



Delayed arm regeneration in the Antarctic brittle star *Ophionotus victoriae*

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ABSTRACT: We describe the levels of natural arm damage in the Antarctic brittle star *Ophionotus victoriae* and the rate of arm regeneration over the period of 1 yr, as measured in our aquarium facilities at Rothera Point, West Antarctic Peninsula. The natural incidence of arm damage in *O. victoriae* is high (97% of individuals examined). The rate of regeneration was 0.44 mm wk⁻¹, which is slow, but within the range of data from temperate brittle stars and also in line with data from the literature showing a general trend of decreasing arm regeneration rate with temperature. The Q_{10} for arm regeneration rate of 2.6 across brittle stars is within the expected biological range. However, *O. victoriae* arm regeneration experiences a lag phase of up to 5 mo before reproducible amounts of new tissue are produced. Such a long lag phase has not been documented for any other brittle star species and produces a range of Q_{10} values from 3.6 (when compared to the slowest documented lag phase for a temperate brittle star) to exceptionally high Q_{10} values of 14.9 and 15.4 (when either fastest or average regeneration times are considered for temperate brittle stars and this Antarctic species). This indicates that the initial process of arm regeneration in *O. victoriae* is either different to that of other brittle stars or it is dependent on factors other than the effects of temperature on enzyme-mediated biochemical reactions, such as gonadal cycles and seasonality.

KEY WORDS: Regeneration · Echinoderm · Ophiuroid · Wound healing · Antarctic

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INTRODUCTION

Echinoderms, in general, represent one of the more important taxa in Antarctic ecosystems, constituting 45% of the large epifauna in terms of both numerical abundance and weight (Moya et al. 2003). Ophiuroids predominate, representing 75% of total numerical abundance and 36% of total weight (Moya et al. 2003). The dominant ophiuroid in coastal waters around the Antarctic Peninsula is *Ophionotus victoriae* (Arnaud et al. 1998). It is found at depths between 5 and 1266 m and, although it has been observed as a primary scavenger, its dietary regime is highly variable, including cannibalism (Fratt & Dearborn 1984, McClintock 1994). Research on this organism so far has been restricted to gross ecological observations (McClintock 1994, Arnaud et al. 1998),

aging (Dahm & Brey 1998) and gametogenic cycles (Grange et al. 2004).

In this paper we expand the studies on *Ophionotus victoriae* to arm regeneration processes. This is a natural phenomenon, often the result of sub-lethal predation or environmental disturbance (e.g. hydrodynamism) and forms a major portion of their secondary metabolic production with significant energetic costs (Bourgoin & Guillou 1994, Sköld & Rosenberg 1996). Echinoderm limb regeneration is used as a biological tool in a number of highly divergent disciplines. These range from ecological, as an estimate of predation pressure (Sköld & Rosenberg 1996), through to *in vivo* models to study the molecular events underpinning cellular regeneration (Dupont & Thorndyke 2006, 2007). In particular, arm regeneration in ophiuroids is a useful and easy-to-use tool to assess the

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impact of an environmental change at cellular, physiological and population level energy allocation, impact on growth and differentiation and fitness (Dupont & Thorndyke 2006).

We have a long-standing interest in the capacity of Antarctic ectotherms to cope with elevated environmental temperatures using physiological metrics such as the ability to perform critical biological functions (Peck 2002, Peck et al. 2004). The work described here will be similarly used, with arm regeneration in *Ophionotus victoriae* used as a cellular metric of this organism's capacity to cope with increased seawater temperatures in line with current predictions for regional climate change of the Antarctic Peninsula. However, firstly we needed to obtain an overview of this process in the natural environment and find a standardised method to assess the effect of temperature change. The main questions for this study were as follows: How frequent is limb damage in natural populations of *O. victoriae*? What is the timescale of the regeneration process? What are the parameters that influence the regeneration rate?

MATERIALS AND METHODS

Study area. The 2 sites (Hangar Cove and South Cove) used for collections are adjacent to the British Antarctic Survey research station at Rothera Point, Adelaide Island, West Antarctic Peninsula (67° 34.5' S, 68° 07.0' W). The sites are 1 km apart and either side of the headland. They have a similar bathymetry with inclines of ca. 30° but differ with regard to substratum composition. Hangar Cove consists of compacted cobbles with an overlying layer of silt, whereas South Cove is a mixture of hard bedrock and areas of compacted cobbles (Smale et al. 2007). The prevailing winds are from the north and as a result small icebergs and brash ice frequently accumulate in Hangar Cove during summer. South Cove has a more sheltered aspect and is often ice-free during summer. Fast ice forms at both sites over winter, but its extent is extremely variable between years and usually forms in Hangar Cove before South Cove (Smale et al. 2007). Iceberg scouring is a relatively common hazard for benthic communities at both sites, with typically 1 iceberg impact per 2 yr period at 10 to 16 m depth (Brown et al. 2004). At the shallower study depths, impacts are less intense but more frequent, as much as 1 impact yr⁻¹ (Smale et al. 2007).

Animal sampling. The brittle stars used in experimental work were collected by SCUBA divers during the austral summer (January). Three separate collections were made over a 2 yr period and assessed during this set of experiments. These were from 3 different

depths: 6 and 13 m in South Cove (South Cove I and II, respectively) and 18 m in Hanger Cove. The animals were immediately taken to the laboratory where they were maintained in a through-flow aquarium with natural seawater under a simulated natural light:dark cycle. Predicted sunrise and sunset times (POLTIPS 3, Proudman Oceanographic Laboratory) were used in conjunction with a mechanical timer to control the lighting regime. During the time the brittle stars were held in the aquarium the water temperature was $-0.5 \pm 0.4^\circ\text{C}$. The brittle stars were fed weekly on chopped white fish meat (*Notothenia coriiceps*) to satiation. Any uneaten food was removed from the tank after 24 h. Only animals with natural damage were used in the survey/experiment, arm damage caused by collection was virtually absent.

Morphometric measurements. After anaesthesia with 3% magnesium chloride solution in seawater, several parameters were measured on each individual: (1) disc diameter (since the disc of *Ophionotus victoriae* is pentagonal, not circular, 3 measurements were taken of the disc diameter using vernier callipers and the average diameter calculated); and (2) each arm was measured and assessed for damage (length of visible regenerative portion and number of scars).

Regeneration experiments. We used 280 brittle stars for this study: one arm was amputated from each individual approximately 10 segments from the disc and the animal was returned to the aquarium. Each month over the course of a year, 15 brittle stars were sampled at random from the aquarium and the length of the regenerating portion of the amputated arm was measured and photographed (cf. Fig. 6). This random sampling technique was developed (rather than measuring all animals each month) to minimise disturbance of the animals, to avoid any potential effects of repeated anaesthesia on limb regeneration and also to avoid damage from handling. Additionally the regenerated limb was amputated and stored for future molecular analyses. The animals were then returned to the sea.

Calculations and statistics. We analysed size-frequency distributions of the disc diameter using the Bhattacharya (1967) method in order to estimate number, mean and standard error of Gaussian components (cohorts) using FISAT II software (FAO-ICLARM Stock Assessment Tools). Regeneration rate (mm wk⁻¹) was calculated as the slope of the significant simple linear regression between the regenerated length (mm) and time (wk). Simple linear regression models were used to test the relationship type between the variables. ANOVA and ANCOVA were used to determine the significance of the observed differences between groups. The Shapiro-Wilk test (Shapiro & 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 1990).

RESULTS

Size and age measurements

Each assemblage was assessed for size frequency of the individuals (Fig. 1). The South Cove I assemblage showed a young dominating cohort (68% of the sampling, mean size \pm SD = 17.4 ± 2.2 mm) and an older cohort (32%, mean size 25.9 ± 1.8 mm); the South Cove II population had only one cohort (mean size 17.7 ± 2.6 mm); and the Hangar Cove population had a young cohort (25%, mean size 21.5 ± 3.2 mm) and an older dominating cohort (75%, mean size 28.9 ± 3.3 mm).

Natural arm damage

Each assemblage was surveyed for the number of arms damaged on each individual (Table 1) with the position of the amputation (proximal, medial or distal) (Table 2). Over 97% of the brittle stars surveyed had some detectable damage, with arms either in the process of regenerating or showing less than ideal arm length. Whilst there were differences between the 3 assemblages with regard to the number of arms damaged per individual (with Hangar Cove having the most individuals with damage to all 5 arms), in general over 50% of individuals surveyed showed damage to all 5 arms. The mean disc size of brittle stars with 5 regenerating arms was significantly greater than that of those with fewer or no regenerating arms, indicating that larger (and presumably older) individuals had more damaged arms (ANOVA: $F = 15.1$, $df = 5$, 273, $p < 0.001$) (Fig. 2). There was no correlation between disc size and the percentage length of arms undergoing regeneration (data not shown). As regards the position of the breaks in each arm, both groups from South Cove showed the majority of the damage to be distal, whilst damage to Hangar Cove animals was concentrated in the central portion of the arm (Table 2). The number of multiple scars on single arms was only noted for the South Cove I and Hangar Cove assemblages. The South Cove animals had significantly fewer arms with multiple scars (3%) compared to the Hangar Cove group (15%).

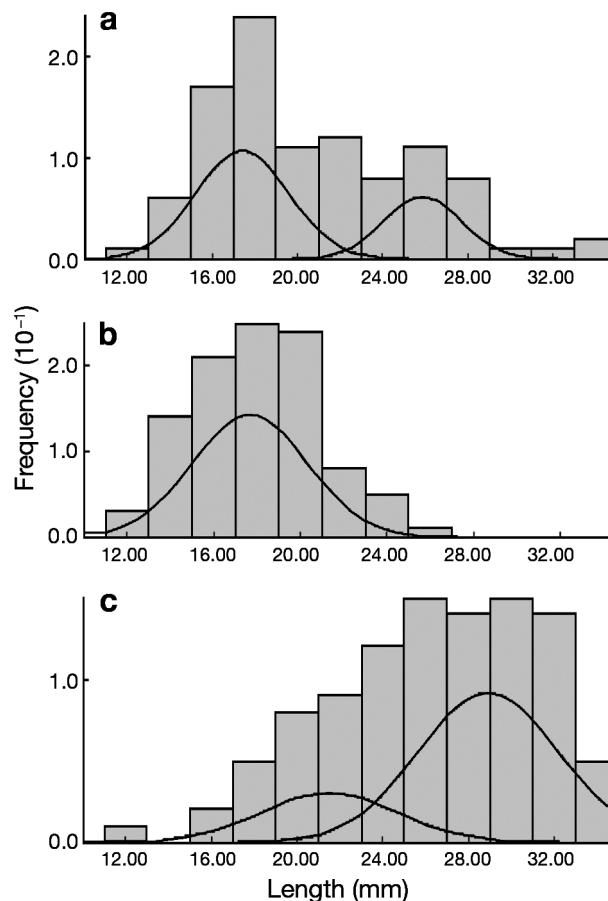


Fig. 1. *Ophionotus victoriae*. Size-frequency distribution (bars) of disc diameter at the 3 studied locations: (a) South Cove I, (b) South Cove II, and (c) Hangar Cove. Curves in (a) and (c) indicate separate cohorts. Sample size = 280 animals

Table 1. *Ophionotus victoriae*. Percentage values for the number of damaged arms per individual in the 3 assemblages

Assemblage	n	Damaged arms ind. ⁻¹					% ind.	% arms	
		0	1	2	3	4			5
South Cove I	100	1	1	2	15	24	57	99	86.2
South Cove II	80	4	4	10	14	20	48	96	86.2
Hangar Cove	100	3	3	2	11	10	71	97	73.0
Overall	280	2.5	2.5	4	13	18	60	98	81.3

Table 2. *Ophionotus victoriae*. Position of damage on arms for the 3 assemblages. Mean and median values are shown as the position index (Bourgoin & Guillou 1994). This is equivalent to the majority of the damage being distal for both the South Cove populations, but medial for Hangar Cove animals

Assemblage	Proximal (%)	Medial (%)	Distal (%)	Mean	Median
South Cove I	6	26	68	0.71	0.77
South Cove II	5	21	74	0.73	0.77
Hangar Cove	13	39	48	0.64	0.69

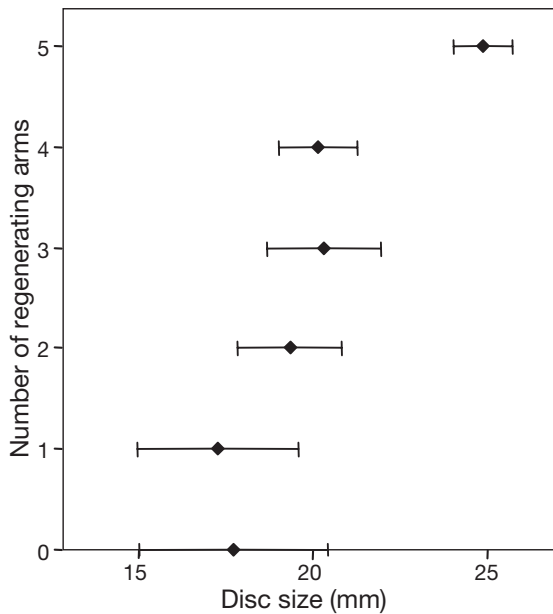


Fig. 2. *Ophionotus victoriae*. Amount of arm damage (total number of damaged arms per animal) compared to mean disc size (± 2 SD). Sample size = 280

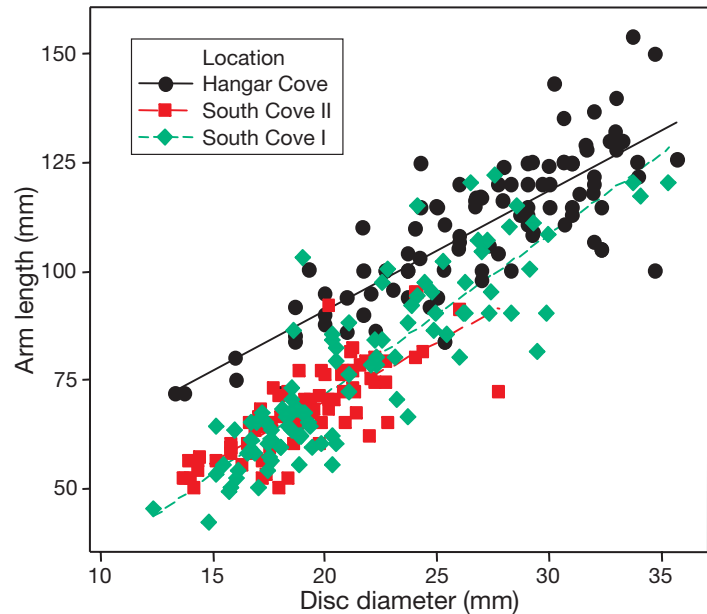


Fig. 3. *Ophionotus victoriae*. Correlation between disc diameter and arm length in the 3 assemblages (see also data in Table 1). Sample size = 280

Table 3. *Ophionotus victoriae*. Fitted line data for arm length and disc diameter in the 3 assemblages (see Fig. 2 for plots). F , p and R^2 values are shown for ANOVA-General Linear Model analyses of fitted lines

Assemblage	Intercept	SE	Slope	df	p	F	R^2
South Cove I	-2.23	4.65	3.69	1	0.000	307.77	75.8
South Cove II	14.79	4.79	2.75	1	0.000	124.11	61.4
Hangar Cove	35.71	5.43	2.76	1	0.000	193.34	66.4

Disc diameter related to arm length

To assess regeneration rates it was important to establish the relationship between disc diameter and the size of an ideal arm (i.e. an arm without any observable evidence of regeneration). The longest arm was measured for each animal and this metric plotted against disc size for all 3 assemblages separately (Table 3, Fig. 3). In all cases, ideal arm length was significantly correlated to disc diameter; however, the analysis of the different groups highlighted some differences. All animals within each assemblage showed the same rate of increase in arm length with disc diameter ($p = 0.997$), but there was strong evidence for a difference in intercepts ($p = 0.001$). Brittle stars of a given disc diameter in Hangar Cove had longer arms than those of a similar disc size from South Cove II; however, the higher rate of increase at South Cove II meant that this disparity decreased with increasing length. The rate of increase in arm length with disc

diameter at Hangar Cove was lower than at South Cove I (ANOVA-General Linear Model test, see Table 3); however, brittle stars at South Cove I with small disc diameters had slightly shorter arms than those at Hangar Cove, whereas in the intermediate sizes, the South Cove I animals' arms were longer, i.e. the fitted lines cross. The analysis described above assumed that the underlying

relationship between arm length and disc diameter was a straight line and not curvilinear. Fitting a quadratic model to test for curvature produced a relatively small increase in R^2 and was not statistically significant ($p > 0.05$). However, the fitted curves all showed a similar reduced rate of increases with increasing disc diameter (data not shown). Combining the 3 datasets, allowing for different slopes and intercepts, including a quadratic term gave a statistically significant effect ($p = 0.036$). However the contribution to the fit was relatively small and did not affect the main conclusions concerning differences in lines for the 3 locations.

Regeneration

Regeneration over the course of the 1 yr study period was slow, with only a single individual showing a hint of a regenerating arm after 3 mo; 2 individuals had

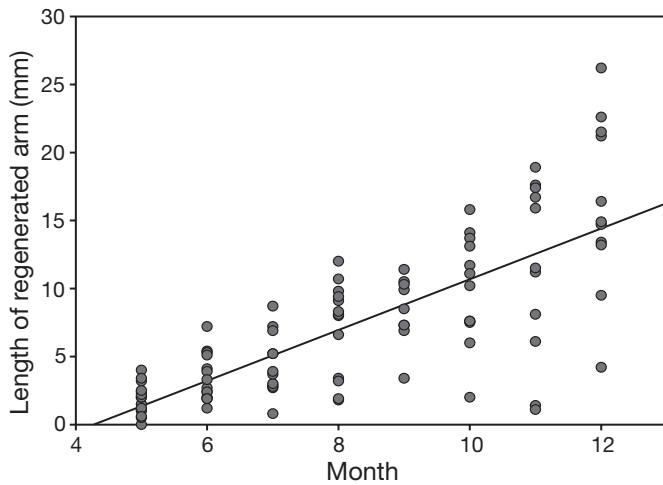


Fig. 4. *Ophionotus victoriae*. Monthly increase in length of regenerated arms. There was no measurable regeneration prior to Month 5. $R^2 = 0.573$. Sample size = 180

measurable arm regeneration at 4 mo, whilst at 5 mo, the process was reproducible with 14 out of the 15 brittle stars sampled that month having measurable arm regeneration. Therefore, regeneration lengths were only plotted for Months 5 to 12 (Fig. 4). A total of 7% showed no arm regeneration, approximately 3% showed damage to their regenerating arm and a further 3% had serious damage to their other arms in addition to their amputation. None of the data for these animals were analysed, but they were recorded as part of the monthly sampling effort. The regeneration rate (mm wk^{-1}) was calculated as the slope of the significant simple linear regression between the regenerated length (mm) and time (mo). Analysis of the slope of the line (ANOVA: $F = 127.29$, $p = 0.000$, $R^2 = 0.573$) produced an average rate of arm regeneration of 0.44 mm wk^{-1} (Fig. 4). However there was a large inter-individual variation in this rate, such that after 1 yr, one individual had only regenerated 4.2 mm of the amputated arm (5.6% re-growth when compared to the length of the longest arm), whilst the fastest regeneration produced 26.2 mm of re-growth (33% of original arm length). To explain this variability, we assessed the impact of the amount of tissue lost ('length lost': a key parameter, which represents the quantity of tissue needed to completely regenerate an intact arm with no previous history of regeneration; Dupont & Thorndyke 2006) on regeneration rate. The regeneration rate was plotted against length of arm removal (length lost). The regeneration rate increased from 0.2 to 0.7 mm wk^{-1} when the length lost increased from 30 to 100 mm ($p < 0.01$; Fig. 5).

The form of regenerative growth was also noted. The process appeared to be similar to other brittle stars, such as *Ophiura ophiura* and *Ophiothrix fragilis* (S.

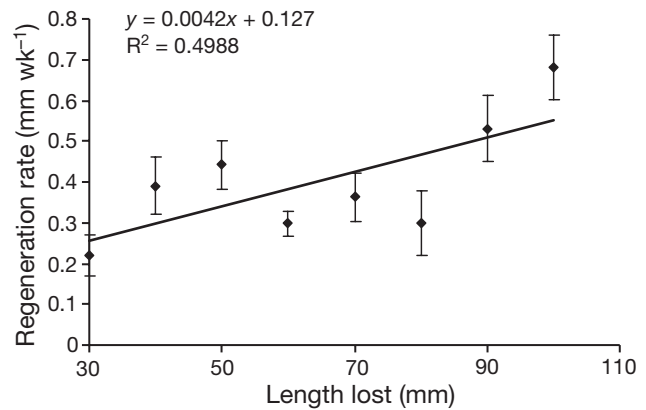


Fig. 5. *Ophionotus victoriae*. Regeneration rate of arms compared to the length of arm lost (with amount of arm amputated collected in 8 bins). Sample size = 280



Fig. 6. *Ophionotus victoriae*. Regenerating arm at Month 10. Photographed at $10\times$ magnification. Note absence of elongation section; the limb is completely differentiated throughout the whole length

Dupont pers. comm.) with an initially undifferentiated structure followed by gradual differentiation (i.e. development of spines and functional podia) of up to 25% by Month 8 onwards and 50% after 12 mo (Fig. 6).

DISCUSSION

Incidence of damage

Species level

Limb damage in ophiuroids is a common phenomenon (Lawrence 1990) and the Antarctic brittle star *Ophionotus victoriae* is no different in this respect; 97% of individuals sampled had some level of damage (Table 1),

with an average proportion of 80% of arms surveyed showing scars. This value is high compared to many ophiuroid species, but is similar to levels observed in *Amphiura chiajei* caught off the west coast of Ireland (Munday 1993) and *Microphiopholis gracillima* (85%; Stancyk et al. 1994). However, a previous study relating the amount of arm damage to ophiuroid lifestyle showed more damage in infaunal suspension- and deposit-feeding species compared to epibenthic suspension feeders and epibenthic carnivores. Brittle stars from the latter category had between 13 and 62% of arms regenerating, depending on the species and location (Sköld & Rosenberg 1996). *O. victoriae* also falls into this latter group, being described as a eurybathic, non-selective species regarding bottom type with a catholic diet (Moya et al. 2003, D. A. Smale pers. comm.), so it clearly has a higher than expected average amount of arm damage (by at least 20%) given its lifestyle and comparable data from related temperate brittle stars.

A literature survey by Fujita (2001) emphasised the problems associated with estimating the average amount of arm damage for any particular brittle star species. He (op. cit.) also divided the ophiuroid species into habitat types, but these do not convey the whole story, as illustrated by epibenthic deposit feeders; e.g. *Ophiura albida* collected from 3 different samplings around Scotland showed arm damage of 14, 13 and 89%, whereas *Ophiura ophiura* collected from Scottish waters, the Kattegat-Skagerrak and the Adriatic showed damage levels of 62, 24 and 27%, respectively. Therefore environment and lifestyle probably play the major roles in incidence of ophiuroid arm damage, rather than such being necessarily species-specific.

Assemblage level

The position of the amputations in the arms (distal, rather than proximal) (Table 2) suggests that at least one of the causes of the damage (that of arm cropping by demersal fish) remains the same in this Antarctic species compared to temperate ophiuroids (Aronson 1991). However, brittle star arms are not commonly found in Antarctic fish gut contents and the only species regularly reported to consume ophiuroid arms, *Gobionotothen gibberifrons*, is rare or absent at our study site (Caseux et al. 2003). Therefore, another cause of damage seems likely. Icebergs have a major effect on shallow polar benthos (Peck et al. 1999, Smale et al. 2007), from minor impacts to destruction of whole communities. Certainly, environmental factors can affect the arm regeneration process in brittle stars (McAlister & Stancyk 2003). A comprehensive survey of arm damage in different *Amphipholis squamata* populations showed differences in damage levels of

between 19.3 and 79.3% (Dupont 2002). This was related to habitat, with protected lagoon animals having a much lower (33.9%) level of damage compared to those from the intertidal (75.8%). Whilst predators exist in all the populations surveyed, there are more abiotic disturbances in the intertidal zone (tides and movement of rocks) compared to the lagoon. The structure of the habitat can then explain the observed differences. These data exemplify the role of the environment in ophiuroid arm damage: abiotic factors (habitat, waves, tides, water chemistry, icebergs etc.) and biotic disturbances (mainly predation) combine with the physiology of the regeneration process in the individual species to produce a complex picture in surveys of natural arm regeneration in brittle stars.

To a certain extent, this environmental effect is supported by the observation that the Hangar Cove brittle stars are bigger and have longer arms than those of South Cove at shallower depths (Table 2, Fig. 3). The level of disturbance due to icebergs at South Cove was 1.5× greater than at Hangar Cove in 2004–2005, principally due to the disparity in longevity of winter fast ice at the 2 sites (Smale et al. 2007), but in the previous 2 yr the fast ice longevity was greater (Brown et al. 2004). Disturbance frequency is also related to depth, and therefore it is not surprising that the Hangar Cove brittle stars at 18 m depth are on average larger, and by inference, older (Dahm & Brey 1998) than those in South Cove (Fig. 1). As brittle stars are highly mobile, it is impossible to monitor and compare the rate of arm damage in the natural environment. Therefore it is only possible to hypothesise that the higher percentage of damaged arms in the Antarctic brittle star may simply be a reflection of the additional problems caused by ice damage. Indeed, the 'older' Hangar Cove brittle stars have more double scars than those 'younger' individuals sampled from shallower depths, and taking the data from all 3 groups, the larger animals are more likely to have 5 regenerating arms (Fig. 2). A greater incidence of damage in Antarctic ophiuroids compared to temperate species also may be a function of slower growth and greater age.

Regeneration rate

The slow growth rate of *Ophionotus victoriae* has already been documented (Dahm & Brey 1998), and therefore the observed slow arm regeneration rate results should not be surprising. Comparing the *O. victoriae* data with those for temperate brittle stars shows a significant correlation ($p < 0.01$) between temperature and regeneration rate. Although there is a lot of variation between species (some of which could be due to sampling regimes), *O. victoriae* provides the lowest

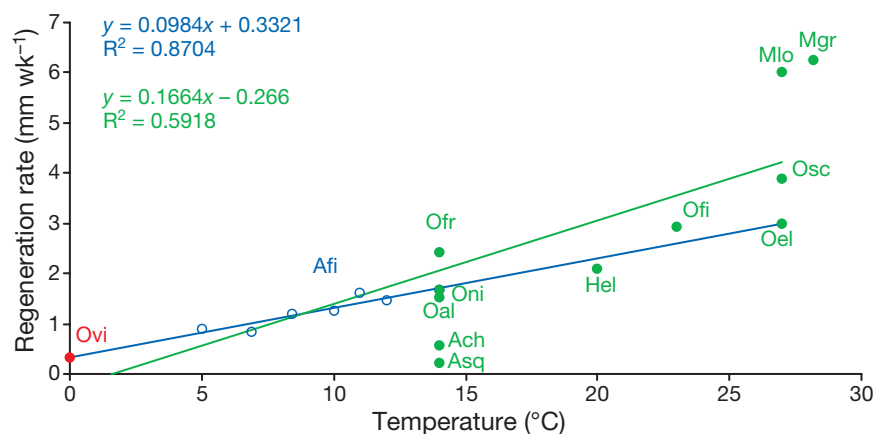


Fig. 7. Correlation between temperature and regeneration rate for *Amphiura filiformis* (Afi, in blue) and other brittle star species. Red: Ovi = *Ophionotus victoriae*. Green: Ach = *Amphiura chiajei*; Asq = *Amphipholis squamata*; Hel = *Hemipholis elongata*; Mgr = *Macrophipholis gracillima*; Mlo = *Macrophipholis longipeda*; Oal = *Ophiura albida*; Oel = *Ophiarthrum elegans*; Ofi = *Ophiophragmus filigraneus*; Ofr = *Ophiothrix fragilis*; Oni = *Ophiocomina nigra*; Osc = *Ophiocoma scolopendrina*. See Table 4 for references

data point on the graph, with a significant linear relationship to temperature (Fig. 7). Growth in Antarctic marine ectotherms is slow, compared to species from lower latitude, and growth rates are generally slowed by 2× to 10×, compared with temperate and tropical species (Peck 2002). Development rates of embryos and larvae are likewise markedly slowed, compared to species from warmer waters, by 5× to 10× in most groups (Pearse et al. 1991, Peck et al. 2006, 2007). In the context of these data, the slowing of regeneration rate in the Antarctic *O. victoriae* is in line with slowing of development and growth processes in Antarctic species.

The *Ophionotus victoriae* data indicate a clear demarcation in the regeneration process, with the initial wound healing stage proceeding at a different rate to that of the actual limb regeneration process. Once limb regeneration has started, the rate is low, compared to that of temperate brittle stars (Fig. 7, Table 4). When the *O. victoriae* regeneration rate is compared to temperate species to identify any temperature effect using the Q_{10} coefficient, a value of 2.6 is obtained, which is within expected biological ranges. Even so, this means that an Antarctic brittle star with an 80 mm arm could take over 3 yr to re-grow a complete arm. This rate is an average value and there is considerable individual variation. The data from Fig. 4 at 12 mo show such variation with one *O. victoriae* having only re-grown 4.2 mm of arm, whilst at the other

extreme, another had re-grown 26.2 mm. Other factors, including intrinsic variability in regeneration rate, food availability and stores, will have an effect here. Similarly long periods of time for whole arm re-growth are also documented for a few temperate brittle stars (e.g. Stancyk et al. 1994). However, comparison of the length of arm lost in *O. victoriae* with regeneration rate produces a correlation ($R^2 = 0.4988$) between the 2 metrics. So the longer the length of arm lost, the faster the regeneration rate. This makes biological sense in that loss of an arm potentially represents an impaired ability to compete for and obtain food.

Table 4. Regeneration rate (RR) of different ophiuroid species measured at different temperatures and relationship with the arm length lost (LL; nt = not tested)

Species	RR (mm wk ⁻¹)	Temp. (°C)	'Length lost' effect	Source
<i>Amphipholis squamata</i>	0.25	14	nt	Dupont et al. (2001)
<i>Amphiura chiajei</i>	0.2 to 1	14	RR = 0.02 × LL	S. Dupont & M. Thorndyke (unpubl.)
<i>Amphiura filiformis</i>	0.8 to 1.5	7 to 12	nt	Thorndyke et al. (2003)
	0.5 to 3.5	14	RR = 0.52 e ^{0.03 × LL}	Dupont & Thorndyke (2006)
<i>Hemipholis elongata</i>	2.1	20	nt	McAlister & Stancyk (2003)
<i>Macrophipholis longipeda</i>	6	26 to 29	nt	Chinn (2006)
<i>Microphipholis gracillima</i>	6.25	28	nt	D'Andrea et al. (1996)
<i>Ophiarthrum elegans</i>	3	26 to 29	nt	Chinn (2006)
<i>Ophiocoma scolopendrina</i>	3.6 to 4.2	26 to 29	nt	Chinn (2006)
<i>Ophiocomina nigra</i>	1.5 to 2	14	RR = 0.01 × LL + 1.30	S. Dupont & M. Thorndyke (unpubl.)
<i>Ophionotus victoriae</i>	0.22 to 0.68	0.75	RR = 0.0042 × LL + 0.127	This study
<i>Ophiophragmus filigraneus</i>	2.94	23	nt	Clements et al. (1994)
<i>Ophiothrix fragilis</i>	2.2 to 2.8	14	RR = 0.01 × LL + 2.03	S. Dupont & M. Thorndyke (unpubl.)
<i>Ophiura albida</i>	1.2 to 1.8	14	RR = 0.01 × LL + 1.13	S. Dupont & M. Thorndyke (unpubl.)

Wound healing and blastema formation

The initial stages of arm regeneration in ophiuroids are poorly understood at the cellular level. They are thought to include morphallactic wound healing processes with initial expansion and migration of epidermal cells, followed by rapid formation of an epimorphic blastemal structure due to accumulation of coelomocytes. Cell division and neurogenesis are also thought to play a role (Candia Carnevali 2006). It is these processes that comprise the 'lag' phase of regeneration. The lag phases in temperate (12 to 14°C average environmental temperature) ophiuroid limb regeneration vary according to species and location; this phase can be minimal, often lasting only 2 to 4 d (S. Dupont pers. obs.), but may be as long as 20 d (Bourgoin & Guillou 1994). The maximum lag phase documented in the literature is 1 mo for *Astrobrachion constrictum*, which lives at 10.5 to 14.5°C (Stewart 1996). *Ophionotus victoriae* is slower than all others, with no reproducibly measurable re-growth in the first 5 mo, although a few animals showed some arm regeneration in Months 3 and 4. On average, the lag phase in *O. victoriae* lasts 4.7 mo (141 d). Q_{10} coefficients can be calculated from these data to estimate the relative rates for this process. At the most conservative, using the slowest times documented for *O. victoriae* of 5 mo (150 d) and for the temperate brittle star *Astrobrachion constrictum* of 1 mo (30 d) at temperatures of 0 and 12.5°C, respectively, produces a Q_{10} of 3.6. However, using data for the fastest regeneration time in *O. victoriae* (3 mo or 90 d) and the fastest time for a temperate brittle star (2 d) with a 14°C temperature difference produces a much higher Q_{10} of 14.9. Similarly an average value of 4.7 mo (141 d) for *O. victoriae* and 3 d for a temperate brittle star with a 14°C temperature difference results in a Q_{10} of 15.4.

Q_{10} coefficients are often used to compare the change in rates of biological processes across temperatures. The coefficient was initially devised to compare single or limited groups of biochemical reactions. However, almost a century of physiological research into the effects of temperature at the individual, tissue or cell level has shown that thermal dependence falls within a narrow range of rates, roughly doubling to tripling with each 10°C rise in temperature, and the overwhelming majority of processes fall within the range $Q_{10} = 1$ (i.e. no change) to 4 for each 10°C. Values within this range are generally taken to show that there is no temperature compensation in the process. Values outside that range either show temperature compensation ($Q_{10} < 1.0$), or that the process being examined is affected by something other than the direct effect of temperature on enzyme-mediated systems. Q_{10} values in the range of 14 to 15 (as calculated

above) indicate that some other factor is important in initial wound healing processes. Some echinoderms show extreme seasonality with very low activity and metabolic rates in winter (Brockington & Peck 2001). Grange et al. (2004) showed that the gut index in *Ophionotus victoriae* decreased between September and November, as energy was diverted into reproductive effort. In parallel, the gonad index of *O. victoriae* peaked between October and November, with spawning in December. Hence, by the time our experiment started, all brittle stars had spawned, and because they were being fed ad libitum, they would be in peak condition when their limbs were amputated. No seasonality in feeding behaviour was observed in the individuals kept in the aquarium; therefore it would be difficult to justify either seasonality or gametogenesis as causal factors for delayed arm regeneration. Also, the lag phase in the experiment ran across the end of summer to the start of winter; therefore, other factors may be causal, for example crossing of a threshold minimum temperature that inhibits progress, or changes in calcium solubility in seawater at low temperatures, which make the regeneration process much more difficult. This prolonged lag phase requires significant research.

SUMMARY

This study documents the extent of natural arm damage and regeneration in Antarctic brittle stars. Arm regeneration rates in *Ophionotus victoriae* are slow, but once re-growth has started, the rate of growth is in line with expected effects of temperature on regeneration in brittle stars, i.e. the lower the temperature, the slower the rate. Why the regeneration process in *O. victoriae* is so clearly partitioned into 2 stages, with such a long lag phase, is unknown. However, because this lag phase is so long, it provides an excellent metric to use as a response to temperature change. It can also provide clues as to the exact nature of the delayed cellular processes, which could potentially be partitioned into cell type and/or cell function.

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