The maturing males were found in August and September, and the mature ones in November. One spent specimen with testes again full of spermatozoa in January was observed. The mature and spent males were found in the same period as the fertilized females (Table 2).

DISCUSSION

In the present paper we have reported reproductive data of the bythitid *Cataetyx alleni* for the southeastern Sardinian Sea (central-western Mediterranean). As reported by Relini Orsi and Relini (1973) for the Gulf of Genoa (Ligurian Sea), this species may be considered a common component of the deep population of the southeastern Sardinia deep waters, though it is not dominant (Follesa et al., 2010).

Total size distribution appeared similar to that of the other Mediterranean data. Our observed ranges of standard length (43-116 mm) were in agreement with Ligurian (57-122 mm, Relini Orsi, 1974) and Balearic data (30-110 mm, Morales-Nin, 1990). However, in the Adriatic Sea larger individuals (90-120 mm TL) than those caught in our area were observed (Ungaro et al., 2001).

The smallest size of mature specimens caught in southeastern Sardinian deep-waters (83 and 77 mm SL for males and females, respectively) was different from that found by Relini Orsi (1974), who stated that males became mature at smaller sizes (65-70 mm SL) and females at larger sizes (only one sampled mature female at 122 mm SL).

Until now the viviparous nature of *C. alleni* had been supported only by the existence of a copulatory organ in males. In this paper, for the first time, the internal fertilization has been demonstrated by the finding of spermatozoa in the ovarian cavity. The spermatozoa, filiform in shape, were found free only in females containing mature eggs, whereas maturing individuals have never shown free spermatozoa in the ovary.

In general, brotulid species are known to possess storage chambers for spermophores in which the sperm is preserved. Few viviparous brotulids of the genera *Calamopteryx*, *Oligopus*, *Microbrotula* and *Brosmophysis* possess permanent storage structures in the posterior portion of the testes. Nielsen et al. (1968) suggest that the development of these structures may be a device ensuring that the spermatozoa are kept alive in the female until the eggs mature. However, our studied species showed a testis type common to other teleosteans and did not have spermophores or similar structures. This aspect, also found in other brotulids such as *Calamopteryx goslinei* (Nielsen et al., 1968), was confirmed by Relini Orsi (1974), who also observed free spermatozoa in the penis of *C. alleni*.

The histological study of oogenesis showed some cellular characteristics typical of viviparous fishes (e.g. *Xiphophorus helleri*, Azevedo, 1974), such as the decrease in thickness of the zona radiata from maturing oocytes to mature ones that seemed to make possible intraovarian nutrition of the embryo. Another observed modification, associated with viviparity in fishes (Takemura et al., 1987) was the scarcity and small size of cortical alveoli. The role of the cortical alveoli in the strengthening of the chorion immediately after the fertilization of the eggs is known (Selman and Wallace, 1989; West, 1990). All these modifications are considered adaptations to the viviparous condition (Wourms, 1976).

Histological examination demonstrated that ovaries of *C. alleni* showed a ‘group-synchronous’ pattern, i.e. a mature female could exhibit at least two contemporaneous yet distinct populations of oocytes: one at the mature stage and a more heterogeneous one consisting of small immature oocytes (Wallace and Selman, 1981). The tendency for many eggs to ripen almost simultaneously is also typical among the brotulid forms (the viviparous genera *Oligopus* and *Dinematichthys* and oviparous *Dicrolene* and *Benthocometes*) (Mead et al., 1964).

Despite the paucity of the samples, the occurrence of mature females in summer (June and September), autumn (November) and winter (January) could suggest a long reproductive period. This result further emphasized the data on the Ligurian Sea reported by Relini Orsi (1974), who observed a maturation of gametes during the summer (June). A prolonged spawning is probably related to seasonal variability in food supply (Coggan et al., 1998) and increases the chances of meeting a mate in low-density populations and the probability of survival of offspring. However, the occurrence of inseminated females only in November and January, in correspondence with the highest maturity of males, suggests a probable reproductive peak with copulation in autumn-winter.

In the evolution of viviparity, there has been a tendency away from a dependency of the embryo on its own yolk reserves (lecithotrophy) to a greater reliance on nutrients supplied by the mother (matrotrophy). The congener *C. laticeps* was clearly classed as an “obligate lecithotrophic livebearer”. In lecithotrophy, a less evolved adaptation, little or no nutrient material crosses to the embryo from the maternal circulation and all, or nearly all, of the substrates for growth and metabolism are obtained from yolk reserves stored in the egg prior to fertilization (Grove and Wourms, 1991, 1994). As in oviparous fishes, in this condition yolk is the sole source of nourishment. In this study no embryos were observed in the histological section of the ovaries analysed. This lack of embryos does not allow us to conclusively establish the kind of dependency of embryos, but the deep-sea species *C. alleni* could probably also be classified as its congener.
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