In situ observation of sexual reproduction of Holothuria tubulosa GMELIN, 1788 (Echinodermata: Holothuroidea) in the Azores (NE Atlantic)

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The aspidochirote *Holothuria tubulosa* Gmelin, 1788 is widely distributed in the Mediterranean Sea and in the Atlantic from Gibraltar to the Bay of Biscay (Tortonese 1965). It lives on rocky substrata, soft sediments and phanaerogam seagrass beds, in depths that vary between 5 m and 100 m, being one of the predominant species of the benthic macrofauna in the *Posidonia oceanica* meadows (Boudouresque and Meinesz 1982).

*H. tubulosa* is one of the four species (*H. sanctori* Delle Chiaje, 1823; *H. forskali* Chiaje, 1841; *Eostichopus regalis* (Cuvier, 1817)) of sea cucumbers in the Azores. Although *H. forskali* Chiaje, 1841, is considered to occur in the area, systematic studies are urgently needed for confirmation.

Since it plays a central role in recycling bottom detritus (Massin 1982; Bulteel et al. 1992), *H. tubulosa* would thus be particularly interesting to complement the set of bioindicators for surveying metal contamination in ecosystems (e.g. the *P. oceanica* meadows) (Warnau et al. 2006).

H. tubulosa, as most of Holothuroidea species, has separate sexes, albeit with no sexual dimorphism, and fertilization is external. It has an annual reproductive pattern, in which different phases of gonadal development are differentiated: reabsorption of the gonad after the post-spawning period; gonad recovery stage; growing stage; maturity stage, spawning stage and post-spawning stage (Despalatovic et al. 2004).

In the Mediterranean, and specifically in the case of *H. tubulosa*, the spawning phenomenon has been observed before in the Adriatic Sea (Despalatovic et al. 2004), on the Spanish Mediterranean coast of Costa Brava (Valls 2004), in the Alboran Sea (Ocaña and Tocino 2005) and the Aegean Sea (peninsula of Chalkidike) as reported by Moosleitner (2006) on the years of 1972, 1994, 1997 and 2003.

In the Azores, *H. tubulosa* spawning occurs in the summer months (so far observed in July and August) during the afternoon, which may follow the annual pattern, during warm sea temperatures (22-26°C) and being synchronous in both sexes, as stated by Despalotovic et al. (2004) in the Adriatic Sea.

Spawning of *H. tubulosa* in the Azores was first recorded by FADC on 16<sup>th</sup> August 1996, at Monte da Guia (Faial Island - Azores), close to Ilhéu Negro (Porto Pim beach) (38°52.29'N

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28°62.90'W) at a depth of 19 m, but due to the low water visibility photo records were compromised.

The second record (AAB and LFM on the  $26^{th}$  July 2007 - three days before full moon -) occurred in the southern coast of Terceira Island (Azores) at Salgueiros rocky-shore ( $38^{\circ}64.85^{\circ}N$  27°09.68°W). Tide was low (0.6 m), water temperature 22°C, depths ranged from 2 to 4 m and bottom was composed of sand areas with sparse boulders. Observation time was during day light from 18:30-19:30, solar time.

For Salgueiros site, spawning was already occurring by the start of observations (18:30), where numerous individuals (>50) were visibly active and by the end of it (19:30) some individuals were already recovering from their spawning vertical position.

Photographic records of the spawning are presented in Figure 1 (male posing vertically on the rock surface, raising half of its body and releasing sperm); Figure 2 (female posing vertically on the rock surface and releasing eggs); Figure 3 (close-up at the sperm release); Figure 4 (close-up at the egg release).

During this 1 hour observation, it was noticed that not only males were in a higher density, but sperm release (Figures 1 and 3) was more constant in short intervals varying from 1 to 3 minutes. On the other hand, a single observed female released eggs (Figure 2 and 4) in intervals of 10 minutes, during 30 minutes observation.

As proposed for other species (see Ocaña and Tocino 2005; Moosleitner 2006), some males begin the spawning event, and their sperm includes informative substances that push other males and females to participate in the spawning. Posing with half (or up to two thirds) of the body vertically provides a maximum dispersion of the gametes in the environment.

A relationship between spawning events and moon phases is not clearly established in the data collected but there seems to be a preference for spawning close to a full moon as already stated by other authors (Despalotovic et al. 2004; Moosleitner 2006).

No predation on the reproductive material was observed. Although *Coris julis* (Linnaeus, 1758) is a common and abundant species in the Azores, no fish were observed feeding on the spawning event, as Moosleitner (2006) did in the Aegean Sea.

## Acknowledgements

Authors would like to thank Fernando Tempera, Jorge Fontes, Michel Warnau, Chantal de Ridder and Claude Massin for their valuable help. AAB benefits from a Brazilian PhD. Grant (CNPq 210231/2006-8).

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Figure 1: Male posing vertically on the rock surface, raising half of its body and releasing sperm.



Figure 2: Female posing vertically on the rock surface and releasing eggs.



Figure 3: Male detail of sperm release.



Figure 4: Female detail of egg release.