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Arm regeneration in two populations of *Ophionereis reticulata* (Echinodermata, Ophiuroidea)

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ABSTRACT. This study compared the arm regeneration frequencies in two different populations of *Ophionereis reticulata* (Say, 1825) in São Sebastião, Southeast Brazil and observed arm regeneration between age classes (juvenile and adults) and sexes (male and female). From the 1,170 individuals sampled, 1,089 (92.2%) showed signs of arm regeneration. The relative frequencies of regenerating arms in the two areas were not different (Baleeiro Isthmus: 91.3% and Grande Beach: 99.5%). Both areas also presented similar values for the number of arms regenerating/individual and in the frequency of regenerating individuals. The major part of the regenerating scars was concentrated in the distal portion of the arm. Sub-lethal predation is most likely the cause to the high rates of arm regeneration in *O. reticulata*. There was no significant differences in the regeneration rates between females (3.57 ± 1.36 arms regenerating/individual) and males (3.47 ± 1.42).

KEYWORDS. Ophiuroid, arm regeneration, sub-lethal predation, Brazil.

RESUMO. Regeneração dos braços em duas populações de *Ophionereis reticulata* (Echinodermata, Ophiuroidea). Este estudo comparou as frequências de regeneração dos braços de duas populações de *Ophionereis reticulata* (Say, 1825), de São Sebastião, sudeste do Brasil. Além disso, foram observadas diferenças nas frequências de regeneração entre classes etárias (juvenis e adultos) e entre machos e fêmeas. Dos 1.170 indivíduos coletados, 1.089 (92,2%) mostraram sinais de regeneração nos braços. A frequência relativa de braços em regeneração entre as duas áreas não diferiu significativamente (Istmo do Baleeiro: 91,3% e Praia Grande: 99,5%). Ambas as áreas apresentaram valores semelhantes no número de braços regenerando/indivíduo e na frequência de indivíduos em regeneração. A maior parte das marcas de regeneração concentraram-se na porção distal dos braços, o que indica a influência de predação subletal como a causa principal da regeneração em *O. reticulata*. Além disso, não houve diferença significativa nos índices de regeneração entre fêmeas (3,57 ± 1,36 braços regenerando/indivíduo) e machos (3,47 ± 1,42).

PALAVRAS-CHAVE. Ofiuróide, regeneração braquial, predação subletal, Brasil.

A clearly advantage of being able to lose a structure is to escape lethal or sub-lethal predation. Regeneration acts to restore the functionality of the lost structure, however, it requires the allocation of energy and matter (Pomory & Lawrence, 1999). Echinoderms are well-known for their propensity to lose body parts as a result of predation, accidental injury or asexual reproduction (Emson & Wilkie, 1980; Fielman *et al.*, 1991). Ophiuroids undergo tissue loss frequently and burrowing brittle-stars present high rates of sub-lethal arm loss to numerous cropping predators, including penaeid shrimp, decapod crustaceans, pleuronectids, bothid flatfishes, and other echinoderms (Buchanan, 1964; Emson & Wilkie, 1980; Bowmer & Keegan, 1983; Duineveld & Van Noort, 1986; Fielman *et al.*, 1991; Bourgoin & Guillou, 1994; Stewart, 1996). Arm regeneration in populations of ophiuroids has been examined by numerous researches (Singletary, 1980; Duineveld & Van Noort, 1986; O'Connor *et al.*, 1983; Munday, 1993; Stewart, 1996; Morgan & Jangoux, 2004), which observed high rates inside the populations and that regeneration scars frequently occur in the distal portion of the arms.

There are no regeneration studies about the family Ophionereididae. Works were focused in the reproductive cycle (Byrne, 1991; Selvakumaraswamy & Byrne, 1995; Yokoyama & Amaral, 2008a), larval development (Medeiros-Bergen & Ebert, 1995; Selvakumaraswamy & Byrne, 2000a,b), physiological aspects (Davis *et al.*, 1985), feeding diet (Yokoyama & Amaral, 2008b), and biological interactions (Rodrigues & Shimizu, 1988). Besides that, the regeneration rates of ophiuroid populations from the South Hemisphere are still unknown and comparisons are difficult to access.

Ophionereis reticulata (Say, 1825) is a common Ophionereididae from Florida (USA), Caribbean Sea and Brazil, occurs in the rock-sediment interface, and usually has arm regeneration scars. Its habitat has potential echinoderm predators such as decapods crustaceans and bothid flatfishes. However, no study assessed the arm regeneration of *O. reticulata* or even the influence of predators and abiotic factors. Therefore, the aim of this work was to compare the arm regeneration frequencies of two different *O. reticulata* populations and the incidence of regeneration between sexes.

MATERIAL AND METHODS

Individuals of *Ophionereis reticulata* were sampled in the Baleeiro Isthmus (23°49'43''S, 45°25'24''W) and in the Grande Beach (23°49'25''S, 45°25'02''W), both located in São Sebastião, north coast of the state of São Paulo, Brazil. The Baleeiro Isthmus (BI), located in the Segredo Beach, has a small sand region mostly covered by rocky boulders of different sizes that

protect the beach from wave action. In contrast, the Grande Beach (GB) has a more exposed area with different inclination degrees to wave action and is composed by small and medium rocky boulders, usually oval or circular shaped. This intertidal region extends itself for approximately 40 m and the zone of occurrence of *O. reticulata*, in both areas, is only exposed during low tide periods.

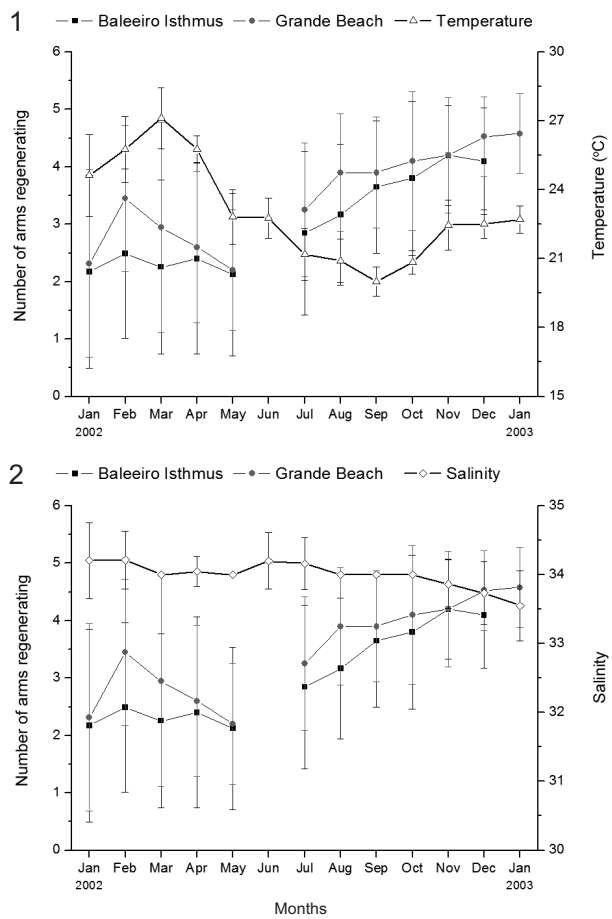
Monthly samples were conducted from January 2002 to January 2003, always under low tide conditions. To avoid sampling the same specimen over the months, the individuals from BI were returned to a different location every month. Since all individuals in the GB were used for a reproductive study, the above procedure was unnecessary. Individuals were handle-collected and stored in plastic buckets with water and sediment from its original place. In the laboratory, prior to the measurement of the disc diameter, the specimens were anaesthetized with a magnesium chloride solution (HENDLER *et al.*, 1995) to avoid arm autotomy during manipulation. Based on the methodology described by MUNDAY (1993), the arms of *O. reticulata* were divided into three equal portions: proximal, middle and distal. This procedure permitted the observation of differences in arm regeneration frequencies for each portion. *Ophionereis reticulata*, as well as *Astrobrachion constrictum* (Farquhar, 1900), has distinctly color patterns of the regenerated portion when compared to the old section (STEWART, 1996), which helped in the identification of the regenerating scars. Each brittle star was placed oral-side up in a petri dish and the number of visible regeneration scars was counted.

Comparison of the mean number of arms regenerating between the two areas was made using a Student T-test. A Kruskall-Wallis analysis was used to verify seasonal variation in the regeneration, after the Shapiro-Wilk and Levene test showed, respectively, no normality and homogeneity of variances for this data set. Differences in the position of the regeneration scar (proximal, middle and distal) were tested with a One-Way ANOVA after a log transformation of the data. Because of the lack of small individuals in the population of GB, only the specimens from BI were used to analyze the relationship between the size of the individual and the number of arms under regeneration. A Student T-test was used to analyze differences in the regeneration between sexes. In this particular case, only specimens from GB were used since they were also sectioned for a reproductive cycle study (YOKOYAMA & AMARAL, 2008a). Furthermore, the values of seawater temperature and salinity, measured daily in the Centro de Biologia Marinha, Universidade de São Paulo, were used to assess the possible influence on the regeneration of *O. reticulata*.

Some specimens of *O. reticulata* (ZUEC OPH 1261) were deposited in the ophiuroid collection of the Museu de Zoologia "Prof. Dr. Adão José Cardoso", Universidade Estadual de Campinas, Campinas, Brazil.

RESULTS

From the 1,170 individuals of *Ophionereis reticulata* sampled, 92% (n = 1,089) showed signs of arm regeneration. The occurrence of regeneration in the two populations was similar with 91% (n = 933) in BI and 99% (n = 237) in GB. The mean number of arms regenerating per individual in BI was 3.01 ± 0.79 (mean ± SD) while in GB was 3.49 ± 0.83 and did not significantly differ between the stations. In BI, low mean numbers of arms regenerating were observed between January 2002 and May 2002 (Figs 1, 2), with sequential increase to the maximum values in November (4.20 ± 0.87). In GB, low numbers of arms regenerating were observed on January 2002, April 2002 and May 2002 (Figs 1, 2) and after this the number of regenerating arms raised and reached the maximum values in January 2003 (4.58 ± 0.69). The Kruskal-Wallis test showed a significant difference in regeneration rates over the period for both areas (BI: $H = 214.08$, $p<0.05$ and GB: $H = 76.90$, $p<0.05$). In BI, an elevation in the mean number of arms regenerating per individual occurs from May 2002 to December 2002 (Figs 1, 2). The same was observed in GB, with an increase in the regeneration rates from May 2002 to January 2003 (Figs 1, 2). Furthermore, in GB there was an oscillation between January 2002 and March 2002, which was not recorded with the same



Figures 1, 2. Mean number of arms regenerating per individual of *Ophionereis reticulata* (Say, 1825) for Baleeiro Isthmus and Grande Beach, São Sebastião, state of São Paulo, Brazil, during the period of January 2002 to January 2003, considering: 1, variation of seawater temperature; 2, variation of salinity. Each vertical bar above and below the points indicates the standard deviation of the mean.

intensity to BI (Figs 1, 2). There was no relation between the number of arms regenerating and the seawater temperature and the salinity for both populations (Figs 1, 2).

Most of the individuals of *O. reticulata* tend to lose the distal portions of their arms in both areas (Fig. 3). Within each area, there was no difference in frequency of regeneration between the middle and proximal portions of the arm. Comparing the results of each portion between the areas, there is also no significant difference. The distribution of arm regeneration frequencies within the portions were significantly different (BI: $F = 32.517$, $p<0.05$; GB: $F = 36.218$, $p<0.05$). For the GB population, there were no statistical differences to this fact between the sexes (male: 3.47 ± 1.42; female: 3.57 ± 1.36). Comparing each arm separately, no significant difference was observed between sexes (Fig. 4), indicating no sexual differences in regeneration.

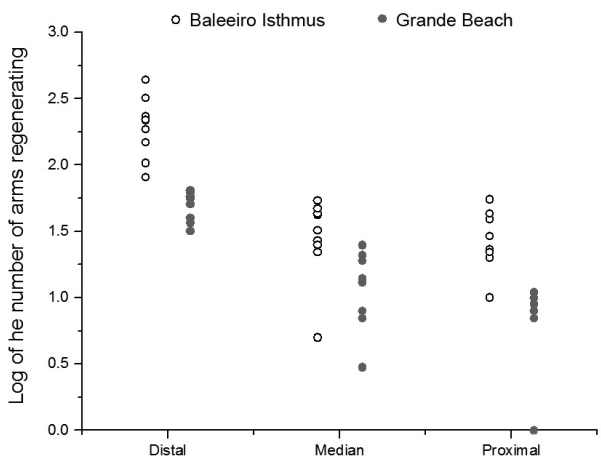


Figure 3. Log of the mean number of arms regenerating of *Ophionereis reticulata* (Say, 1825) for each regeneration portion (distal, median and proximal) over the period of March 2002 to January 2003 for Baleeiro Isthmus and Grande Beach, São Sebastião, state of São Paulo, Brazil.

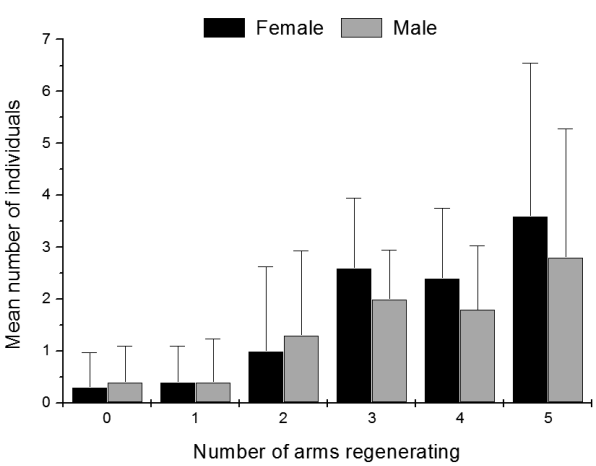


Figure 4. Number of arms regenerating for females and males of *Ophionereis reticulata* (Say, 1825) for the population of Grande Beach, São Sebastião, state of São Paulo, Brazil. The bars above the histograms indicates the standard deviation of the mean.

DISCUSSION

The two populations of *Ophionereis reticulata* had high frequencies of regenerating individuals (BI: 91% and GB: 99%), which was also observed for other ophiuroid species (Tab. I). These frequencies for *O. reticulata* reached high values, as registered for *Amphioplus coniortodes* Clark, 1918 by Singletary (1980) and *Ophiophragmus filograneus* (Lyman, 1875) by Clements *et al.* (1994). Generally, most of the literature of arm regeneration in ophiuroids observed frequencies of regenerating individuals ranging from 50 to 80% (Tab. I). Furthermore, there was no significant difference in regeneration between the Baleeiro Isthmus and the Grande Beach, with very similar pattern of arm breakage.

Accentuated differences in regeneration rates are related to variations in the habitat composition, which could offer differential protection (Pomory & Lawrence, 2001). *Amphiura filiformis* (Müller, 1776) occurs in areas without vegetal cover and presents a great percentage of arm regeneration (Bowmer & Keegan, 1983). Although living in areas with algae cover, species like *A. coniortodes*, *Microphiopholis gracillima* (Stimpson, 1852), and *Ophionephtys limicola* Lütken, 1869 showed great number of individuals with arm scars (Singletary, 1980). Thus, higher regeneration rates in well-protected areas could be caused by hydrodynamic regimes that interfere in the skeletal growth or in the food availability, which could alter the ophiuroids calcification rates. The two areas occupied by *O. reticulata* in this study are composed by small rock boulders, forming numerous shelters, without conspicuous vegetal cover. Individuals always live under the stones, buried in the sediment and during the period analyzed, no hydrodynamic modifications were noted, which could reduce growth or food availability. Even though these facts point to a low level of arm regeneration, the species presented a high number of arm breakage. The habitat structure can influence the amount of injury incurred in ophiuroids either by attracting predators or providing protection (Pomory & Lawrence, 2001; Brooks *et al.*, 2007). For *O. reticulata*, behavior aspects seem to play a role in the loss of tissue. The specimens usually occur buried in the sediment, under stones and only the distal parts of their arms are exposed, which are easily accessed by predators.

Our results of high incidence of regeneration do not match the influence of abiotic factors, such as temperature, salinity and wave action. To the latter, there was no drastic change in the hydrodynamic regimes for both areas during the period analyzed. *Ophionereis reticulata* presented a mean of 3.01 and 3.49 arms regenerating per individual in the BI and GB, respectively. These high percentages of regenerating individuals were common during August 2002 to January 2003. Buchanan (1964) observed a mean of 1.8 arms regenerating per individual to *Amphiura chiajei* Forbes, 1843 in the Northumberland region, England. On the other hand, Munday (1993) registered a mean of 4.21 arms regenerating per individual for the same species in Killary Harbour, Ireland. In this case, where there is a large probability to find an individual regenerating a great number of arms, the sub-lethal predation can be the main cause to arm loss (Munday, 1993).

Table I. Arm regeneration frequencies for various ophiuroid species.

Species	Arm regeneration(%)	Location	References
Amphioplus coniortodes Clark, 1918	87	Florida, USA	SINGLETARY, 1980
Amphiura filiformis (Müller, 1776)	78.3	Galway, Ireland	BOWMER & KEEGAN, 1983
Microphiopholis gracillima (Stimpson, 1852)	77	Florida, USA	SINGLETARY, 1980
	20 - 70	South Carolina, USA	STANCYK *et al.*, 1994
Ophiacantha bidentata (Retzius, 1805)	60	Southeastern USA	BROOKS *et al.*, 2007
Ophionephtys limicola Lütken, 1869	79	Florida, USA	SINGLETARY, 1980
Ophionereis reticulata (Say, 1825)	92.2	Southeast Brazil	Present study
Ophiophragmus filograneus (Lyman, 1875)	52 - 94	Florida, USA	CLEMENTS *et al.*, 1994

Regenerating arms are usually considered to be an indication of sub-lethal predation (LAWRENCE & VASQUEZ, 1996). For *O. reticulata*, predation could act to maintain the levels in the arm regeneration rates inside the population. If predation is an important cause of sub-lethal tissue loss, an increase in the frequency of regenerating arms might be expected during the times of year when putative predators are abundant (STANCYK *et al*., 1994). A diet study for the goby *Bathygobius soporator* (Valenciennes, 1837) by Mariene M. Nomura and Valeria F. Hadel (pers. comm.), which occurs in the same area of the ophiuroid population, did not found any signals of arm fragments from this ophiuroid in their stomachs. However, we observed some *B. soporator* cropping on distal portions of *O. reticulata* arms during the sampling.

Ophionereis reticulata showed high proportion of individuals with arms breakage scars in both populations, a common feature for ophiuroids. Variations in the seawater temperature and salinity do not seem to be the cause for the regeneration differences between the beaches. Other factors, such as sub-lethal predation and hydrodynamics factors might have some importance to the high regenerations rates, especially during the last semester of 2002. The lack of regeneration differences between contrasting areas and sexes (males and females) could be a consequence of the action of a variety of factors, such as differential predation pressure or food avaiability.

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REFERENCES

BOURGOIN, A. & GUILLOU, M. 1994. Arm regeneration in two populations of *Acrocnida brachiata* (Montagu) (Echinodermata: Ophiuroidea) in Douarnenez Bay, (Brittany: France): an ecological significance. **Journal of Experimental Marine Biology and Ecology 184**(1):123-139.

BOWMER, T. & KEEGAN, B. F. 1983. Field survey of the occurrence and significance of the regeneration in *Amphiura filiformis* (Echinodermata: Ophiuroidea) from Galway, West coast of Ireland. **Marine Biology 74**(1):65-71.

BROOKS, R. A.; NIZINSKI, M. S.; ROSS, S. W. & SULAK, K. J. 2007. Frequency of sublethal injury in a deepwater ophiuroid, *Ophiacantha bidentata*, an important component of western Atlantic *Lophelia* reef communities. **Marine Biology 152**(2):307-314.

BUCHANAN, J. B. 1964. A comparative study of some of the features of the biology of *Amphiura filiformis* and *Amphiura chiajei* (Ophiuroidea) considered in relation to their distribution. **Journal of the Marine Biological Association of the United Kingdom 44**(3):565-576.

BYRNE, M. 1991. Reproduction, development and population biology of the Caribbean ophiuroid *Ophionereis olivacea*, a protandric hermaphrodite that broods its young. **Marine Biology 111**(3):387-399.

CLEMENTS, L. A. J.; BELL, S. S. & KURDZIEL, J. P. 1994. Abundance and arm loss of the infaunal brittlestar *Ophiophragmus filograneus* (Echinodermata: Ophiuroidea), with an experimental determination of regeneration rates in natural and planted seagrass beds. **Marine Biology 121**(1):97-104.

DAVIS, J. P.; STEPHENS, G. C. & RICE, M. A. 1985. Net entry of amino acids into the brittle star *Ophioneresis annulata*. **Comparative Biochemistry and Physiology Part A: Physiology 81**(4):899-903.

DUINEVELD, G. C. A. & VAN NOORT, G. J. 1986. Observations on the population dynamics of *Amphiura filiformis* (Ophiuroidea: Echinodermata) in the southern North Sea and its exploitation by the dab, *Limanda limanda*. **Netherlands Journal of Sea Research 20**(1):85-94.

EMSON, R. H. & WILKIE, I. C. 1980. Fission and autotomy in echinoderms. **Oceanography and Marine Biology, an Annual Review 18**:155-250.

FIELMAN, K. T.; STANCYK, S. E.; DOBSON, W. E. & CLEMENTS, L. A. J. 1991. Effects of disc and arm loss on regeneration by *Microphiopholis gracillima* (Echinodermata: Ophiuroidea) in nutrient-free seawater. **Marine Biology 111**(1):121-127.

HENDLER, G.; MILLER, J. E.; PAWSON, D. L. & KIER, P. M. 1995. **Sea stars, sea urchins, and allies – Echinoderms of Florida and the Caribbean**. Washington, Smithsonian Institution. 390p.

LAWRENCE, J. M. & VASQUEZ, J. 1996. The effect of sublethal predation on the biology of echinoderms. **Oceanologica Acta 19**(3-4):431-440.

MEDEIROS-BERGEN, D. E. & EBERT, T. A. 1995. Growth, fecundity and mortality rates of two intertidal brittlestars (Echinodermata: Ophiuroidea) with contrasting modes of development. **Journal of Experimental Marine Biology and Ecology 189**(1):47-64.

MORGAN, R. & JANGOUX, M. 2004. Assessing arm regeneration and its effect during the reproductive cycle in the gregarious brittle-star *Ophiothrix fragilis* (Echinodermata). **Cahiers de Biologie Marine 45**(3):277-280.

MUNDAY, B. W. 1993. Field survey of the occurrence and significance of regeneration in *Amphiura chiajei* (Echinodermata: Ophiuroidea) from Killary Habrour, west coast of Ireland. **Marine Biology 115**(4):661-668.

O'CONNOR, B.; BOWMER, T. & GREHAN, A. 1983. Long-term assessment of the population dynamic of *Amphiura filiformis* (Echinodermata: Ophiuroidea) in Galway Bay (west coast of Ireland). **Marine Biology 75**(2-3):279-286.

POMORY, C. & LAWRENCE, J. M. 1999. Energy content of *Ophiocoma echinata* (Echinodermata: Ophiuroidea) maintained at different feeding levels during arm regeneration. **Journal of Experimental Marine Biology and Ecology 238**(1):139-150.

___. 2001. Arm regeneration in the field in *Ophiocoma echinata* (Echinodermata: Ophiuroidea): effects on body composition and its potential role in a reef food web. **Marine Biology 139**(4):661-670.

RODRIGUES, S. DE A. & SHIMIZU, G. Y. 1988. *Ophionereis reticulata*

e *Petrolisthes armatus*: disputa pelo abrigo fisico na baixa-mar? **Ciência e Cultura 40**(7):691-692.

SELVAKUMARASWAMY, P. & BYRNE, M. 1995. Reproductive cycle of two populations of *Ophionereis schayeri* (Ophiuroidea) in New South Wales. **Marine Biology 124**(1):85-97.

___. 2000a. Vestigial ophiopluteal structures in the lecithotrophic larvae of *Ophionereis schayeri* (Ophiuroidea). **Biological Bulletin 198**(3):379-386.

___. 2000b. Reproduction, spawning, and development of 5 ophiuroids from Australia and New Zealand. **Invertebrate Biology 119**(4):394-402.

SINGLETARY, R. 1980. The biology and ecology of *Amphioplus coniortodes*, *Ophionephthys limicola*, and *Micropholis gracillima* (Ophiuroidea: Amphiuridae). **Caribbean Journal of Science 16**(1-4):39-55.

STANCYK, S. E.; GOLDE, H. M.; PAPE-LINDSTROM, P. A. & DOBSON, W. E. 1994. Born to lose. 1. Measures of tissue loss and regeneration by the brittlestar *Microphiopholis gracillima* (Echinodermata: Ophiuroidea). **Marine Biology 118**(3):451-462.

STEWART, B. 1996. Growth dynamics of the radial shields of the euryalid snake star *Astrobrachion constrictum* (Echinodermata: Ophiuroidea). **Invertebrate Biology 115**(4):321-330.

YOKOYAMA, L. Q. & AMARAL, A. C. Z. 2008a. Reproductive cycle of *Ophionereis reticulata* (Ophiuroidea, Echinodermata) on the Southeast coast of Brazil. **Invertebrate Reproduction and Development 51**(2):111-118.

___. 2008b. The diet of *Ophionereis reticulata* (Echinodermata: Ophiuroidea) in Southeastern Brazil. **Revista Brasileira de Zoologia 25**(3):576-578.

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