Cah. Biol. Mar. (2004) 45 : 277-280

Assessing arm regeneration and its effect during the reproductive cycle in the gregarious brittle-star *Ophiothrix fragilis* (Echinodermata)

Raphael MORGAN[1] and Michel JANGOUX[1,2]
(1) *Laboratoire de Biologie Marine, Université Libre de Bruxelles, 50 Av F. Roosevelt, 1050 Brussels, Belgium.*
E-mail: rmorgan@ulb.ac.be
(2) *Laboratoire de Biologie Marine, Université de Mons-Hainaut, 20 Place du Parc, B7000 Mons, Belgium.*

Abstract: Arm regeneration has been assessed in the field in a population of *Ophiothrix fragilis* situated in the Oosterschelde (Netherlands). Small individuals are less likely to be regenerating than larger ones. Few individuals have 4 or 5 arms undergoing regeneration at any one time, possibly to optimise suspension feeding. Arm regeneration has a small but significant effect on energy storage and gonadal growth.

Keywords: Oosterschelde, *Ophiothrix fragilis*, ophiuroid, regeneration, reproduction.

Introduction

Echinoderms have the ability to regenerate most of their organs and appendages. Such regeneration can affect important biological functions such as maintenance, somatic growth and reproduction (Lawrence & Vasquez, 1996). Energy otherwise devoted to these major functions might instead be reallocated to regeneration potentially reducing fitness in the short term.

Arm regeneration was seen to occur in every investigated population of the temperate suspension feeding brittle star *Ophiothrix fragilis* (Aronson, 1989). The species, moreover, has a well documented reproductive cycle (Gounin & Richard, 1992; Morgan & Jangoux, 2002). All this makes it a good candidate for a field study of the effect of arm regeneration on reproduction.

Reçu le 24 octobre 2003 ; accepté après révision le 1er avril 2004
Received 24 October 2003; accepted in revised form 1st April 2004

Our goals were to assess the prevalence of arm regeneration in a population of *O. fragilis* from the Oosterschelde (Netherlands) and to determine the effect of regeneration on individuals' body compartments and reproductive cycle.

Materials and Methods

To assess arm regeneration, 251 individual *Ophiothrix fragilis* were observed in February 2002 and 200 in May 2002 from a population situated in Wemeldinge (Zeeland, Netherlands). The following measurements were taken: (1) the number of arms undergoing regeneration [Nr]; (2) the diameter of the disk [Dd]; (3) the length of the longest intact arm [Li]; (4) the length of the regenerate [Lr]; (5) the length of the arm stump [Ls]; (6) the 'position index of the breakage point' according to the method of Bourgouin & Guillou (1994).

Investigations on the effect of arm regeneration on different body compartments and on the reproductive cycle were done on individuals with a disk diameter between 9 and 11 mm. All considered regenerating individuals had two regenerating arms with a position index between 0 and 0.3 and regenerates measuring between 5 and 10 mm in length.

To assess the effect of arm regeneration on the body compartments, four groups of 20 individuals (10 males and 10 females) were considered, two in February and two in May. At each month, one group consisted of intact individuals (control) and the other of regenerating individuals. Every individual was dissected, only the disk and gonads were considered. Disk and gonads were dried separately for 48 h at 60°C and weighed.

To assess the effect of arm regeneration on the reproductive cycle, four groups of 30 individuals were investigated, two in February and two in May. At each month, one group consisted of intact individuals (control) and the other of regenerating individuals. The gonads of every individual were dissected, fixed in Bouin's fluid with acetic acid (5%), embedded in paraffin, sectioned at 7 µm and stained with Masson's trichrome. Measurements of oocytes and spermatocyte layer, identification of individuals gonadal stages and calculation of the maturity index value for each group were done according to Morgan & Jangoux (2002).

Results

Arm regeneration in the field

In February 2002, 49% of the sampled individuals were undergoing regeneration compared to 65% in May of the same year (Fig. 1A), the May sample having a higher percentage of short regenerates (Fig. 1B). Both in February and in May, the majority of regenerating individuals had one or two arms undergoing regeneration (Fig. 1A). A higher percentage of intact individuals occurred in the smaller size classes (< 7 mm disk diameter) (Fig. 2). For the two months combined, the majority of breakage points occurred in the first three quarters of the arms starting from the disk. Also, no correlation seems to exist between the position of the breakage point and the number of arms undergoing regeneration per individual.

Effect of arm regeneration on body compartments

Comparisons between males and females, with either all arms intact or some regenerating arms in February or May, did not show any significant difference in the dry weights of any of the body compartments (ANOVA, $p > 0.05$) except for the disk of some male individuals (Fig. 3). Indeed, the dry weight of the disk in regenerating males in May is significantly higher than that of intact males at the same month (ANOVA, $p < 0.05$).

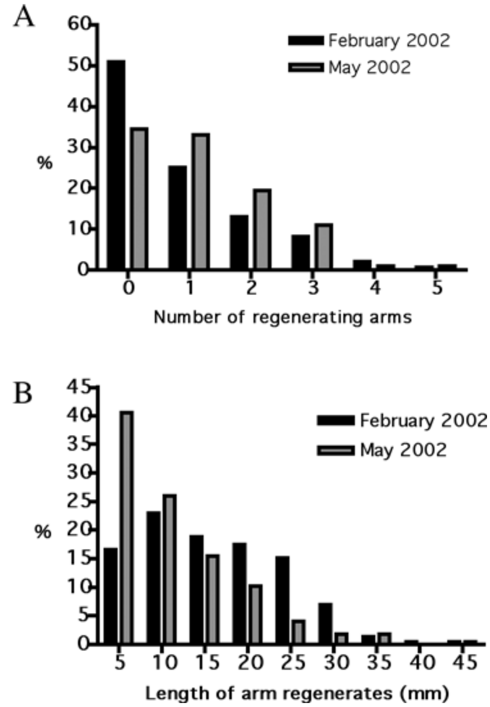

Figure 1. *Ophiothrix fragilis*. A) Number of regenerating arms per individual in the Wemeldinge population in February 2002 (n = 251) and May 2002 (n = 200) [n = number of individuals]. B) Length of arm regenerates in the Wemeldinge population in February 2002 (n = 219) and May 2002 (n = 227) [n = number of regenerating arms].

Figure 1. *Ophiothrix fragilis*. A) Nombre de bras en régénération par individu dans la population de Wemeldinge en février 2002 (n = 251) et en mai 2002 (n = 200) [n = nombre d'individus]. B) Longueur des régénérats dans la population de Wemeldinge en février 2002 (n = 219) et mai 2002 (n = 227) [n = nombre de bras en régénération].

Effect of arm regeneration on the reproductive cycle

In February 2002, the gonads of both males and females, either with all arms intact or regenerating some arms, were at the resting stage (stage I of Morgan & Jangoux, 2002). The sampled individuals were developmentally very similar with a value of maturity index between 1 and 1.1. In May 2002 female gonads became premature (stage III) while male gonads reached the growth stage (stage II); this was true whether individuals had all arms intact or were regenerating some arms.

Measurements made in May showed no significant difference between the thickness of primary spermatocyte layer in males with intact [38.92 µm (S.D. = 16.42)] and regenerating [41.47 µm (S.D. = 17.16)] arms (ANOVA, $p > 0.05$). On the contrary, oocytes in the gonads of females with all arms intact were significantly larger [54.13 µm

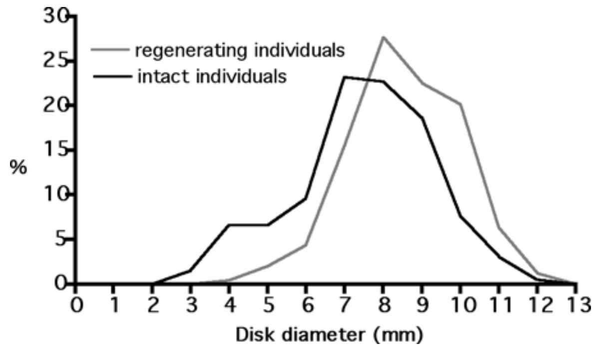

Figure 2. *Ophiothrix fragilis*. Distribution of disk diameters in intact (n = 198) and regenerating (n = 253) individuals (samples from February and May 2002 have been pooled).

Figure 2. *Ophiothrix fragilis*. Distribution des diamètres discaux chez des individus intacts (n = 198) et en régénération (n = 253) (les individus de février et mai 2002 ont été rassemblés).

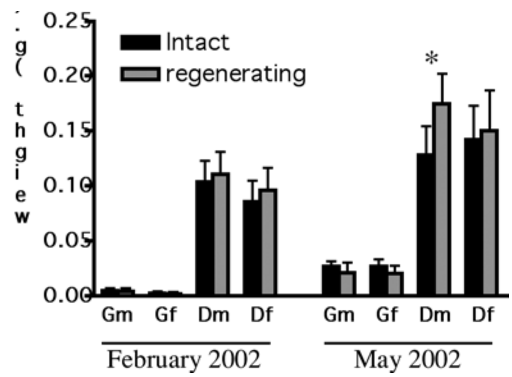

Figure 3. *Ophiothrix fragilis*. Dry weights (grams) of the gonads and disk in intact and regenerating individuals in February 2002 and May 2002. Gm: testes, Gf: ovaries, Dm: male disk, Df: female disk. The bars represent the standard deviations. The asterisk denotes a significant difference ($p < 0.05$).

Figure 3. *Ophiothrix fragilis*. Poids secs (grammes) des gonades et du disque chez des individus intacts et en régénération en février 2002 et mai 2002. Gm : gonades mâles, Gf : gonades femelles, Dm : disque mâle, Df : disque femelle. Les barres représentent les écarts-types. L'astérisque indique une différence significative ($p < 0,05$).

(S.D. = 14.75)] than those from females with regenerating arms [49.53 µm (S.D. = 13.39)] (ANOVA, $P < 0.05$).

Discussion

Arm regeneration in the field

The 49% and 65% of individuals undergoing arm regeneration we observed in the Wemeldinge population is in accordance with other *Ophiothrix fragilis* populations (Aronson, 1989). Regenerating arms are usually considered to be an indication of sub-lethal predation (Lawrence & Vasquez, 1996). Aronson (1989) found a lower percentage of regeneration occurring in high density brittlestar beds compared to populations living in lower densities on rocky reefs and attributed this to the near absence of predators in the first case. Similarly high levels of regeneration for *O. fragilis* living on a rocky reef were given by Skold & Rosenberg (1996). While habitat appears to play a role in limiting or enhancing predation, the situation should be more complex. Indeed, the Wemeldinge population, while living in high densities in an area where potential predators commonly occur (Sheridan & Massin, 1998), shows a rather low level of predatory pressure.

Autotomy mostly occurs in the proximal part of the arm. A disadvantage of having such a large part of the arm autotomized would be a reduction of suspension feeding efficiency. In *O. fragilis* this is compensated by rarely having 4 or 5 arms regenerating simultaneously suggesting the importance of having at least some of the arms intact. Limiting the number of arms undergoing regeneration at any one time to optimise feeding has been observed in other ophiuroids (Stewart, 1996; Bourguoin & Guillou, 1994). Interestingly, our results showed that individuals below 7 mm do not suffer as much arm breakage as larger individuals. Stewart (1996) also showed that small *Astrobrachion constrictum* were less likely to have regenerating arms than larger ones. The individuals' small size could make them more cryptic thus limiting autotomization due to predation.

Effect of arm regeneration

During the study period, whether the individuals were regenerating or not, all body compartments gained weight. The fact that in *O. fragilis* a rise in the dry weights of the disk was observed could be an indication these organs are used to stock reserves. This has been shown in *Microphiopholis gracillima* whose disk, oral frame and arm tips are preferentially resorbed when the animals are maintained below maintenance levels (Dobson et al., 1991). Here, the fact that practically no difference occurs in the dry weights of the body compartments between regenerating and intact individuals and that these gain weight over time, clearly shows that the individuals have above maintenance levels of food available.

Regenerating males accumulate more reserves than those with all arms intact. Reserves stored in the disk could therefore be used for both gonadal growth and/or regeneration. Salzwedel (1974) has shown that at least part of the material needed for regeneration comes from the disk in *Amphiura filiformis*. In this study, no difference were found between the dry weights of the disk in intact and regenerating females in May 2002. This could be because the disk has reached its full capacity in terms of storage. We should as a result see a difference in either regeneration rates, gonadal growth or both.

In the case of gonadal growth, regenerating females produce smaller eggs than intact ones even though intact and regenerating females accumulate equivalent reserves in the disk. Therefore, we can safely say that some of the nutrients that were allocated to gonadal growth has been translocated to regeneration. Thus, when food conditions are optimal, it seems that individuals are limited in the quantity of reserve material that they can store. This was already presumed by Pomory & Lawrence (1999) for *Ophiocoma echinata*. A certain equilibrium seems to occur where the total amount of energy stored is distributed either to gonad production in intact individuals or to gonad and regeneration in regenerating individuals. In males the energy needed for gonad production is small compared with females (Pomory & Lawrence, 2001) so that surplus energy can be used for regeneration without any effect on gonad production. It should however be noted that males were at an earlier stage of gonadal development than females. This stage might have a lower energy demand than the next which would explain why no difference could be detected between intact and regenerating males in terms of gonadal development.

Further study on regeneration and its effect at different times of the reproductive cycle should be carried out to see if the allocation of resources changes with the availability of food and the physiological state of the brittle star. It seems clear, however, that an equilibrium between storage of material, regeneration and gonadal growth occurs so as to maintain fitness at its optimum.

Acknowledgements

We are grateful to Ph. Pernet for help in collecting specimens. This study was made possible by a F.R.I.A. grant attributed to R. Morgan. This is a contribution to CIBIM (Centre Interuniversitaire de Biologie Marine).

References

Aronson R.B., 1989. Brittlestar beds: low-predation anachronisms in the British Isles. *Ecology* **70**: 856-865.

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**: 123-139.

Dobson W.E., Stancyk S.E., Clements L.E. & Showman R.M., 1991. Nutrient translocation during early disc regeneration in the brittlestar *Microphiopholis gracillima* (Stimpson) (Echinodermata: Ophiuroidea). *Biological Bulletin* **180**: 167-184.

Gounin F. & Richard A., 1992. Cycle reproducteur chez *Ophiothrix fragilis* (Abildgaard) dans le détroit du Pas-de-Calais (France): description et évolution saisonnière des gonades. *Bulletin de la Société Zoologique de France*, **117**: 321-328.

Lawrence J.M. & Vasquez J., 1996. The effect of sublethal predation on the biology of echinoderms. *Oceanologica Acta* **19**: 431-440.

Morgan R. & Jangoux M., 2002. Reproductive cycle and spawning induction in the gregarious brittle star *Ophiothrix fragilis* (Echinodermata) in the Oosterschelde (Netherlands). *Invertebrate Reproduction and Development* **42**: 145-155.

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**: 139-150.

Pomory C. & Lawrence J.M., 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**: 661-670.

Salzwedel H., 1974. Arm-regeneration bei *Amphiura filiformis* (Ophiuroidea). *Veröffentlichungen des Instituts für Meeresforschung in Bremerhaven* **14**: 161-167.

Sheridan R. & Massin C., 1998. *Guide de la faune et flore sous-marine de Zélande*. (Sheridan and Massin eds) 320 pp.

Skold M. & Rosenberg R., 1996. Arm regeneration frequency in eight species of Ophiuroidea (Echinodermata) from European sea areas. *Journal of Sea Research* **35**: 353-362.

Stewart B., 1996. Sub-lethal predation and rate of regeneration in the euryalinid snake star *Astrobrachion constrictum* (Echinodermata, Ophiuroidea) in a New Zealand fiord. *Journal of Experimental Marine Biology and Ecology* **199**: 269-283.

Warner G.F., 1971. On the ecology of a dense bed of the brittle-star *Ophiothrix fragilis*. *Journal of the Marine Biological Association of the United Kingdom*. **51**: 267-282.