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Regeneration as a novel method to culture marine ornamental sabellids

J.M. Murray [a,b,*], G.J. Watson [b], A. Giangrande [c], M. Licciano [c], M.G. Bentley [d]

[a] Centre for Environment, Fisheries and Aquaculture Science (Cefas), Pakefield Road, Lowestoft, Suffolk NR33 0HT, UK
[b] Institute of Marine Sciences, School of Biological Sciences, Ferry Road, Eastney, Portsmouth PO4 9LY, UK
[c] Department of Biological and Environmental Sciences and Technologies, University of Salento, 1-73100 Lecce, Italy
[d] Dove Marine Laboratory, School of Marine Science and Technology, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU, UK

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ABSTRACT

Collection of live invertebrates from coral reefs has increased dramatically over the past two decades in response to the growing marine aquarium industry, and currently, more than 500 species (excluding corals) are traded globally. Aquaculture of ornamental species is deemed a priority solution in mitigating the effects of wild collection but expanding the range of species is limited by bottlenecks at key life history stages. A novel culture method for ornamental sabellids, which utilises their outstanding regenerative capacity in a process similar to coral 'fragging', has been developed and survivorship after regenerative development assessed. *Sabella pavonina*, a temperate species found around the UK, was used as a model to develop a culture technique which was subsequently transferred to a tropical species of *Sabellastarte*. Survivorship of *S. pavonina* was high (≥80%) in individuals which had been cut into as many as eight fragments and all fragments completed regenerative development within a four week period. *Sabellastarte* species exhibited ≥75% survivorship when cut into just two fragments, but higher mortality was recorded with increasing number of cuts, with only 20% of fragments from individuals cut into eighths surviving the duration of the experiment. Both test species were capable of regenerating cephalically and caudally within a four week period. Caudal regeneration involved the healing of the cut surface, reconstruction of the pygidium and subsequent segment addition, while cephalic regeneration was a more complex process of wound healing, reconstruction of a new mouth and the development of the branchial crown structure. It is concluded that differences in survivorship between *S. pavonina* and *Sabellastarte* sp. could be attributed to either infection due to sub-optimal water quality in the test tanks, or species-specific differences in the area of wound size in relation to the length of the fragment. Optimisation of survivorship and the speed of regenerative growth could be improved with the enhancement of the culture system.

1. Introduction

The majority (90–99%) of ornamental species which supply the marine aquarium trade are obtained from coral reefs, with Indonesia and the Philippines dominating the export market (Tlusty, 2002; Wood, 2001b). The trade is of low volume but high market value (*e.g.* reef fish harvested for food from one country were valued at US$6000 per tonne compared with the US$496,000 per tonne harvested for the aquarium trade (Bunting et al., 2003; Cohen et al., 2013)) and has the potential to provide economic stability for many rural, low-income coastal communities (Baquero, 1999). However the trade is controversial and has been implicated as a major contributor to the demise of coral reef ecosystems (Green, 2003).

There is growing demand for improved research and development of captive bred aquarium organisms to relieve pressure on coral reefs (Bruckner, 2000) and the aquaculture sector is often seen as a priority solution in reef habitat conservation (Pomeroy and Balboa, 2004). Despite approximately 90% of all ornamental freshwater fish being reared in captivity, only 1–10% of marine ornamental fish are bred commercially (Cohen et al., 2013; Moorhead and Zeng, 2010; Olivotto et al., 2011). Even fewer invertebrates have been reared successfully and are limited to corals (Arvedlund et al., 2003), shrimps (Calado et al., 2003, 2007, 2008) and giant clams (Bell and Gervis, 1999; Bell et al., 1997). Expanding the range of invertebrate species cultured using current technologies is problematic and constrained by bottlenecks at key stages in their life histories (Olivotto et al., 2011). Still, it is recognised that development of aquaculture projects would be most valuable to countries where livelihoods are reliant upon the collection and provision of ornamentals, and where the benefits of integrating conservation and sustainability of coral reef resources would be greatest (Bell et al., 1997; Parks et al., 2003; Wabnitz et al., 2003). Therefore, there is a requirement for simple

* Corresponding author at: Centre for Environment, Fisheries and Aquaculture Science (Cefas), Pakefield Road, Lowestoft, Suffolk NR33 0HT, UK. Tel.: +44 15025 24584.
E-mail address: Joanna.Murray@cefas.co.uk (J.M. Murray).

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and cheap culture technologies which can be transferred to local communities in developing countries.

Ornamental fan worms (Polychaeta, Sabellidae) and coco worms (Polychaeta, Serpulidae) are routinely collected to supply the aquarium trade, with recent estimates suggesting as many as 18,500 individuals sold annually in the UK alone (Murray et al., 2012). Sabellids build their own mucoid-sediment tubes, whereas serpulids secrete tubes of calcium carbonate, from which, brightly coloured branchial crowns (block colours of yellow, red, purple and white to combinations of 2 or more) extend. Both tend to attach to hard substrates, often within a crevice or burrow on the surrounding reef framework. Removal is consequently a delicate procedure that can damage both the worm and the surrounding reef habitat (Bybee et al., 2006).

Efforts to understand aspects of sabellid reproduction and life history are underway to facilitate the future culture of this group (Bybee et al., 2006, 2007; Tamaru et al., 2008, 2011). Such efforts include manipulating the timing of spawning, improving larval survivorship and reducing the time taken to reach market size. In addition to manipulating sexual reproduction, the regenerative capacity of sabellids could provide an alternative method of culture. Sabellids can regenerate both caudally and cephalically from any segment (Berrill and Mees, 1936; Licciano et al., 2012), with some species being capable of regenerating an entire individual from a single segment taken from the mid-body within a matter of weeks (Bely, 2006). Such powers of regeneration could be exploited to supply the ornamental trade.

In this investigation, the boreal sabellid species *Sabella pavonina* was used as a model for the development of a simple culture technique exploiting the process of regeneration. Developed methodologies have also been adapted for the tropical *Sabellastarte* sp. By optimising this regenerative ability, the techniques can be transferred to low-income coastal communities reliant on supplying the marine ornamental trade.

2. Materials and methods

2.1. Specimen collection and maintenance

Specimens of *S. pavonina* were collected from Sword Sands, an intertidal soft sediment shore in Langstone Harbour, Portsmouth, UK. In the laboratory aquarium, worm tubes were partially buried in boxes of sediment and placed in flow-through tanks supplied with seawater from Langstone Harbour (filtered through a settlement tank and sand filters) at ambient conditions. Imported specimens of *Sabellastarte* sp. from the Batam Island area (Riau Archipelago) 20 km off Singapore's south coast, were obtained from the Tropical Marine Centre (TMC) Ltd, London, in bags of artificial seawater and oxygen. Specimens were acclimatised to stock tank conditions at least three days prior to the experiments to allow recovery and assess their quality and health. Stock tanks were held in quarantine facilities at 25 °C. Water from each tank was filtered using an under-gravel filter system with medium size coral gravel (44% >4 mm, 54% 2–4 mm, and 1% 1–2 mm). Stock tank cultures were fed 3–4 times per week on a diet of Marine Snow® (Two Little Fishies, Inc.).

2.2. Sectioning

Prior to sectioning, individuals were removed from their tubes by applying gentle pressure with the finger tips from the most posterior end of the tube towards its anterior region. Specimens were subsequently bisected anterio-posteriorly using dissecting scissors, resulting in an anterior and a posterior fragment (Fig. 1a). This first cut was applied at the point equidistant from the first thoracic segment and the last posterior segment of the individual (not including the branchial crown in overall length). To investigate regenerative capabilities of worms cut into multiple fragments, two further conditions were introduced, quarters (Fig. 1b) and eighths (Fig. 1c). Mid-body fragments contained roughly the same number of segments as the anterior and posterior fragments, and were composed of abdominal segments only.

2.3. Assessment of survivorship and regenerative development

Anterior and posterior fragments were required to heal and regenerate from a single cut surface, whereas mid-body fragments were required to heal and regenerate along both anterior and posterior cut surfaces in order to survive. An assessment of survivorship was completed daily. Live individuals were identified as those contracting and relaxing with structurally intact chaetae and an open branchial crown (anterior fragments only). Regeneration and reorganisation were assessed visually at seven-day intervals during a four-week regeneration period (*i.e.*, 7, 14, 21 and 28 days after sectioning), using a dissection microscope and image capture software. A series of images were taken at the regenerating surfaces of all individuals as a record of weekly growth. Using two morphological keys – one for posterior ends regenerating cephalically (Table 1) and another for anterior ends regenerating caudally (Table 2) – each worm was assigned to a numbered stage of regenerative development. It should be noted that due to the low survival of *Sabellastarte* sp. in Experiment 2, all treatments were combined when assessing key stage analysis.

2.4. Experimental set-up

Experiments were maintained in temperature and photoperiod controlled rooms. *S. pavonina* trials were held at 15 °C and *Sabellastarte* species at 25 °C with constant light conditions (L:D 12:12; intensity, 40 μmol m^{-2} s^{-1}). Additional food was not provided during the experimental period to ensure consistent conditions across treatments, as 'crownless' worms would be unable to feed. All experimental treatments were matched with two control groups, one comprising an equal number of whole, uncut specimens of each species within their original tubes, the other comprising an equal number of whole uncut specimens of each species removed from their tubes. Anterior, posterior and mid-body fragments are collectively referred to as regenerating fragments; halves, quarters and eighths are collectively referred to as treatments, and no tube and in tube are referred to as the controls.

2.4.1. Experiment 1 — Regeneration of worms cut into two fragments (Fig. 1a)

Twelve specimens of each test species were cut in half, producing 24 anterior and 24 posterior fragments (12 sets of half fragments per species). Each fragment and each control specimen was kept separately in glass Petri dishes and placed in biologically filtered seawater tanks using under-gravel filter grids, airlifts attached to twin outlet pumps and coral gravel, for four weeks. Tanks were treatment-specific so that physicochemical variables of the water could be measured and related to each treatment (see Section 2.5).

2.4.2. Experiment 2 — Regeneration of worms cut into two, four and eight fragments (Fig. 1a, b, c)

Specimens of both test species were cut in two (n = 10 per species), four (n = 10 per species) and eight (n = 10 per species) fragments. All treatments (*i.e.*, anterior, posterior, mid-body fragments and control specimens) were kept in individual 500 ml plastic beakers with daily water changes.

2.5. Physiochemical variables

Physiochemical conditions of supplied seawater were measured daily. Temperature and salinity were measured using a WTW microprocessor conductivity meter (LF 196), pH using a Jenway 3505 pH

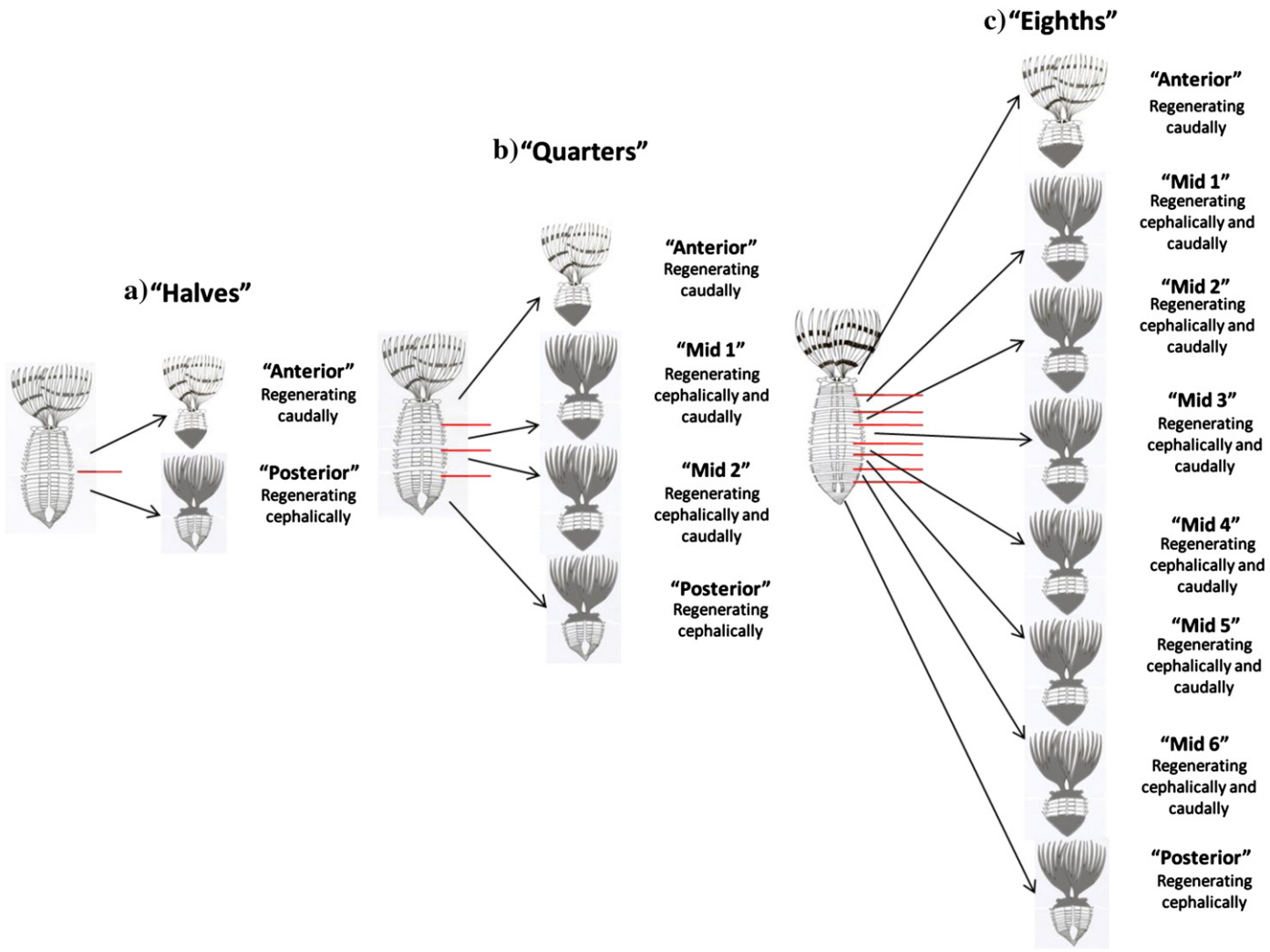

Fig. 1. Schematic drawing of the process of sectioning. Specimens removed from their tube were cut using dissecting scissors into: (a) halves, (b) quarters and (c) eighths, from the first thoracic segment to the last abdominal segment. Fragments containing the head (prostomium) are referred to as anterior fragments, those containing the tail (pygidium) are referred to as posterior fragments, and all other fragments are referred to as mid-body fragments. Anterior fragments regenerated caudally, posterior fragments regenerated cephalically, and mid-body fragments, containing only abdominal segments, regenerated both cephalically and caudally. Tissue replaced by regeneration is shaded grey.

meter with ATC probes, dissolved oxygen using a WTW Oxi 315i probe and complete ammonia (ammoniacal nitrogen) using a Palintest Photometer 7000se and associated reagents. Because of the large number of replicates in Experiment 2, three representative beakers of anterior, posterior and mid-body fragments were chosen at random for daily sampling of water quality parameters.

2.6. Statistical analysis

The Kaplan–Meier estimator was used to estimate the survival function of each treatment during Experiments 1 and 2 for both *S. pavonina* and *Sabellastarte* sp. This is based on a simple estimate of the probability of surviving from time 1 (t^1) to time 2 (t^2): $1 - d / n$, where d is the number of fragments failing between t^1 and t^2 and n is the number that could have failed in the interval. A log-rank test was used to test the null hypothesis that the survival curves of all the experimental treatments were the same, overcoming the limitations of comparing survival at a single point in time. A G-statistic was then calculated using the unplanned test of homogeneity of replicates for pair-wise comparisons (Sokal and Rohlf, 1995), to compare overall survivorship in different treatments. All data were assessed for normality and equality of variance. Daily physiochemical measurements from Experiment 1 were used to produce a tank mean (to avoid repeated measures) with standard error of the mean (±SE), and between-treatment differences were tested using a one-way ANOVA. All randomly sampled beakers from Experiment 2 were used in the ANOVA model.

3. Results

3.1. Experiment 1

Survivorship of *S. pavonina* was ≥80% at the end of the four-week regeneration period. No significant difference in the survival curves of the controls and regenerating fragments was observed (Fig. 2). Additionally, there were no differences in the water quality measurements between control and regenerating treatment tanks.

All surviving fragments restored missing body parts within the experimental period. At day 7, both cephalic and caudal wound-healing and blastema formation were observed, but by day 14, variability between individuals regenerating cephalically had increased, with 9% at key stage 3 (formation of rudimentary radioles), 36% at key stage 4 (development of 3 segments and appearance of pinnules on branchial crown radioles) and 55% at stage 6 (eye spot development on the crown) (Table 1). Inter-individual variability decreased by day 21, with

Table 1
Percentage of fragments at each key stage of posterior regeneration reached by anterior fragments of *Sabella pavonina* and *Sabellastarte* sp. from Experiments 1 and 2 measured at days 7, 14, 21 and 28 after sectioning. Key stages are defined as: (1) wound healing — smooth covering of the cut surface with wound epithelium, (2) blastema — formation of a bilobed blastema around the mouth, (3) rudimentary radioles — definition of future radioles on the blastema, (4) 3 segments — formation of the head, collar and pro-thoracic segments with addition of new chaetae already in thoracic position and serrations for future pinnules upon the extending radioles, (5) eye spot development — the addition of between 1 and 4 eye spots at equal distance along the length of the extending radioles, (6) 5 eye spots — addition of 5 eye spots in total; only after prolonged growth are eye spots added to the radioles.

		Days post surgery	Key stage of development					
			1	2	3	4	5	6
Experiment 1		7	91	9				
S. pavonina		14			9	36	55	
		21					100	
		28					64	36
Experiment 2	1/2s	7	10	40	50			
S. pavonina		14			20	60	20	
		21				10	90	
		28				10		90
	1/4s	7		59	41			
		14				62	38	
		21					100	
		28					24	76
	1/8s	7	41	51	8			
		14			7	82	11	
		21				3	97	
		28				3	24	73
Experiment 1		7	72	28				
Sabellastarte sp.		14		9	18	55		
		21				9	91	
		28					100	
Experiment 2 *Sabellastarte*		7	73	21	6			
sp.		14		6	12	70	12	
		21			3	6	91	
		28					100	

Table 2
Percentage of individuals at each key stage of anterior regeneration reached by posterior fragments of *Sabella pavonina* and *Sabellastarte* sp. from Experiments 1 and 2 measured at days 7, 14, 21 and 28 after sectioning. Key stages are defined as: (1) wound healing, covering of the cut surface with wound epithelium and invagination to form the anus; (2) formation of the pygidium, a cone-like projection around the anus; (3) initial 6–8 segments defined along the extending mesoderm; (4) emergence of chaetae on newly defined segments; (5) additional segments, the merging of regenerated segments into normal growth.

		Days post surgery	Region	Key stage of development				
				1	2	3	4	5
Experiment 1		7		83	17			
S. pavonina		14			83	17		
		21			17	42	42	
		28					33	67
Experiment 2	1/2s	7		38	62			
S. pavonina		14				100		
		21				38	38	24
		28						100
	1/4s	7		18	82			
		14			14	86		
		21				18	21	61
		28				4	4	92
	1/8s	7		30	70			
		14			13	83	3	1
		21				18	17	70
		28				1	1	98
Experiment 1		7	B	50	50			
Sabellastarte sp.		14	B	18	55	9	18	
		21	B	9	18	9	36	27
		28	B			12	33	55
Experiment 2		7		57	43			
Sabellastarte sp.		14		14	62	17	7	
		21		3	7	3	48	39
		28			3	3	18	76

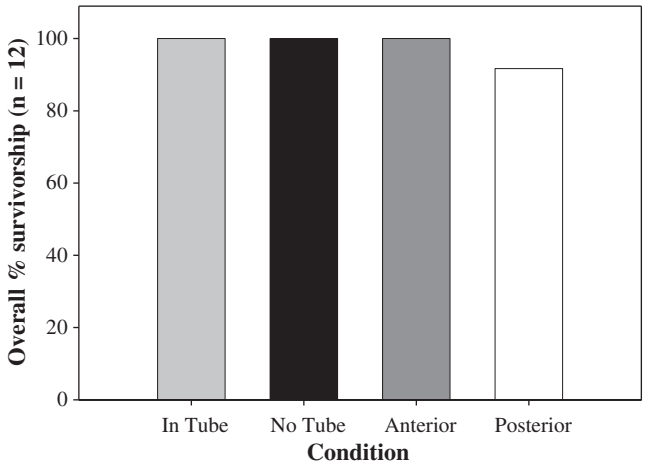

Fig. 2. Percentage survivorship of *Sabella pavonina* at the end of Experiment 1 and 28 days of regeneration for experimental conditions: in tube, no tube, anterior and posterior (n = 12).

all individuals (posterior fragments) showing at least one eye spot on the regenerated branchial crown (stage 5), and 36% showing complete regenerative development at the end of the experiment. Variability in caudal development was greatest at day 21, with individuals (anterior fragments) spanning 3 stages of development, from blastema formation (stage 2) to the pigmentation of developed chaetigers (stage 4) (Table 2). Sixty-seven percent of individuals had completed regenerative caudal development by day 28.

The log-rank test showed a significant difference in the survival curves of the four treatments of *Sabellastarte* sp. (*i.e.*, anterior, posterior, in tube and no tube) (Fig. 3). When overall survivorship was tested, anterior fragments had significantly higher mortality than the controls ($F_3 = 5.63$, $P = 0.003$), although survivorship did not differ between either of the regenerating treatments. In addition, water in the tank containing anterior fragments had significantly ($F_{3,141}$ 2.74, $P = 0.045$) higher concentrations of complete ammonia (0.04674 mg $l^{-1} \pm$ 0.15754) compared with the tank containing posterior fragments (0.01840 mg $l^{-1} \pm 0.06115$), and that containing the control specimens (0.01825 mg $l^{-1} \pm 0.04295$). This observation was the highest mean measurement of complete ammonia across all trials.

At day 7, 72% of *Sabellastarte* sp. individuals had completed cephalic wound healing (stage 1). Variability in development between regions

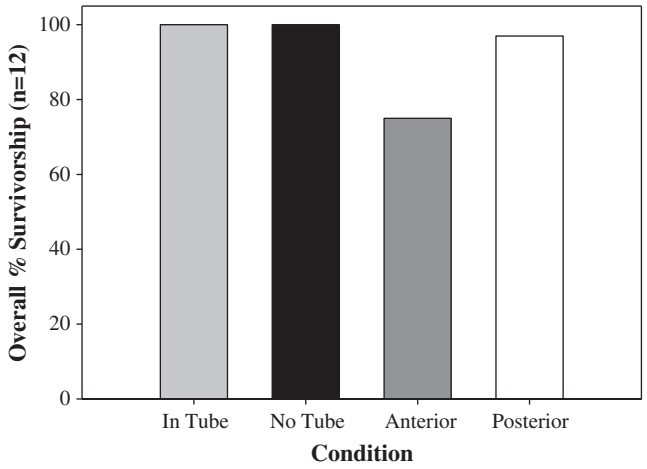

Fig. 3. Overall percentage survivorship of *Sabellastarte* sp. at the end of Experiment 1. The four experimental conditions: in tube (n = 12), no tube (n = 12), anteriors (n = 12) and posteriors (n = 12) following a regeneration period of 4 weeks are shown.

was greatly reduced by day 21 and at the final day of assessment 100% of individuals had reached key stage 5. No individuals from any region had completed cephalic development at the end of the four week regeneration period based on key stage analysis.

Caudal development was a variable process in *Sabellastarte* sp. following initial wound healing and blastema formation at day 7 (Table 2). On day 14, formation of the pygidium around the anus (stage 2) was the most commonly observed stage of development (55%), although wound healing to chaetae formation were observed concurrently. All five key stages of development were recorded in individuals at day 21, although key stage 4 was the most frequently observed. At the end of the experiment, only 55% of individuals had completed tail regeneration.

3.2. Experiment 2

Survivorship of *S. pavonina* cut into multiple fragments exceeded 80% at the end of the regeneration period, however, a significant difference was observed between the survival curves of the control and regenerating treatments (Fig. 4). Lowest survivorship (80%) was observed in the anterior fragments. The water associated with this treatment also exhibited a significantly higher mean concentration of complete ammonia (0.6929 mg $l^{-1} \pm 0.3859$, $F_{2,237} = 72.61$, $P = <0.001$), the lowest mean concentration of dissolved oxygen (5.8476 mg $l^{-1} \pm 0.3927$, $F_{2,237} = 35.33$, $P = <0.001$) and the lowest mean pH (7.96 ± 0.24, $F_{2,237} = 16.78$, $P = <0.001$) when compared with the other treatments. Temperature and salinity remained constant (25 °C ± 1 °C and 34 psu ± 1) across all treatments.

All surviving fragments of *S. pavonina* regenerated missing body parts. Mid-body segments were grouped according to treatment (quarters or eighths) and the direction of regeneration (cephalic, caudal or both) was assessed. At day 7, posterior halves had the highest percentage (50%) of individuals at stage 3 of cephalic development (the most developed stage observed), followed by the mid-body quarter fragments (41%) and the mid-body eighth fragments (8%) (Table 1). On day 14, ≥60% of fragments from all treatments had reached stage 4, having regenerated 3 segments and pinnules on branchial crown radioles. By day 28, 90% of posterior halves, and 76% of quarter and 73% of eighth mid-body fragments had completed cephalic development, with the regeneration of 5 pigmented eye spots on the branchial crown.

During the first week of caudal development ≥62% of *S. pavonina* fragments in all treatments had healed the posterior cut surface and formed the blastema (Table 2). Development was synchronised in the anterior halves on day 14, with 100% of individuals at stage 3 (6–8 segments formed). Also by day 14, there was a high variability in the development of the mid-body eighth fragments, all exhibiting morphological characteristics ranging from stage 2 (blastema formation) to stage 5 (complete regenerative development). A high proportion of fragments from all treatments attained complete regenerative development (stage 5) by day 28 (halves, 100%; quarters 92%; eighths 98%).

Fragments of *Sabellastarte* sp. experienced high levels of mortality, however significant differences were observed between the survival curves of all treatments. Only the in tube specimens had 100% survivorship at the end of the experimental period (Fig. 5). The next highest survival rate was recorded for the half fragments (75%). Survivorship for the quarter fragments and for the eighth fragments was significantly lower than in other treatments, with ≥60% surviving to the end of the experiment. Survival decreased with increasing number of fragmentation, with only 35% of mid-body quarter fragments surviving and 19% of mid-body eighth fragments (Fig. 4). Within each regeneration treatment (Fig. 4), the posterior fragments always exhibited a higher survivorship relative to the anterior fragments. Mid-body fragments displayed different rates of

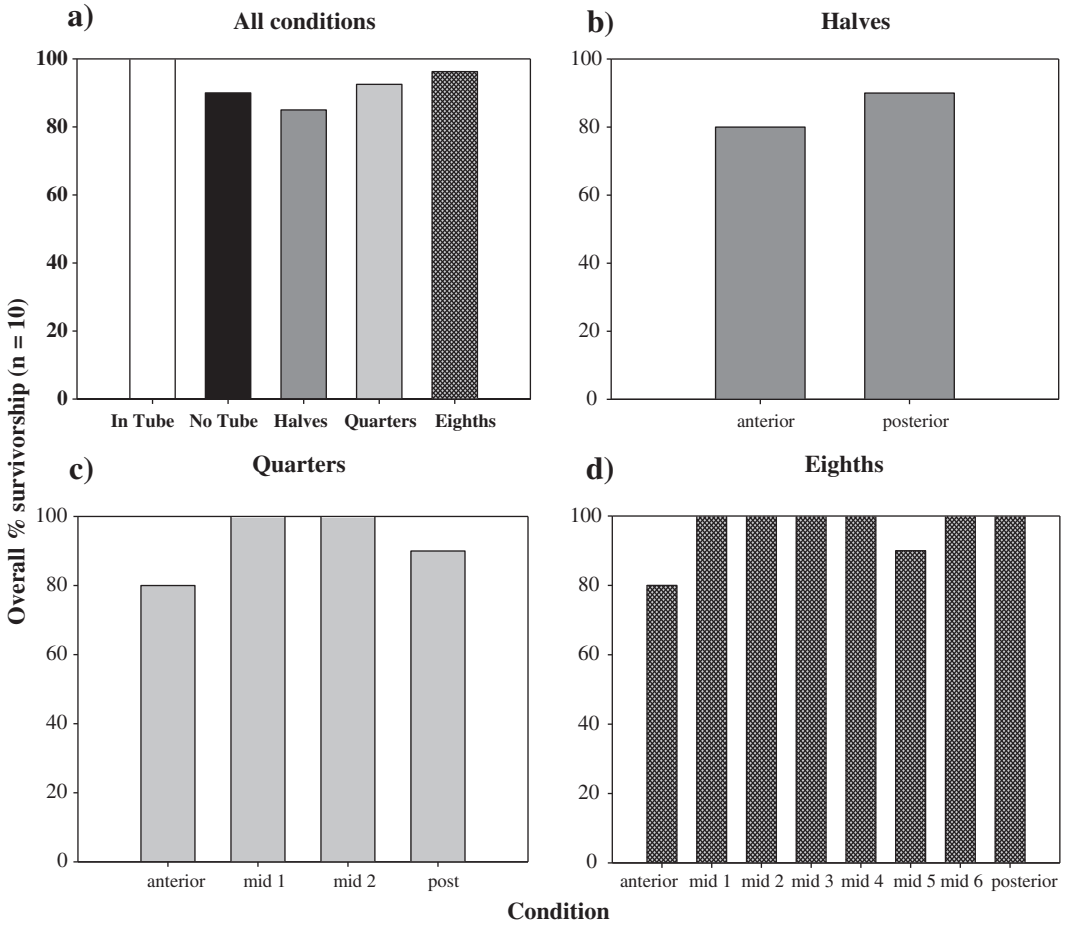

Fig. 4. (a) Percentage survivorship of *Sabella pavonina* at the end of Experiment 2 for experimental conditions: no tube (n = 10), halves (n = 20), quarters (n = 40) and eighths (n = 80) and specific overall percentage survivorship of all treatments (anteriors, posteriors and mid sections) within each condition: (b) halves, (c) quarters and (d) eighths.

survivorship and regeneration depending on where in the 'parent' body they originated. For specimens cut into eighths, mortality was 100% in fragments Mid 1, Mid 2, Mid 3 and Mid 4, but from Mid 5 onwards, survivorship increased. The rearmost segment of all 8 (the one with the pygidium) exhibited the highest level of survivorship (70%).

Water temperature and salinity levels remained constant between all treatments. Beakers containing only anterior fragments and control specimens correlated with the highest mean values of complete ammonia (1.3024 mg l^{-1} ± 0.8919 and 1.5093 mg l^{-1} ± 0.7127 respectively) and were significantly higher ($F_{3,111} = 25.99$, $P = <0.001$) than those measured in the beakers containing only posterior and mid-body fragments. The anterior fragment and control treatments also had the lowest pH (7.87 ± 0.2034 and 7.76 ± 0.1048 respectively), which was significantly lower than the treatments comprising posterior and mid-body fragments ($F_{3,111} = 19.56$, $P = <0.001$). The lowest concentration of dissolved oxygen was recorded in the anterior fragment treatment (4.2318 mg l^{-1} ± 0.4873), which was significantly lower ($F_{3,111} = 6.69$, $P = <0.001$) than in the posterior fragment treatment (4.7038 mg l^{-1} ± 0.5449).

All surviving fragments of *Sabellastarte* sp. regenerated missing body parts and were grouped as described for *S. pavonina*. At day 7, most posterior halves regenerating cephalically (73%) had healed the wound surface and 6% had reached stage 3 with the appearance of rudimentary radioles (Table 3). Variability reached a maximum at day 14, with posterior fragments ranging from blastema formation (6%) to pigmentation of the branchial crown (12%), although 70% of fragments were restricted to the formation of 3 segments and the definition of pinnules. At the end of the regeneration period, 100% of posterior fragments had a pigmented branchial crown but complete regeneration to stage 6 was not observed in any individual. Caudally regenerating anterior fragments exhibited signs of wound healing (57%) and blastema formation (43%) 7 days after sectioning (Table 2). Variability reached a maximum at day 21, with anterior fragments recorded at all 5 key stages of caudal development. At the end of the four-week regeneration period, 76% of anterior fragments had reached the final stage of development, with segment regeneration merging with normal growth.

4. Discussion

This study demonstrates the potential of utilising the regenerative capacity of sabellids as a novel method of aquaculture to supply the marine ornamental trade. The procedure was deliberately 'low-tech' (*e.g.* no anaesthetics or antibiotics were used) to achieve a simple method which can easily be transferred to communities that depend on the trade, where only basic equipment may be available, but where its implementation would be most beneficial (Wabnitz et al., 2003).

4.1. Regeneration and survivorship

Both sabellid species examined were found to be very robust, surviving and regenerating missing appendages within 4 weeks. Individuals of *S. pavonina* and *Sabellastarte* sp. which had been sectioned only once exhibited the highest rate of survival. Survivorship remained above 80% in individuals of *S. pavonina* which were sectioned up to eight times, however this was not the case for *Sabellastarte* sp. Species of *Sabellastarte* are shorter in length but

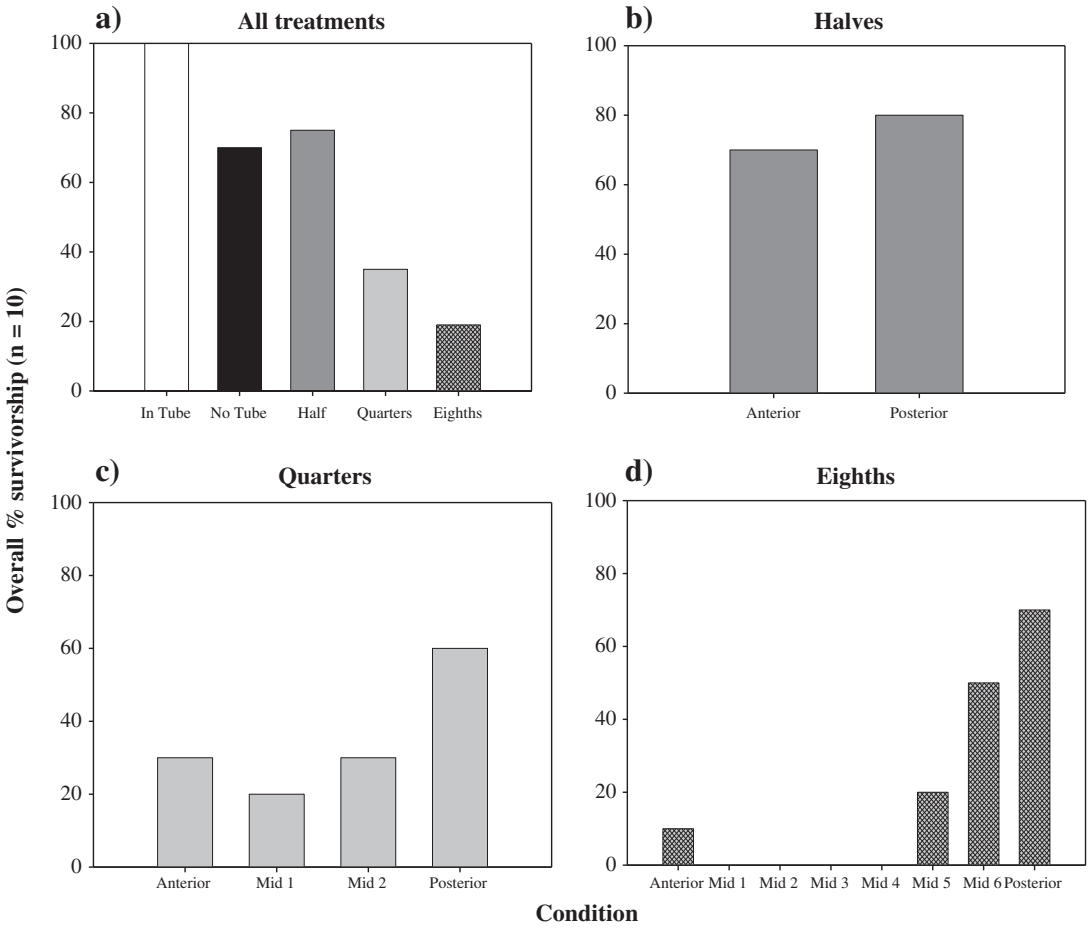

Fig. 5. Percentage survivorship of *Sabellastarte* sp. at the end of experiment 2. Experimental conditions: (a) in tube (n = 10), no tube (n = 10), halves (n = 20), quarters (n = 40) and eighths (n = 80) are shown and specific overall percentage survivorship of all treatments (anteriors, posteriors and mid fragments) within each condition: (b) halves, (c) quarters and (d) eighths.

have a much higher body mass compared with *S. pavonina*, consequently, an increase in the number of fragments also increases the wound surface area to fragment length ratio, resulting in wound surface area exceeding the size of fragment length, further reducing the fragment's ability to effectively close the wound. The higher survivorship rate of posterior fragments supports the theory that the tapering pygidium results in a reduced wound surface area to fragment length and therefore limited fluid and tissue loss.

Contrary to previous studies in sabellids, which found no anterior–posterior gradient with regard to regenerative ability (Berrill, 1931; Fitzharris, 1973), results presented here suggest a survival advantage in the most posterior fragments. Anterior fragments demonstrated a higher mortality compared to posterior fragments. Initially it was thought that the energetic demands of cephalic regeneration (by posterior fragments) involving new tissue growth and rearrangement would have been greater, however, individuals with an intact branchial crown regenerating caudally showed greater mortality. In some cases this increased mortality rate of anterior fragments was linked with loss or degradation of the branchial crown — a stress indicator in sabellids (Fitzsimons, 1965). The crown was lost in one of three ways: (i) shedding of the branchial crown at the collar segment/thorax junction (as described by Kennedy and Kryvi, 1980), (ii) the gradual degeneration of radioles over time leaving an intact collar segment with dorsal lips and ventral sacs, and (iii) the extensive degeneration of radioles and the collar segment resulting in subsequent death. Berrill (1931) explained crown degeneration in worms regenerating posteriorly by proposing that the structure was reabsorbed to obtain energy reserves stored in those tissues, however the subsequent survival of those individuals was not documented. In the present study, shedding did not occur in every individual, nor did the process and when it did occur, it always results in death. It is possible that those individuals demonstrating crown degradation/loss may have had fewer initial energetic reserves to offset the cost of subsequent posterior regeneration.

Higher mortality in the anterior treatment was associated with the deterioration of the test tank water conditions and included the highest recorded levels of complete ammonia and the lowest concentrations of dissolved oxygen. This is a process of negative feedback where crown degradation or shedding resulting from the

Table 3
Assessing the commercial feasibility of regeneration as a method to propagate ornamental sabellids. Data shown includes: the number of individuals imported annually by the Tropical Marine Centre from three exporting regions: Batam Islands (Indonesia), the Philippines and Kenya using values obtained from Murray et al. (2012); the number of individuals required per month assuming equal distribution of the annual total; the number of worms required from an initial collection of wild stock (based on a 75% crop rate at full term) and the number of worms sold and maintained for re-cutting per week.

Region	No. individuals imported annually to TMC	No. required per month	No. collected initially	No. sold and re-cut each week
Batam Islands (Indonesia)	4448	371	250	94 re-cut 94 to sell
Philippines	1063	92	62	23 re-cut 23 to sell
Kenya	4284	357	240	90 re-cut 90 to sell

reabsorption of energy stored within the crown increases the concentration of ammonia, and reduces the availability of oxygen which in turn, promotes deterioration of condition often leading to death. The next step in the development of this technique is to take the methodologies to field-trials, where the limitations of water quality and nutrition would be avoided.

4.2. Commercial application

A preliminary commercialisation model to assess the commercial feasibility of culturing sabellids using regeneration is presented. The model described is based on using *Sabellastarte* sp. individuals sectioned once (into halves) for which survivorship exceeded 75% (Table 3). For the purpose of predicting a 'time to market' using regenerative propagation, the development of a coloured ornamental branchial crown was used to determine a marketable size in the absence of consumer opinion data. Branchial crown measurements (Murray, 2010) revealed that a maximum of 16 weeks was required to achieve a crown length equivalent to those currently for sale in the UK (4.27 cm). This period is almost half the time taken for individuals to be propagated using conventional sexual reproduction. Optimisation of water quality and the introduction of a feeding regime into the regeneration tanks would further reduce the time to market. This crown grow-out phase would also allow for tube re-construction; regenerated individuals can construct a new tube within ten days (Murray, 2010).

The number of individuals imported into the UK by TMC from three of the major export regions (Murray et al., 2012) was used as the number of individuals required by the market (*i.e.*, demand), and monthly demand based on the equal distribution of this annual import total. Production would commence with the initial collection of wild stock each week, for 16 weeks in the set-up phase. Given an expected 75% survival rate to marketable size, this would result in achieving the monthly demand just 16 weeks after initial stock collection, with batches being harvested every week thereafter.

The business plan described is based on a number of assumptions from the developmental stage of the culture methodology. We predict that by improving water quality parameters and providing additional nutrition, survivorship would further be enhanced to maximise productivity. The next step would be to take this methodology to field-based trials (in flow-through holding tanks (*ex-situ*) or within protective frames which are put back into the sea (*in-situ*)), which is likely to obviate water quality issues.

The proposed commercialisation plan should be implemented within the context of a community-based management (CBM) approach. Small-scale community led projects have had proven success with continued efforts for such projects like the post larval capture and culture of the giant clam *Tridacna maxima* in French Polynesia where 8 spat collecting stations have been set up to provide local farmers with a sustainable livelihood for export to the marine aquarium trade or reared to a larger size for the meat market (http://www.spc.int/aquaculture accessed 5th May 2013). The demand for cultured fan worms may not alone prove to be economically viable. Small coastal communities could propagate fan worms as an alternative to wild collection and alongside other activities, such as coral or live rock farming, maintaining a livelihood for local communities which may otherwise be shifted to developed locations. The CBM approach would encourage ownership of local coral reef systems and promote education of local collectors, including methods of sustainable collection and industry monitoring, as suggested by Murray et al. (2012).

Efforts to achieve artificial sexual propagation of *Sabellastarte spectabilis* (another ornamental sabellid) on a commercial scale are underway in Hawai'i (Tamaru et al., 2008, 2011), but the venture requires investment and infrastructure beyond the reach of many low-income coastal communities. The technique also relies on a supply of sexually mature individuals from the wild and subsequent induction of spawning, which ordinarily is limited to a 2 month window each year. The regenerative propagation techniques described here offer a 'low-tech', low cost method with the potential of a year-round supply of organisms. Collectors could also target more desirable specimens as stock-supply (*e.g.* popular colours) to maximise profits.

4.3. Additional benefits

Like a number of coral reef rebuilding projects (*e.g.* Bowden-Kerby, 2001), propagation of sabellids could be used to repopulate areas of reef where numbers are particularly low. Collection of Sabellidae and Serpulidae is prohibited in Sri Lanka in a government action to prevent their over-exploitation and reduce the indirect effect of their removal; they are an important food source and act as filter-feeders of suspended organic matter (Wood, 2001a). Regenerative propagation could provide an alternative to wild collection, allowing the ban on fan worms to be lifted, and used to repopulate over-exploited reefs. However, regenerated worms are essentially genetic clones of one individual, so the long-term implications of this to the sabellid population structure must first be considered.

Sabellids are also excellent bioremediators of fish aquaculture systems and can effectively remove solid waste from the water column (Giangrande et al., 2005). Future exploration of regenerating ornamental sabellids within an ornamental fish rearing system may prove beneficial for growing both organisms.

Aquaculture of ornamental species is a key priority in the future of the marine aquarium trade. Widespread development of aquaculture practices such as those presented here would reduce possible impacts of wild collection on reef ecosystems and potentially improve the livelihoods of communities at the supply end of the chain.

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