

# Growth or differentiation? Adaptive regeneration in the brittlestar *Amphiura filiformis*

Samuel Dupont\* and Michael C. Thorndyke

Kristineberg Marine Station, 566 Kristineberg, 45034 Fiskebäckskil, Sweden

\*Author for correspondence (e-mail: samuel.dupont@kmf.gu.se)

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## Summary

*Amphiura filiformis* is a burrowing brittlestar, which extends arms in the water column when suspension feeding. In previous studies, unexpectedly high variability was observed in regeneration rate between individuals even when experiments were performed under identical conditions. The aims of this work were to understand this variability and interpret the observed variability in terms of adaptation to sublethal predation. Our experiments on the dynamics of arm regeneration in *A. filiformis* revealed that the developmental program during regeneration is well adapted to its burrowing life style. We demonstrate that there is a trade-off between regeneration in length and functional recovery for feeding (differentiation index). The amount of tissue lost (length lost), which represents

the quantity of tissue needed to completely regenerate an intact arm with no previous history of regeneration, determines whether the arm will invest more energy in growth and/or in differentiation, which must be a reflection of the ability to differentially regulate developmental programs during regeneration. We show that combining regeneration rate with differentiation index provides an ideal tool for the definition of a standard temporal framework for both field and laboratory studies of regeneration.

Key words: development, adult regeneration, echinoderm, differentiation, adaptation.

## Introduction

The brittlestar *Amphiura filiformis* (Echinodermata) is remarkably well adapted to its habitat. It is the dominant species on many sublittoral soft bottoms down to 200 m depth in the North Sea and the Mediterranean (Rosenberg, 1995). In this environment, predation is the main selective pressure and several crustacean and fish species prey mainly on *A. filiformis* (Baden et al., 1990; Duineveld and Van Noort, 1986; Pihl, 1994). This brittlestar possesses several adaptations to face this biotic disturbance [i.e. removal of energy from an organism (Lawrence, 1990; Lawrence, 1991)] leading to a trade-off between feeding efficiency and predation avoidance (Rosenberg and Lundberg, 2004).

When suspension feeding, *A. filiformis* generally lives with its disc 4–8 cm below the sediment surface (Solan and Kennedy, 2002) and two arms extended into the water column (Woodley, 1975; Loo et al., 1996). When inactive, arm tips are kept at the sediment–water interface where chemo- and photoreceptors are thought to detect conditions for feeding: tidal currents, food concentration, etc. (Rosenberg and Lundberg, 2004).

During feeding, arms extended in the water column are easy prey for visual predators commonly present in the same habitat, e.g. *Limanda limanda* and *Nephrops norvegicus*. *A.*

*filiformis* has developed several adaptations to reduce predation (Wilkie, 1978; Bowmer and Keegan, 1983; Herring, 1995; Rosenberg and Selander, 2000; Rosenberg and Lundberg, 2004).

Nevertheless, sublethal predation is common in *A. filiformis* and more than 84% of individuals show signs of having been injured with more than 80% of arms showing at least one scar (Sköld and Rosenberg, 1996). Since arms are needed for suspension feeding (Woodley, 1975), ventilation of the burrow (Nilsson, 1998; Nilsson, 1999) and as sensory organs (Rosenberg and Lundberg, 2004), regeneration of this lost body part is essential for survival. It is probable that the most damaged arms are withdrawn inside the burrow and are replaced at the sediment surface by less damaged arms [theory of arm rotation (Makra and Keegan, 1999)].

*A. filiformis* has high regenerative capacity and new functional tissues appear in only a few days following amputation (Mallefet et al., 2001; Thorndyke et al., 2003). A review of the literature reveals an unexpectedly high variability in the observed growth rate of the regenerate, even in experiments performed under similar temperature conditions. This rate ranges between 0.08 and 0.45 mm day<sup>-1</sup> (Salzwedel, 1974; Andreasson, 1990; Nilsson and Sköld, 1996; Sköld, 1996; Sköld and Gunnarsson, 1996; Sköld and Rosenberg,

1996; Gunnarsson et al., 1999; Mallefet et al., 2001; Thorndyke et al., 2003; Selck et al., 2004).

The energy costs for regenerating a new arm are likely to be significant (Salzwedel, 1974; Bowmer and Keegan, 1983; Fielman et al., 1991; Stancyk et al., 1994; Pape-Lindstrom et al., 1997; Pomory and Lawrence, 1999; Pomory and Lawrence, 2001) and this energy can be allocated to two main processes: (1) growth in length with little differentiation and (2) differentiation of the regenerate (segmentation and development of podia and spines). We hypothesize that in a single arm there will be differential allocation of energy to these processes according to the length lost and thus the quantity and quality of tissue needed to regenerate that arm to its original intact length. This combination of parameters has never been taken into account in previous studies.

From an evolutionary and adaptive perspective, it must be important to have arms functional for feeding as soon as possible after autotomy. Some authors argue that in organisms such as a brittlestars, which need to reach the surface to feed (Salzwedel, 1974; Stancyk et al., 1994), regeneration might sacrifice length to restore function as quickly as possible. However, the strategy may be different according to the level of autotomy. If autotomy occurs close to the disc, the individual will need to regenerate a complete full-length arm and might therefore invest more energy in growth rather than in differentiation (high growth rate and low differentiation rate) since a short functional arm is useless for feeding because it cannot extend far enough into the water column. However, if only the arm tip is lost, the energy should be invested in differentiation rather than in growth (low growth rate and high differentiation rate) because of the importance of the tip as a sensory organ.

In order to test these hypotheses and try to explain the high variability observed in previous studies, we investigated the influence of disc diameter, length lost and the ratio of length lost to the original intact length and on both growth and differentiation rates in *A. filiformis* arms.

Moreover, there has been an increasing interest in regeneration, largely because of potential clinical applications. Echinoderm models such as *A. filiformis* offer a unique opportunity, in an adult deuterostomian, to study differentiation of stem cells and the factors that induce or repress the expression of genes that control fate decisions during the process. Our results may guide future experimental designs by defining standard conditions for proteomic and genomic studies.

## Materials and methods

### Sampling

Sediment containing *Amphiura filiformis* O. F. Müller was collected at 25–40 m depth, using a Petersen mud grab, in the vicinity of Kristineberg Marine Station, Sweden in January 2004. Individuals were immediately sampled from the sediment cores by gentle rinsing to avoid breaking arms and maintained in natural flowing seawater at 14°C with 1 cm of

sieved sediment taken from the collection site. Animals were used 1 month after collection for the experiments.

### Experiments

The experiments were carried out on intact specimens by selecting those individuals that showed no evidence of recent regeneration events and no apparent gonads. Experimentally induced amputations were performed on one or several arms after anaesthesia by immersion in 3.5% w/w MgCl<sub>2</sub> in artificial seawater. Experimental arm amputation was achieved by gently applying a scalpel blade across a natural inter-vertebral autotomy plane. Two types of preparation were used in this study. (1) Whole animal, in which one arm was cut off at a measured distance from the disc. Animals were then kept for 4 weeks in a PVC aquarium supplied with flowing deep water at 14°C and containing sieved sediment from the sampling site. (2) The double amputated arm explant, which is a valuable model for studying regenerative mechanisms (Candia Carnevali et al., 1998). Explants are sections of arm isolated from the individual that are able to survive and regenerate for several months. Explants were kept in small aquaria containing a thin layer of sieved sediment in circulating deep seawater for 9 weeks.

Experiments were designed to test the influence of disc diameter, length lost and the ratio of length lost to the original intact length of the arm on regeneration rates (growth and differentiation).

### Experiment 1 (whole animal)

One repetition with 100 intact individuals with disc diameters ranging from 3 to 6.2 mm was used. All arms possess the same regenerative capabilities (similar regeneration and differentiation rates if cut at the same distance from the tip; S. Dupont, personal observation) and for practical reasons, the first arm clockwise (oral side) to the madreporite was cut off at a distance (length lost, LL) between 5 and 60 mm from the arm tip (Fig. 1). Two arms of the same individual were cut off at 5 and 50 mm, respectively, and pictures of the regenerate were taken at 0, 3, 6, 12 and 19 days of regeneration.

### Experiment 2 (explant)

Five explants each of 10 mm length were removed from the same arm of 10 intact animals at distances between 15 and 65 mm from the tip (length lost, LL) (Fig. 2). This experiment was repeated once.

Regular (weekly) checks were made to ensure that no spontaneous autotomy occurred in arms or explants during experiments.

### Measurements

Several measurements were made on intact individuals using a graduated ocular in binocular microscope (0.1 mm accuracy): disc diameter, intact arm length and length of each segment. At the beginning of the experiment, LL was also measured (see above). When the regenerate started to differentiate it was possible to divide it into two distinct parts: the proximal

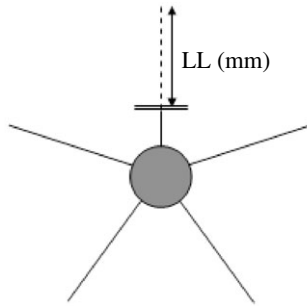


Fig. 1. *Amphiura filiformis*. Diagram of the whole animal experiment; LL, length lost, which ranged between 5 and 60 mm.

differentiated part, comprising fully formed segments with clearly developed ossicles, podia and spines, and the distal part that remained undifferentiated with no, or only poorly defined, spines or ossicles (Fig. 3). In both explants and whole animal regenerates, the total regenerated length (RL in mm) and the differentiated length (DL in mm) of each regenerate was measured each week. See Table 1 for details and summary of abbreviations used.

#### Calculations and statistics

Regeneration rate (RR, in  $\text{mm week}^{-1}$ ) was calculated as the slope of the significant simple linear regression between the regenerated length (RL in mm) and time (in weeks). A differentiation index, (DI as a percentage), was calculated as the proportion in length of the regenerate that is completely differentiated (0 if the regenerate is completely undifferentiated and 100% if the arm is completely differentiated):  $\text{DI} = (\text{DL}/\text{RL}) \times 100$ . According to morphological and physiological studies, DI is a good indicator of functional recovery of the tissue, this index being correlated to the timing of neuropeptide expression and physiological recovery of the nervous system (S. Dupont, personal observation).

Two types of differentiation rate can be calculated: DR1 (in  $\text{mm week}^{-1}$ ), calculated as the slope of the significant ( $P < 0.05$ ) simple linear regression between length of the regenerate

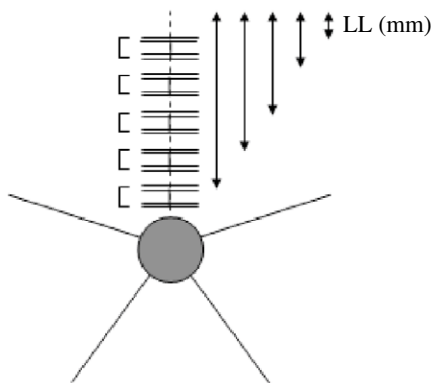


Fig. 2. *Amphiura filiformis*. Diagram of the explant experiment; LL, length lost, which ranged between 15 and 65 mm. All scale bars represent 10 mm.

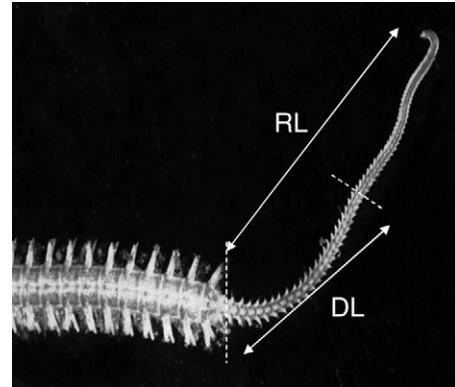


Fig. 3. Regenerating arm of *Amphiura filiformis*. The new arm has extended in length and is clearly distinguishable from the older stump. The demarcation between the proximal differentiated part (with ossicles, podia and spines) and the distal undifferentiated part is indicated by a dotted line. DL, differentiated length (in mm); RL, regenerated length (in mm).

completely differentiated (DL in mm) and time (in week); and DR2 (in  $\% \text{ week}^{-1}$ ), calculated as the slope of the significant ( $P < 0.05$ ) simple linear regression between differentiation index (DI in %) and time (in weeks). Simple linear, power, logarithmic and exponential regression models were used to test the relationship type between the variables. The Shapiro–Wilk test (Shapiro and Wilk, 1965) was used to check that the data were normally distributed and the Levene test was used to check that variances were homogenous. All statistical analyses were performed using SAS/STAT® software (SAS Institute Inc., 1990).

#### Results

Intact individuals (without any scar or colour difference along the arm, which indicate recent autotomy or regeneration events) with larger discs possessed longer arms, and a significant linear regression ( $P < 0.01$ ) was observed between the disc diameter and the size of an intact arm before amputation (Fig. 4). A brittlestar arm comprises a series of segments and the length of each segment varies according to its position on the arm. Segments closer to disc are larger than those at the tip. In *A. filiformis*, the size of segments increases significantly from distal to proximal position following a significant power regression ( $P < 0.01$ ; Fig. 5).

All cut arms and all explants regenerated following the same sequence. The initial response involved the formation of a wound epidermis followed by a blastema of undifferentiated cells. In the ensuing days, the regenerating arm tips increased in length to produce a thinner replicate arm with ossicles developing proximally while the distal tip remained undifferentiated (Fig. 3). Regenerative processes only occurred in the distal part of the explants. No regeneration was observed proximally.

Table 1. Summary of measured and calculated parameters

Parameter	Unit	Description
Measured		
DL	mm	Differentiated length: length of the regenerate completely differentiated (with podia and spines fully formed)
LL	mm	Length lost: length of the removed part of an intact arm between the tip and the amputation plane
RL	mm	Regenerated length; total length of the regenerate
Calculated		
DI	%	Differentiation index: ratio between DL and RL, representing the percentage of the regenerate length, which is completely differentiated (0, no differentiation; 100, completely differentiated). Indicator of functional recovery
DR1	mm week <sup>-1</sup>	Differentiation rate 1: length of arm completely differentiated (with podia and spines fully formed) regenerated per week
DR2	% week <sup>-1</sup>	Differentiation rate 2: percentage of the regenerate length completely differentiated each week or functional recovery of the regenerate per week
RR	mm week <sup>-1</sup>	Regeneration rate: length of arm regenerated per week

Experiment 1

Significant variability was detected at each observation period each week for the regenerate length (RL), the length of the regenerate completely differentiated (DL) and the differentiation index (DI). Three parameters were analysed in order to explain this variability: disc diameter, length lost (LL) and the ratio of LL to original intact arm length. No significant relationship was found between RL, DL or DI and disc diameter, ratio of LL to intact arm length or time ( $P>0.05$ ; not shown). At each observation period (each week from 1 to 4 weeks), a significant linear relationship ( $P<0.05$ ) was observed between the LL and RL. Therefore, the regeneration rate (RR) was estimated as the slope of the significant linear correlations observed between the time (in weeks) and regenerate size (in mm) for different LL. This RR increased exponentially from 0.6 to 3.3 mm week<sup>-1</sup> when the LL increased from 5–60 mm ( $P<0.01$ ; Fig. 6). DR1 (in mm week<sup>-1</sup>) increased with LL following a logarithmic curve ( $P<0.01$ ; Fig. 7). The observed variability for DR2 (in % week<sup>-1</sup>) can also be explained by a significant negative linear regression ( $P<0.01$ ) with the LL (Fig. 8).

In consequence, the size and differentiation of an arm will

differ according to the LL. The relationship between growth and differentiation is summarized in Fig. 9. This figure indicates that for the same regenerate size, each can have very different degrees of differentiation according to the LL. For example, the DI of a 5 mm regenerate varied between 25 and 100% if the LL ranged between 60 and 5 mm, respectively. On the other hand, the same DI can be observed for regenerates of very different sizes. Arms with a 50% DI can have a size between 2 and 10 mm if the LL varies between 5 and 60.

To illustrate this point, two arms from each of two animals with the same disc diameter (4.5 mm) were cut off, one at 5 mm and the other at 50 mm from the tips. Photographs were taken after 3, 6, 12 and 19 days (Fig. 10).

Experiment 2

10 mm explants cut from different regions of the same arm were followed for 9 weeks. Even though the RR were 10 times lower than those observed in the whole animal experiments, a similar significant exponential correlation ( $P<0.01$ ) was observed between LL and RR [calculated as the slope of the significant correlation ( $P<0.05$ ) between the size of the regenerate and time for different classes of size to regenerate;

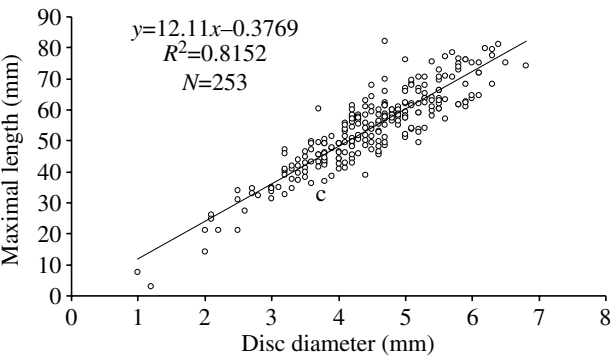


Fig. 4. Non-regenerating arm of *Amphiura filiformis*. Relationship between the disc diameter (in mm) and the maximal length (in mm) of the intact arm before amputation.

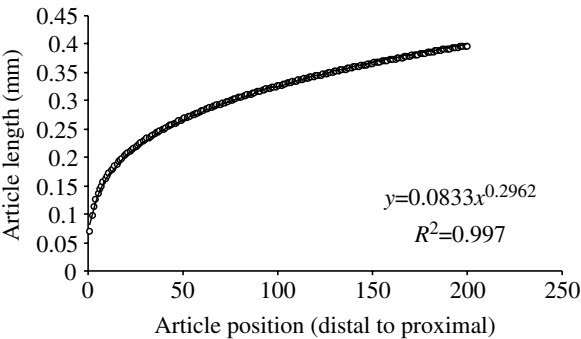


Fig. 5. Relationship between the position (number of segments from distal to proximal) and the length (in mm) of a segment on a non-regenerating arm.

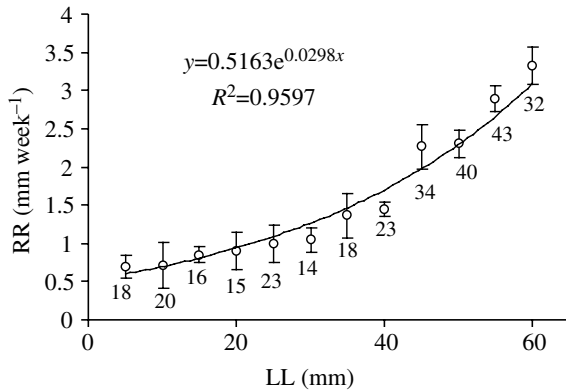


Fig. 6. Relationship between the length lost (LL; in mm) and regeneration rate (RR; in mm week<sup>-1</sup>).

Fig. 11]. Thus explants cut closer to the disc regenerated more rapidly than those cut close to the tip. No clear differentiation was observed after 9 weeks. This indicated that even when isolated from the individual, positional information (LL) is present in the arm.

### Discussion

#### *Adaptation to sublethal predation as a biotic disturbance*

Our experiments on dynamic arm regeneration in *A. filiformis* indicate that the length lost (LL) is a key factor that can explain the significant variability observed for both growth and differentiation rates of the regenerates. We have found a positive exponential relationship between length lost and regeneration rate (RR), a positive logarithmic relationship between length lost and differentiation rate 1 (DR1), an indicator of the speed of differentiation and a negative exponential relationship between the length lost and differentiation rate 2 (DR2), an indicator of the speed of functional recovery (S. Dupont, unpublished). In consequence, arms autotomized close to the disc regenerate faster in length (both regenerated length, RL, and differentiated length, DL) than those cut close to the tip, although overall functional recovery of the arm (measured as DR2) is delayed.

This variation in regeneration and differentiation rates could

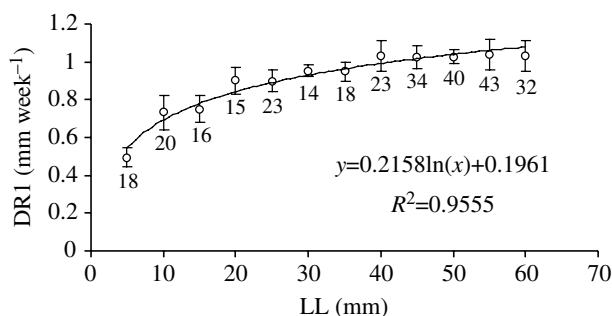


Fig. 7. Relationship between the length lost (LL; in mm) and the differentiation rate (DR1; in mm week<sup>-1</sup>).

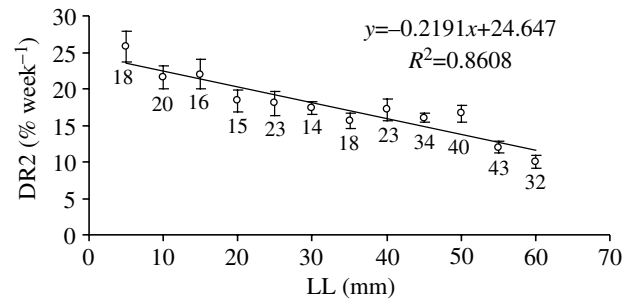


Fig. 8. Relationship between the length lost (LL; in mm) and the differentiation rate (DR2; in % week<sup>-1</sup>).

reflect a valuable adaptation to biotic disturbance: according to the amount of tissue lost, the arm will invest more energy either in growth or in differentiation. An arm cut at the tip is still able to extend into the water column for feeding but lacks essential sensory organs located at the tip (Rosenberg and Lundberg, 2004). Investing energy in rapid differentiation leads to a rapid recovery of this functionality and the autotomized arm is quickly functional for feeding. By contrast if an arm is cut close to the disc, rapid differentiation is useless since a short functional arm is not able to reach the water column for feeding. It is then of more adaptive value to invest energy for growth in length rather than differentiation to functional recovery.

This repartition of energy represents a trade-off between two different adult developmental programmes (growth *per se* and differentiation/maturation). A similar trade-off was observed in nutrient-free condition for another burrowing brittlestar, *Microphiopholis gracillima*. Fielman et al. showed that in starved animals, allocation of energy and the pattern of regeneration are affected by both the quantity and type of tissue lost (Fielman et al., 1991). If enough tissue (including disc) is removed, animals will adopt a 'minimal functional configuration' to allow construction of its respiration and feeding burrows and to digest food.

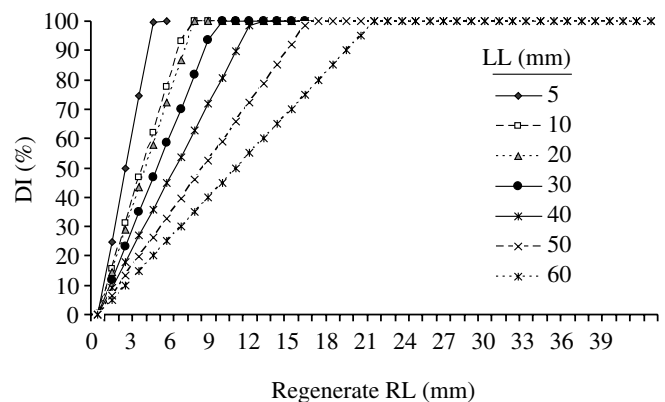


Fig. 9. Relationship between the size of the regenerate (in mm) and its differentiation index (DI; in %) for different lengths lost (LL; ranged between 5 and 60 mm).



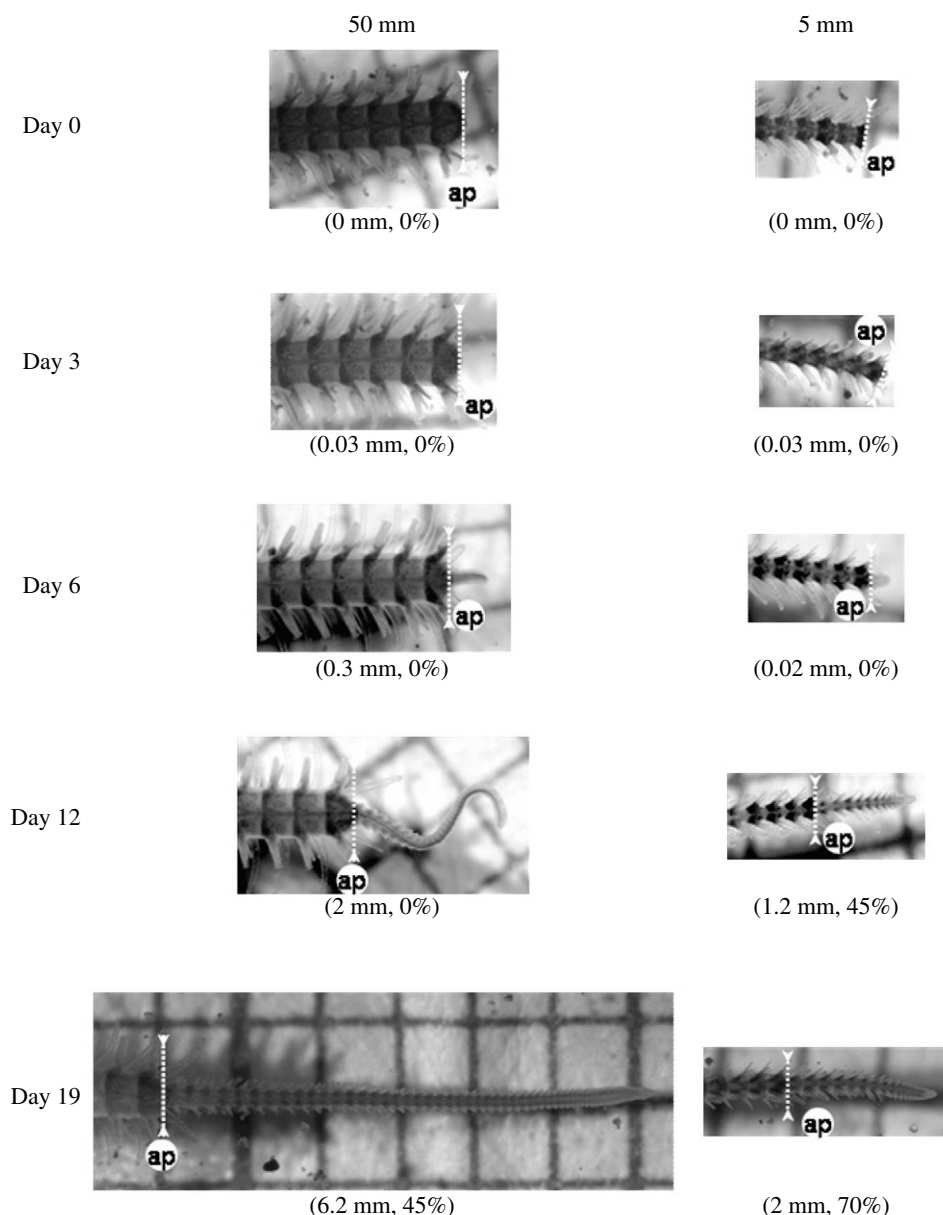


Fig. 10. Comparison of regenerate at different times of regeneration and two different amputation levels (5 and 50 mm; measured as length lost; LL). The differentiation index (DI; in %) is given below each image. ap, amputation plane. The first difference was observed on day 6, when the blastema was longer for the arm amputated at 50 mm from the tip. After 12 days, important differences in both size and differentiation were observed. The arms cut at 5 mm from the tip regenerated a smaller arm (1.2 mm), which had already started to differentiate complete segments with spines and tube feet while arms cut at 50 mm from the tip regenerated a longer arm (2 mm) but no complete segments were formed. These differences were amplified after 19 days. The regenerate was three times longer for the arm cut at 50 mm from the tip but less differentiated (DI of 45% *versus* 70%) than the one cut at 5 mm from the tip.

*A. filiformis* appears to be well adapted to predation in both quantitative and qualitative aspects of energy allocation. Quantitatively in natural conditions, energy allocation is not dependent on the number of arms lost. The same quantity of energy is allocated (from both the remaining proximal part of the arm and the disc) irrespective of whether one or more arms are lost (Nilsson, 1998; Nilsson, 1999). Nevertheless, we have demonstrated that a qualitative difference in how this energy is allocated (growth *versus* differentiation) is correlated with the quantity of tissue lost (LL) in a single arm. From this qualitative point of view, the observed trade-off between growth and differentiation during regeneration is a perfect balance between costs and benefits that has been selected by a long history of sublethal predation in *A. filiformis*.

#### Origin of the variability

An important question raised from the observation that length lost has a huge impact on both growth and differentiation of the regenerating tissue is the origin of the information, or signal, that relates to the size of a lost body part?

The normal sequence of fundamental repair/regenerative events (cell proliferation, migration and differentiation) in the majority of animal models appears to depend on a crucial contribution from the nervous system (Brockes, 1987; Ferreti and Géraudie, 1998). In echinoderms, the nervous system plays several roles in regeneration: (1) promoter/inducer of regenerative process; (2) source of cells (many of which, although non-neural, are associated with the nervous system); and (3) source of regulatory factors (Candia Carnevali and

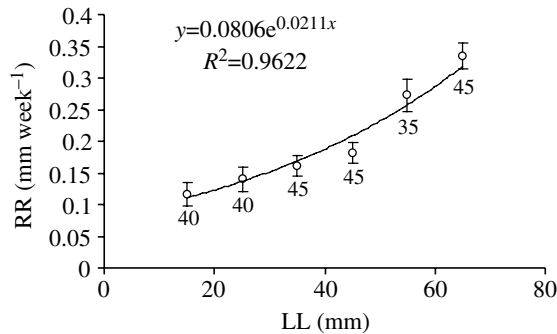


Fig. 11. Relationship between the length lost (LL; in mm) and the regeneration rate (RR; in mm week<sup>-1</sup>) on growing explants.

Bonasoro, 1994; Candia Carnevali et al., 1995; Candia Carnevali et al., 1996; Candia Carnevali et al., 1997; Candia Carnevali et al., 1998). If neurally secreted factors are responsible for our observed differences in growth *versus* differentiation, it is possible to hypothesize that growth and/or differentiation rates could be proportional to the concentration and/or identity of one or several of these factors. Concentration may be simply linked to the size of the neural cord at the position of autotomy, more tissue being able to produce more growth factors (morphological gradient hypothesis). We have shown that in *A. filiformis* the size of a segment is correlated to its distance from the tip on a non regenerating arm (Fig. 5). Moreover, the volume of the internal structures (e.g. radial nerve) in a segment is directly proportional to the size of the segment (S. Dupont, unpublished). Based on current data, we can infer that, (1) growth rate is exponentially related to the size of the segment at the position of autotomy and (2) differentiation rate is correlated to the size of the segment at the position of autotomy following a linear function. These relationships suggest that the differentiation is linked to the size of internal structures such as nerve cord, coelom or muscles in the non-regenerating segments close to the amputation plane and then the quantity and/or quality of secreted growth factors (Thorndyke and Candia Carnevali, 2001).

Another hypothesis to explain the origin of the observed differences in growth and differentiation rates according to the size to be regenerated is the presence of a proximal–distal chemical gradient in the arm induced and maintained by one or several specific sites (organizers) such as that observed in *Hydra* (Holstein et al., 2003).

Arm explants are a simplified and controlled regenerating system which may be useful in regeneration experiments by providing a valuable test of our hypothesis in terms of mechanisms and processes (Candia Carnevali et al., 1998). In *A. filiformis*, isolated explants underwent similar differential energy allocation to growth as those observed with whole individuals. Explants cut closer to the disc regenerated more rapidly than those cut close to the tip. In explants, growth rates were 10 times slower than those observed in whole animal experiments. This observation is not surprising since explants

are not able to acquire energy from food or receive allocation of stored reserve from disc and/or arms as is observed for whole individuals (Nilsson, 1998; Nilsson, 1999). All the energy involved in regeneration is limited to the stored energy of the explant itself. Moreover, in crinoids, regeneration is largely dependent on migratory stem cells (coelomocytes and amoebocytes) than can originate far from the regenerating site (Candia Carnevali and Bonasoro, 1994). In consequence, the number of cells available for the development of a blastema is likely to be far less in an explant than in complete individuals. This limitation leads to the recruitment of myocytes (Candia Carnevali et al., 1998), although this alternative mechanism does not compensate for the difference in growth rate between explant and whole individuals. As also observed in crinoids (Candia Carnevali et al., 1998), *A. filiformis* explant blastemal regeneration appears to be directional and a strict proximal–distal axis is maintained. The isolated explant underwent regenerative processes similar to those of its respective donor arm on the distal part but not on the proximal part, where processes stopped after the repair stage and no blastema is formed. This too must have significant implications for the presence of developmental factors that regulate positional information such as segment polarity genes.

#### Consequences on further research

Our results have several implications for regeneration research in general (standardization, plasticity, etc.) and it seems important to re-visit regeneration in *A. filiformis* and other brittlestars.

An unexpectedly high variability in the observed regeneration rate, is the rule in brittle star regeneration (Salzwedel, 1974; Andreasson, 1990; D'Andréa et al., 1996; Nilsson and Sköld, 1996; Sköld, 1996; Sköld and Gunnarsson, 1996; Sköld and Rosenberg, 1996; Gunnarsson et al., 1999; Mallefet et al., 2001; Thorndyke et al., 2003; Selck et al., 2004) and can mask many of the differences among the treatments (D'Andréa et al., 1996) or lead to contradictory results (Gunnarsson et al., 1999; Selck et al., 2004; Granberg, 2004). Our results demonstrate that taking into account the length lost on one arm is a simple and tractable way to standardize experiments and thus significantly decrease the variability of studied parameters (e.g. regeneration rate). Moreover, differentiation rate (DR1 or DR2) is also a parameter that can be influenced by environmental factors and therefore this too should be integrated in further studies; for example, acute and chronic toxicity tests recently developed using echinoderm regeneration (Walsh et al., 1986; Gunnarsson et al., 1999; D'Andréa et al., 1996; Novelli et al., 2002; Candia Carnevali et al., 2001a; Candia Carnevali et al., 2001b; Candia Carnevali et al., 2003; Selck et al., 2004; Granberg, 2004; Barbaglio et al., 2004).

Time of regeneration is the classical parameter used in molecular, cellular, histological, dynamics and ecological studies of this capacity in *A. filiformis* (Mallefet et al., 2001; Patruno et al., 2001; Thorndyke et al., 2001; Thorndyke et al., 2003; Bannister et al., 2005). A further consequence of this

trade-off between growth and differentiation is that a regenerate of the same size and/or same regeneration time can present very different characteristics in terms of differentiation and functional recovery according to the position of autotomy along the arm. In consequence, the use of time of regeneration is inappropriate, especially in dynamic studies. This can be illustrated by studies on the dynamics of functional regeneration using natural bioluminescence (i.e. the emission of visible light by living organisms). A huge and unexpected variability was observed between the percentage of bioluminescence recovery and time of regeneration (Mallefet et al., 2001; Thorndyke et al., 2003). Similar experiments taking into account our results in the analysis lead to a significant decrease of the variability and more consistent results (S. Dupont, personal observation).

Our results are currently guiding future experimental designs by defining standard conditions for proteomic and genomic studies in progress in our laboratory. They provide a valuable tool for further molecular studies. Manipulation of the length lost will allow the study of regeneration in different cellular and tissue environments (regulation of the trade-off between proliferative growth and differentiation) and the assessment of the impact of individual growth/regulatory factors on this phenomenon. Our results give, for the first time a temporal framework for the analysis of regeneration dynamics.

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