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PROOF RESEARCH PAPER

First Evidence of Artificial Fission in two Mediterranean Species of Holothurians: *Holothuria tubulosa* and *Holothuria polii*.

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Abstract

Holothurians, or sea cucumbers, are a common group of echinoderms occurring in most benthic marine habitats in temperate and tropical oceans. Sea cucumbers, already known as bioactive compounds producers, are increasingly seen as a new source of food worldwide. Actions to foster a sustainable development of the resource they represent are increasingly encouraged. Fission is a way of asexual reproduction reported in these organisms. This natural ability to split their body into two parts is generally shown by various tropical species of holothurians, but it has been never reported for Mediterranean species from Italian waters. Inducing fission in order to double individual numbers would be a promising option for culture and restocking purposes. The present study reports the first evidence of transverse induced fission, after experiments of fission stimulation, in two edible holothurian species from the Tyrrhenian Sea (Italy). Survival and regeneration times after being forced to undergo transverse fission are reported.

Keywords: Echinoderms, induced fission, regeneration, sea cucumber, commercial important species, sustainable exploitation.

Introduction

The sea cucumber or holothurians are belongs to the class Holothuroidea, a common and diverse group of worm-like and usually soft-bodied echinoderm. Ecologically, these important deposit feeders are a key component in many littoral ecosystems playing an important functional role as bioturbators, in recycling nutrients and mixing the upper sediment layers redistributing food resources (Coulon and Jangoux 1993; Mac Tavish, Stenton-Dozey, Vopel, & Savage, 2012). Sea cucumbers are increasingly seen as a new source of food worldwide and they are also harvested to provide bio-extracts for pharmaceutical, nutraceutical, and cosmetic products (Bordbar, Anwar, & Saari, 2011; Purcell, 2014). As a consequence, holothurian stocks have been overfished in many countries as a result of ever-increasing market demand, uncontrolled exploitation and/or inadequate fisheries management (Conand, 2004). This is particularly true in tropical regions (Indo-Pacific). In the Mediterranean, sea cucumbers are a marine resource with a very low exploitation, except in Turkey where holothurian species (*Holothuria spp.)* are already commercially exploited (González-Wangüemert , Aydin, & Conand, 2014 and ref. therein). More recently, in Italy, too, commercial fishermen have launched small-scale collections and transformations of sea cucumbers for export to Asiatic markets (Sicuro and Levine, 2011). In view of the growing economic importance of these organisms, it is necessary to undertake actions for the sustainable development of the resource they constitute (such as restocking strategies and farming projects), to counteract and/or prevent the damage of over-exploitation and the inevitable negative effects on the marine ecosystem at the local level. Some holothurians have long been known for their ability to reproduce asexually by fission (fissiparity), which brings about posterior and anterior parts through self-division (Conand, 1996; Crozier, 1917). After fission, anterior part of body complete with mouth and tentacles, as well as the posterior part with anus, regenerate lacking internal organs, such as intestine and respiratory tree, becoming new individuals (Dolmatov, 2014 and ref. therein). Because of the high commercial value of holothurians, researchers attempt to use their regenerative property and fission ability to develop cultivation methods and increase natural populations. In the last two decades literature have been enriched with data on the capacity of holothurians of fission, both naturally (asexual reproduction by fission) and induced (artificial fission) (Reichenbach and Holloway, 1995; Purwati and Dwiono, 2005; Laxminarayana, 2006; Purwati and Dwiono, 2007; Razek, Rahman, Mona, El-Gamal, & Moussa, 2007; Dolmatov, 2014; Hartati, Widianingsih, & Endrawati, 2016). Fission is of particular importance for commercially important species that are exposed to widespread exploitation of fisheries (Dolmatov, 2014). Inducing fission in order to double individual numbers would be a promising alternative for holothurians re-population, as formerly suggested by Lokani, Polon, & Lary (1996). *Holothuria tubulosa* and *H. polii* are among the most common sea cucumbers species in Mediterranean Sea (Tortonese, 1965), and are considered as a suitable seafood for human consumption (Aydin, Hüseyin, Bekir, Yilmaz, & Sevim, 2011; Sicuro *et al.*, 2012). This paper shows for the first time the fission capability of *H. tubulosa* and *H. polii*, which should represent a potential for multiplying individuals to be used for aquaculture and restocking strategies, strengthening the sustainable exploitation prospect of these species.

Materials and Methods

Transverse induced fission was being carried out on 10 specimens of *Holothuria tubulosa* (body weight 132,9 g ± 33,3 - mean ± SD) and 10 specimens of *Holothuria polii* (body weight 82,9 g ± 23,8 - mean ± SD) collected by scuba-diving at 5-10 meters depht, from a rocky site of the Gulf of Naples (40°47’50’’N and 14°12’04’’E), along the southern Tyrrhenian coasts of Italy in September 2016. The sea cucumber were collected from locations that are not privately-owned nor protected in any way, in compliance with the regulations of the Merchant Navy, now called the Ministry of Agriculture, Food and Forestry (DPR 1639/68, 09/19/1980 confirmed on 01/10/2000). On their arrival in the laboratory, the sea cucumbers were kept in a tank with running sea water (open system) and acclimatized for 1 week to confined rearing conditions, before starting the fission experiment.

A peristaltic silicone tube of 3 mm in diameter and an electrical cable tie 2.6 mm in width were being used to tighten up the animal’s body to the middle portion of the body. Individuals were then placed into suspended basket over the first stage of experimentation pending the fixation. No food was added. When the fission occurred and wounds were healed, the anterior (A) and posterior (P) portions of sea cucumbers were transferred to a floating basket with a sand layer about 3 cm thick on the bottom. Daily observation documented all regenerating phases. When a new anal or mouth aperture was observed, respectively in the A and P, and the presence of fecal strings was detected, the functional recovery was considered completed. By that time, feeding was necessary and rearing techniques had to be changed. New generated sea cucumbers were being moved to the bottom of a bigger tank (500 L) with a sand layer of substrate 5 cm thick. This tank was part of a semi-closed system sustained by a centralized Life Support System (LSS) to maintaining optimal sea water conditions. This consisted of a reservoir equipped with cartridge filter, protein skimmer, ultraviolet sterilizer and refrigerator; a centrifugal pump recirculated natural seawater at a rate of 7.5 L min^{-1} to each tank. Aeration in the tanks provided additional water movement and air supply. Dissolved oxygen (> 90% saturation), pH (8.0 ± 0.1), and salinity (38.0 ± 0.2‰) were measured 3 times a week by a multi-parameter probe (YSI-85, USA). Seawater temperature was maintained at 19 °C ± 1 and the photoperiod was set for 12 light : 12 dark. Ammonia, nitrite, nitrate and phosphate concentration were checked every week by a spectrophotometer (HACH USA, DR/2500) and values matches parameters required for a healthy recirculation system (Huguenin and Colt, 2002). Food items (commercial sea food powder) were being added to the bottom once a week for the enrichment of the substrate.

Results and Discussions

The main events of fission process and regeneration under experimental stimulation are shown in Figure 1. The entire process was divided into three consecutive phases, on the basis of observations on the external morphology of the holothurians: the fission, that is the stimulation and the following division of the body; the regeneration, covering the period of the restoration of tissues, wound healing and regeneration of lacking internal organs; the functional recovery, which marks the end of the fission process and was considered the starting point of the new holothurians’ life cycle (see also Table 1). Initially, some trials of stimulation were being performed using two tools to tighten the animal’s body, peristaltic silicone tubes (2 mm in diameter) and nylon cables ties (2,6 mm in width), in order to assessing the more suitable technic to be used. Narrow cable ties resulted more suited to our purpose and were then used for the trials following. Basically, the cable tie, although made of a more rigid material with respect to the silicon tube, enabled us to tighten firmly the body of the holothurians (Figure 1a). Moreover, they cover a very narrow surface of the body at the clamping level. The final result was the separation into the two portions A and P with very small wounds (Figure 1b, c). The bindings with silicon tube, on the contrary, tended to slacken more easily and forced us to intervene at later time points to tighten them. This caused further manipulation, likely causing stress and extending the fission time. Accordingly, almost all fission stimulations were performed using the cable ties. Clear signs of regeneration that were morphologically visible after the fission were the emergence of a depigmented area of dermis and then a protrusion of the fission plane, as shown in holothurians in Figure 1d and 1e. Functional

Figure 1. Main morphological events of fission process and regeneration under experimental stimulation in *H. tubulosa* and *H. polii*. Tying with cable ties (a; *H. polii*), and result of fission showing the wound closure (b; *H.polii*) and the separation into the two portions A and P (c; *H. tubulosa*). Protrusion structure on fission plane in P individuals of *H. tubulosa* (d; e) and tentacles and mouth develop in P individual of *H. polii* (f). New anal pore and fecal strings in A individuals of *H. tubulosa* (g). Complete morphological recovery, with the regeneration of the papillae and the restoration of coloration and natural size in P individual of *H. polii*. (h).

recovery was considered completed when a new mouth or anal aperture was observed, respectively in the P and A, and lastly the presence of fecal strings was detected (Figure 1f, g). The complete morphological recovery, with the regeneration of the papillae and the restoration of coloration and natural size, requires a longer time that was estimated to be not less than 3 months, after which regenerated portions become almost indistinguishable (Figure 1h).

Table 1 shows the time needed for sea cucumbers to complete the fission and regeneration period of part A and P. Survival rate (%) are also shown. In our trials, the separation occurred after at least 3 days after ligatures and after a maximum of 9 days for *H. tubulosa* and 11 days for *H. polii*. Body twisting and stretching, which usually occurs in specimens undergoing natural fission (Uthicke, 2001), were never observed in induced individuals. The minimum regeneration time of new functional structures (mouth and anus) was 45 days, observed in *H. tubulosa* for the functional regeneration of the anus (part A); the maximum time of 70 days was observed for the functional recovery of the same region in *H. polii* (see table 1). The survival rate of new holothurians was 85% in *H. tubulosa* and 75% in *H. polii*. A slight discrepancy between the survival rates of the A and P parts was, however, observed and in survivorship between the two species *H. tubulosa* and *H. polii*. Failures occurred during the first days (3-5) after completed fission. Table 1 shows the percentage

Table 1. Time needed for experimental fission and regeneration of *H.tubulosa* and *H. polii*

Species (n°individuals)	New generated portions	Fission period (days) min-average-max	Functional recovery (days) min-average-max	Survivors%	New holothurians
	anterior (A) : 10		45 – 52.5 - 60	A : 80	
H. tubulosa (10)		3 – 6.5 - 9			17
	posterior (P) :10		50 – 55 - 60	P : 90	
	anterior (A) : 10		50 – 60 - 70	A : 70	
H. polii (10)		3 – 7.5 - 11			15
	posterior (P) : 10		47 – 56 - 65	P : 80	

of survival after the fission for both species, from which the rates of failure, that is the unexpected death, are deduced. Failure rate was 15% in *H. tubulosa* (20% for the anterior parts and 10% for the posterior ones) and 25% in *H. polii* (30% for the A parts and 20% for the P parts). In all cases of failure, a disease affecting the skin was observed. A whitish patch close to the constriction zone has been noted as first sign of the following integument degeneration. The affected zone extended progressively on the whole body leading individuals to the death in 3-6 days, and was associated with the presence of generous mucus production. This disease is similar to the "skin ulceration disease" frequently affecting integument of cultivated holothurians and resulting from a severe bacterial infection that causes death within few days (Becker, Gillan, Lanterbecq, Jangoux, Rasolofonirina, Rakotovao & Eeckhaut, 2004). Evisceration was frequently observed (50% in *H. tubulosa* and 80% in *H. polii*), some days after ligatures. It usually occurred close to the fission and apparently did not alter the course of the process. In general, comparing the results for the two species, *H. polii* showed a higher time of regeneration, a bigger sensitivity to manipulation (as greater tendency to evisceration) and a slight difference in the percentage of survival. This would suggest that this latter species appeared less resistant to the handling related to the stimulation of induced fission with respect to *H. tubulosa*. Further in-depth analysis are nevertheless needed to confirm these observations and to establish the optimal procedure to be applied for also assessing which species could be more feasible for commercial exploitation.

Conclusions

Sea cucumber fishery is not currently an active industry in the Mediterranean. However, edible species found in the Mediterranean could be the basis for a future sea cucumber farming industry. The growing sea cucumber fisheries has already created new market for temperate species and body organs, and in some cases fishing has been directly encouraged by Asian traders in search of new species and fishing areas. Industrialized fisheries is well established in Turkeys and has more recently emerged in Spain, Greece and other parts of the Mediterranean, included Italy, where small-scale fisheries are emerging (see also Purcell, Mercier, Conand, Hamel, Toral-Granda, Lovatelly, & Uthicke, 2011 and ref. therein). In particular, in Italy they are nevertheless difficult to evaluate and often not reported because involve more frequently fishermen rather than industrialized fisheries, or refer to pilot scale plants that are not yet operational for real commercial exploitation. This first experimental evidence of induced artificial fission can potentially be also applied to Mediterranean holothurians in order to increase individual numbers. It could be well considered a low cost tool for the sustainable stock management, both for aquaculture and for restocking.

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References

Aydin, M., Hüseyin, S., Bekir, T., Yilmaz, E., & Sevim, K. (2011). Proximate composition and fatty acid profile of three different fresh and dried commercial sea cucumbers from Turkey. *International Journal of Food Science and Technology*, 46, 500-508.

Becker, P., Gillan, D., Lanterbecq, D., Jangoux, M., Rasolofonirina, R., Rakotovao, J., & Eeckhaut, I. (2004). The skin ulceration disease in cultivated juveniles of *Holothuria scabra* (Holothuroidea, Echinodermata). *Aquaculture*, 242, 13 - 30.

Bordbar, S., Anwar, F., & Saari, N. (2011). High-Value Components and Bioactives from Sea Cucumbers for Functional Foods - A Review. *Marine Drugs*, 9, 1761-1805. https://dx.doi.org/10.3390/md9101761.

Conand, C. (1996). Asexual reproduction by fission in *Holothuria atra:* variability of some parameters in populations from the tropical Indo-Pacific. *Oceanologia Acta*, 19(3-4), 209-216.

Conand, C. (2004). Present status of world sea cucumber resources and utilisation: an international overview. In: Lovatelli, A., Conand, C., Purcell, S., Uthicke, S., Hamel, J.F., Mercier, A. (Eds.), *Advances in Sea Cucumber Aquaculture and Management*. FAO, Rome, pp. 13 - 24. FAO Fisheries Technical Paper, No. 463.

Coulon, P., & Jangoux, M. (1993). Feeding rate and sediment reworking by the holothuroid *Holothuria tubulosa* (Echinodermata) in a Mediterranean seagrass bed off Ischia Island, Italy. *Marine Ecology Progress Series*, 92, 201-204.

Crozier, W.J. (1917). Multiplication by fission in Holothurians. *The American Naturalist*, 51 (609), 560-566.

Dolmatov, I. Y. (2014). Asexual Reproduction in Holothurians. *The Scientific World Journal,* 2014, 13 pages. https://dx.doi.org/10.1155/2014/527234.

González-Wangüemert, M., Aydin, M., & Conand, C. (2014). Assessment of sea cucumber populations from the Aegean Sea (Turkey): First insights to sustainable management of new fisheries. *Ocean & Coastal Management*, 92, 87-94.

Hartati, R., Widianingsih, & Endrawati, H. (2016). The Growth of Sea cucumber *Stichopus herrmanni* After Transverse Induced Fission in Two and Three Fission Plane. *ILMU KELAUTAN*, 21 (2), 93-100.

Huguenin, J.E., & Colt, J. (2002). Design and Operating Guide for Aquaculture Seawater Systems. Amsterdam, The Netherlands, Elsevier Science Publishers, 264pp.

Laxminarayana, A. (2006). Asexual reproduction by induced transverse fission in the sea cucumbers *Bohadschia marmorata* and *Holothuria atra*. *SPC Beche-de-Mer Information Bulletin*, 2, 35-37.

Lokani, P., Polon, P., & Lary, R. (1996). Management of beche-de-mer fisheries in the Western Province of Papua New Guinea. *SPC Beche-de-mer Information Bulletin,* 8, 7-11.

MacTavish, T., Stenton-Dozey, J., Vopel, K., & Savage, C. (2012). Deposit-Feeding Sea Cucumbers Enhance Mineralization and Nutrient Cycling in Organically Enriched Coastal Sediments. *PLoS ONE* 7(11), e50031.
https://dx.doi.org/10.1371/journal.pone.0050031.

Purcell, S.W., Mercier, A., Conand, S., Hamel, J.F., Toral-Granda, M.V., Lovatelly, A. & Uthicke, S. (2011). Sea cucumber fisheries: global analysis of stocks, management measures and drivers of overfishing. *Fish and Fisheries*, 14, 34-59.

Purcell, S.W. (2014). Value, Market Preferences and Trade of Beche-De-Mer from Pacific Island Sea Cucumbers. *PLoS ONE* 9(4), e95075.
https://dx.doi.org/10.1371/journal.pone.0095075.

Purwati, P., & Dwiono, S. A. P. (2005). Fission inducement in Indonesian holothurians. *SPC Beche-de-mer Information Bulletin*, 22, 11-13.

Purwati, P., & Dwiono, S. A. P. (2007). Experiment on fission stimulation of *Holothuria atra* (Holothuroidea, Echinodemata): Changing in Body Weight and Morphology. *Marine Research Indonesia*, 32(1), 1-6.

Razek, F.A., Rahman, S.A., Mona, M.H., El-Gamal, M.M., & Moussa, R.M. (2007). An observation on the effect of environmental conditions on induced fission of the Mediterranean sand sea cucumber, *Holothuria arenicola* (Semper, 1868) in Egypt. *SPC Beche-de-Mer Information Bulletin*, 24, 45-48.

Reichenbach, N., & Holloway, S. (1995). Potential for asexual propagation of several commercially important species of tropical sea cucumber (Echinodermata). *J. World Aquacult. Soc*., 26(3), 272278.

Sicuro, B., & Levine, J. (2011). Sea Cucumber in the Mediterranean: A Potential Species for Aquaculture in the Mediterranean. *Reviews in Fisheries Science*, 19 (3), 299-304.
https://dx.doi.org/10.1080/10641262.2011.598249.

Sicuro, B., Piccinno, M., Gai, F., Abete, M.C., Danieli, A., Dapra, F., & Vilella, S. (2012). Food Quality and Safety of Mediterranean Sea Cucumbers *Holothuria tubulosa* and *Holothuria polii* in Southern Adriatic Sea. *Asian Journal of Annual and Veterinary Advances*, 7 (9), 851-859.
https://dx.doi.org/103923/ajava.2012.851.859.

Tortonese, E. (1965). Fauna d'Italia, Echinodermata. Ed. Calderini, Bologna, pp. 419.

Uthicke, S. (2001). The process of asexual reproduction by transverse fission in *Stichopus chloronotus* (greenfish). *SPC Beche-de-Mer Information Bulletin*, 14, 23-25.