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Mantle-edge regeneration after cropping in the fluted giant clam, *Tridacna squamosa* Lamarck, 1819

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Abstract. Mantle-edge cropping in giant clams is a common phenomenon, but little is known regarding their wound-healing capacity. This study provides a brief account of the healing and regeneration processes after the mantle edges of the fluted giant clam, *Tridacna squamosa* were cropped. Tissue recovery periods were estimated using photographs taken before and after the mantle edges were wounded. We provide observations on two types of wounding: artificial cropping (in a single adult clam) and cropping by three species of fish (in 20 juvenile clams). Observations showed that the adult clam's mantle took ~60 days to regenerate and return to its original state but regeneration took much longer for juvenile clams (up to 90 days). Scarring occurred in the juvenile clams, but was not observed in the only adult involved in the experiment. The ability of *Tridacna squamosa* individuals to survive and regenerate their injured tissues within three months provides preliminary insights into their responses to natural predation and can inform giant clam mariculture and restocking operations.

Key words. giant clam, mantle regeneration, mantle tissue, tissue cropping, Tridacninae, wound healing

INTRODUCTION

Wound healing and regeneration have been well documented among bivalves, particularly commercially important species such as the Pacific oyster *Crassostrea gigas* (Ruddell, 1971), hard clams *Mercenaria mercenaria* (Coen & Heck, 1991) and pearl oysters *Pinctada* spp. (Acosta-Salmón & Southgate, 2005, 2006; Mamangkey & Southgate, 2009). The process of wound healing in bivalve mantle involves the rapid accumulation of haemocytes at the injured region. This prevents excessive haemolymph loss through the formation of a mechanical plug to seal the wound; connective tissue then accumulates around the lesion and facilitates the regeneration of new epithelial cells (Ruddell, 1971; Hodgson, 1982a, 1982b; Acosta-Salmón & Southgate, 2005, 2006; Mamangkey & Southgate, 2009). After excision, the mantle tissue of both *Pinctada fucata* and *Pinctada margaritifera* healed within the first three days and the mantle completely healed and regenerated to their original extent within 90 days (Acosta-Salmón & Southgate, 2005).

Large and conspicuous, giant clams are ecologically significant species on tropical and subtropical Indo-Pacific coral reefs (Neo et al., 2017): they are important as producers of calcium carbonate, providers of food and as reef builders (Mingoa-Licuanan & Gomez, 2002; Vicentuan-Cabaitan et al., 2014; Neo et al., 2015). With their large mantle tissue mass, it is unsurprising that at least 75 predators are known to prey on giant clams—including nonlethal cropping of mantle tissue (Neo et al., 2015). Giant clams are also a popular mariculture species (Heslinga & Fitt, 1987). It is common practice to cut away small portions of their mantle tissue and use them (after some preparation) for zooxanthellae inoculation of early tridacnine larvae (Ellis, 1998). Tissue cropping of the exposed giant clam mantle by fish is a natural and common phenomenon, but wound healing abilities in the clams have not been documented—although Nakayama et al. (1997: p. 110) noted that, "The mantles which harbour symbiotic zooxanthellae must be exposed to external seawater in order to receive sunshine for photosynthesis. Since this requires keeping the shell open and exposing the giant clam to the possibility of external injury, the giant clam must possess a well-developed hemostatic system which includes the agranular cells and the morula-like cells." The present study attempts to fill this knowledge gap by providing a brief account of the healing and regeneration processes of mantle tissue in the fluted giant clam, *Tridacna squamosa* Lamarck, 1819.

MATERIAL & METHODS

We made a posteriori observations after two types of wounding were made on the mantle edges of *T. squamosa* maintained in flow-through marine aquaria at the St. John's Island National Marine Laboratory located on St. John's Island (Pulau Sakijang Bendera), Singapore. The first type of wound was due to artificial cropping: three pieces of mantle tissue (~1 cm^2 each) were excised from the edges of outer mantle using surgical scissors and haemostat forceps for zooxanthellae extraction from a single mature specimen (shell length, SL=348 mm). The second type was due to cropping by reef fish:

three species were introduced at once into a single tank as biological controls of fouling algae. The fishes unexpectedly cropped mantle tissue from a batch of 20 cultured juvenile clams (mean SL=97.8 ± 5.6 mm; mean ± S.E.). The three fish species introduced were: two-spot surgeonfish, *Ctenochaetus binotatus* (n=4), blue-spotted spinefoot, *Siganus corallinus* (n=5) and fox-face rabbitfish, *Siganus puellus* (n=5). All fishes introduced were approximately 4–5 cm long (from snout to tail). The fishes and clams were together for approximately one week. To allow the clams to recover, the fish were removed immediately after cropping was noticed. The adult and juvenile clams were held in two separate tanks located in an outdoor aquarium, exposed to similar seawater conditions (mean salinity of ~33‰ and mean seawater temperature of ~30.5°C). For both wound types, the recovery of mantle tissues was compared and estimated visually for all clam individuals. Only a subset of juvenile clams was photographed to capture the mantle regions before and after mantle cropping (Olympus Tough TG-1 iHS). Any mortality that ensued was also noted.

RESULTS

Shortly after the mantle was cropped, the mantle border of the adult *Tridacna squamosa* at both ends of the wounds rolled inwards towards the centre of the wound. The mantle borders of all three wounds on the adult *Tridacna squamosa* grew back to their original extent within 60 days of excision, including the formation of new hyaline organs and zooxanthellal tubular systems (Fig. 1). However, compared to the original mantle periphery, these regenerated regions appeared to have less zooxanthellae (and these only occurred in small clusters with light brown colouration). Furthermore, regenerated tissue did not appear to follow the previous mantle colour pattern in the adjacent areas of outer mantle (Figs. 1a, 1b).

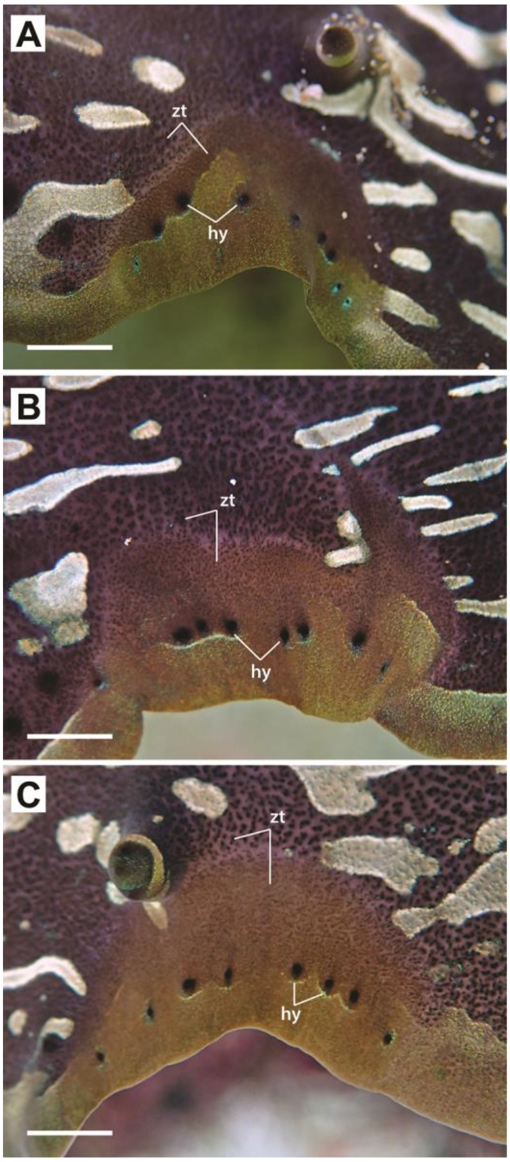

Fig. 1. Regenerated regions of three mantle-clipping wounds (A, B, C) observed on a single adult fluted giant clam, *Tridacna squamosa* (SL=348 mm) after 60 days. Scale bars = 2.5 mm. Abbreviations: zt = zooxanthellar tubules; hy = hyaline organs.

Two days after the introduction of fish, the 20 juvenile *Tridacna squamosa* appeared highly stressed with their mantles partially retracted (Fig. 2). Albeit to varying degrees, tissue cropping was observed to have occurred on all the exposed tissues (mantle edges, exhalant and inhalant siphons) of all clams (Figs. 2a, 2b, 3a, 3c). Based on observations, survival of juvenile clams was 80% at the end of monitoring, with four individuals dying within five days after spending most of this period with their valves fully closed. There were no non-cropped control clams to make a direct mortality comparison. Cropped tissues of surviving clams took ~90 days to heal completely, but some of the tissues showed scarring (Figs. 3b, 3d), that is, the mantle edge did not fully recover to its original extent. Subsequent monitoring revealed no further healing of wounded tissues, but clams had begun depositing new shell material—a sign of good health.

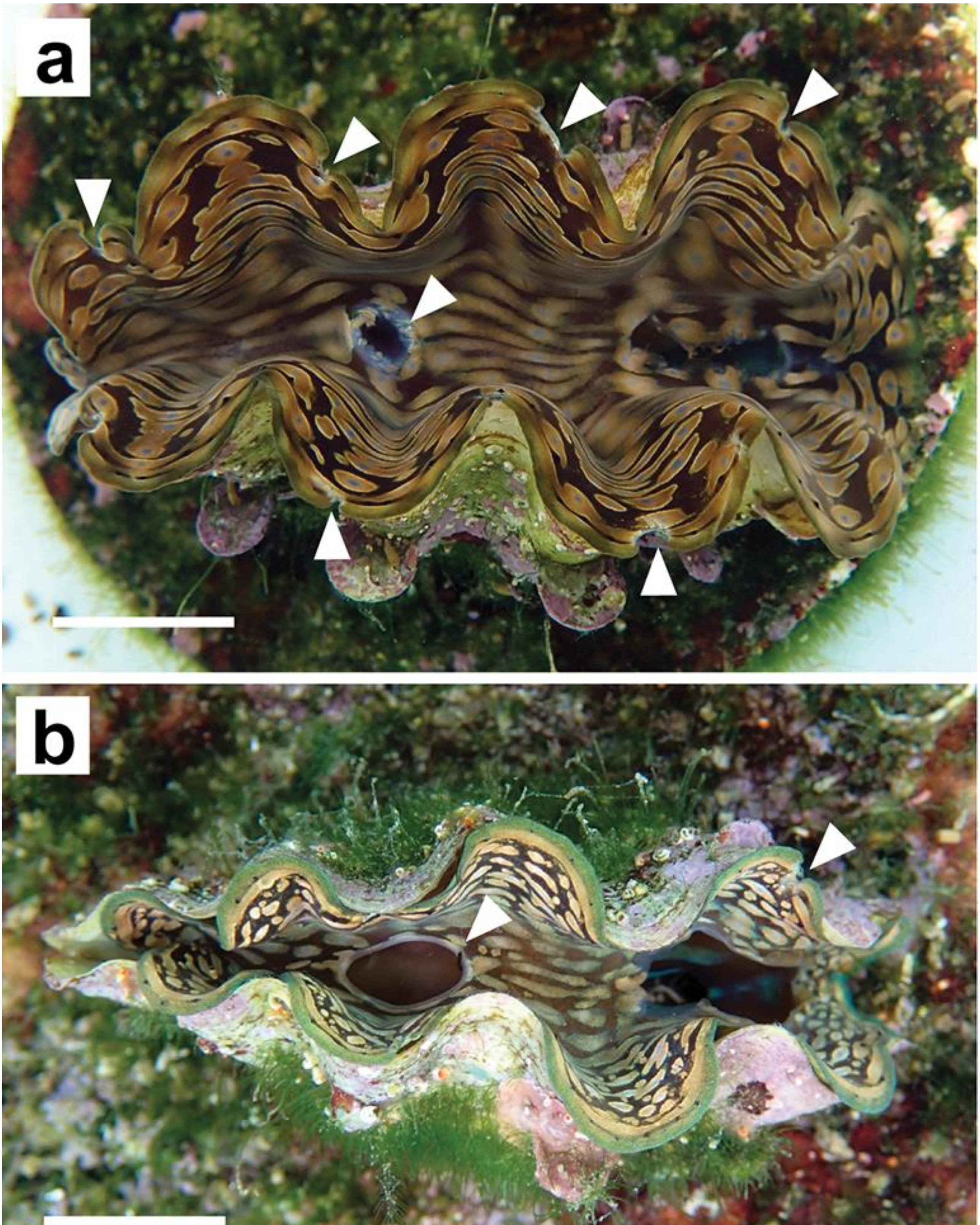

Fig. 2. Varying degrees of tissue cropping injuries in juveniles of the fluted giant clam, *Tridacna squamosa*. White arrows indicate the wound areas. Scale bars = 17 mm [a]; 13 mm [b].

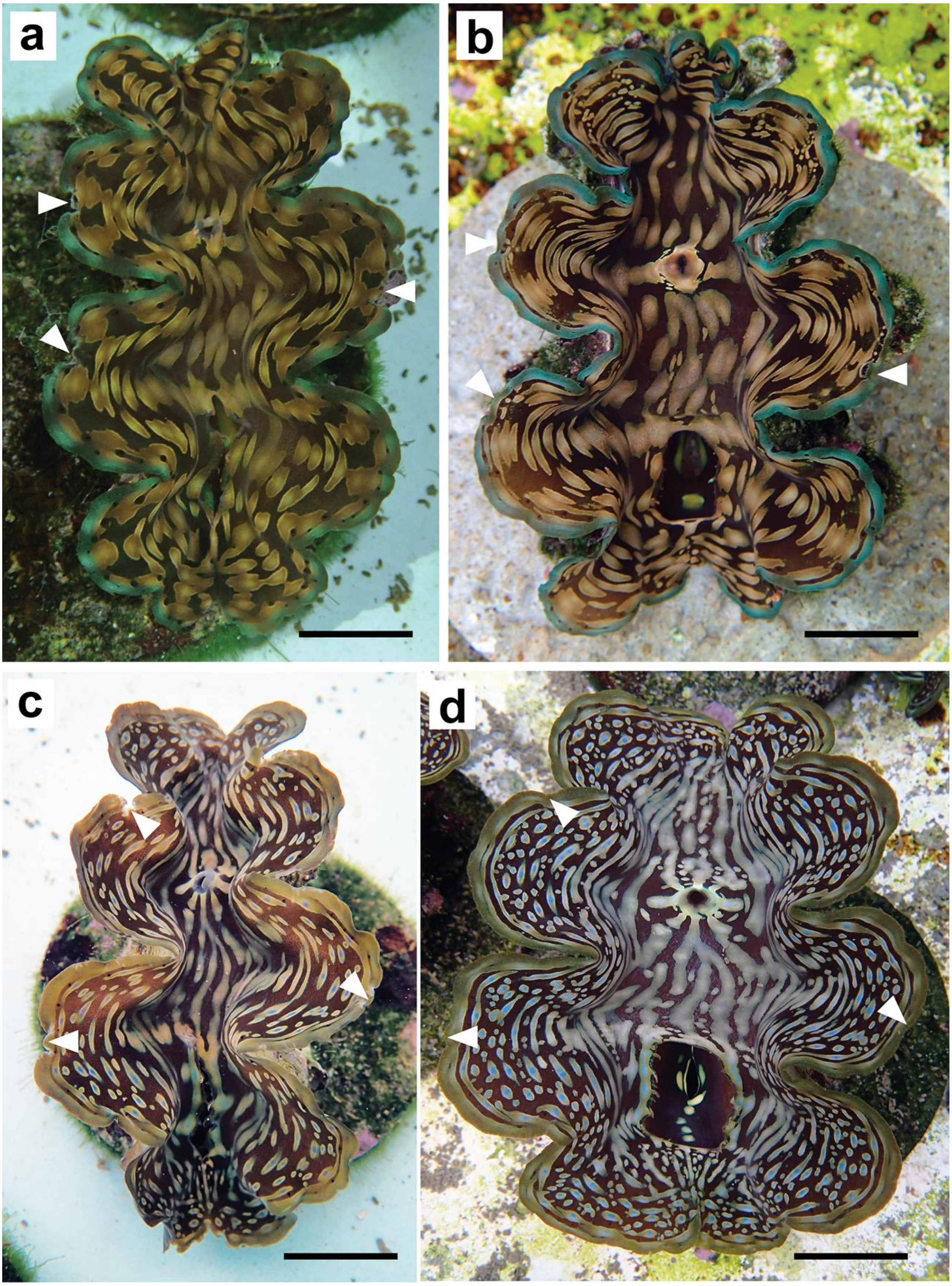

Fig. 3. Wound healing in juveniles of the fluted giant clam, *Tridacna squamosa*. White arrows indicate the wound areas. Two juveniles (a and c) are shown just after fish had cropped their mantle edges and the same two individuals (b and d, respectively) after 90 days. Scale bars = 15 mm [a]; 18 mm [b]; 20 mm [c]; 26 mm [d].

DISCUSSION

The majority of *Tridacna squamosa* individuals in this study appeared to have survived their mantle wounds. It took ~60 days for the single adult *Tridacna squamosa* to regenerate its cropped tissues to the original mantle edge but possibly with a reduced density of zooxanthellae, which was visibly distributed in smaller clusters compared to the surrounding intact tissues (Fig. 1). The inward rolling of the mantle wound observed in the adult giant clam here has previously been observed for *Pinctada* spp. (Acosta-Salmón & Southgate, 2005). This type of muscular response is thought to reduce the size of wound and contain the haemorrhage more rapidly while using fewer haemocytes and less connective tissue for healing. The juvenile clams took a longer time (up to 90 days) to heal and this period of recovery is comparable to that of pearl oysters *Pinctada* spp. (Acosta-Salmón & Southgate, 2005). A plausible explanation of the disparity in healing time between juvenile and adult giant clams could be that the total haemocyte count in the adult clam was much higher than in the juvenile clams. However, the size, type and number of wounds are also likely to have had an effect. The three wounds on the adult clam were relatively small (in relation to its body size) and excised with a sterile scalpel, compared to the multiple bites taken by fish from the juvenile clams.

The introduction of reef fishes as biological controls (of fouling algae or ectoparasitic snails, for example) is a common practice in giant clam mariculture operations (Mingoa-Licuanan & Gomez, 2007) but, to our knowledge, there are no records of such fish directly damaging the giant clams. Of the three species introduced, the acanthurid surgeonfish feeds almost exclusively on macroalgae and is rarely observed to feed on corals and other invertebrates (Fatherree, 2009). Hence the two *Siganus* spp. (rabbitfish) were most likely responsible as they have been reported to nip off coral tissues for the symbiotic microalgae that they contain (Kurtz, 2007) and may similarly be feeding on the zooxanthellae in the giant clam mantle. Tissue cropping of giant clams by fish is a common occurrence in natural populations (e.g., Irlandi & Mehlich, 1996; Sasaki et al., 2002; Lindsay, 2010), but it can affect bivalve reproduction and somatic growth (Hodgson, 1982b; Coen & Heck, 1991), as well as filter feeding rates (Irlandi & Mehlich, 1996). On the other hand, giant clams are known to exhibit a wide array of defensive strategies (Soo & Todd, 2014), including rapid mantle withdrawal (McMichael, 1974), camouflage (Todd et al., 2009), aggregation (Huang et al., 2007; Sim et al., 2018), shell ornamentation (Ling et al., 2008) and squirting jets of water (Stasek, 1965; Neo & Todd, 2011). Nevertheless, despite their defences giant clams still face predation pressures, even in adulthood, where mantle tissues often bear cropping scars (Neo et al., 2015).

Although the mortalities among juvenile *Tridacna squamosa* cannot be directly attributed to the cropping wounds, they could have contributed based on the following reasons: 1) the severity of injuries as seen in Fig. 2b, where both siphons became unnaturally enlarged after cropping, could have affected the clams' filter-feeding activities (Irlandi & Mehlich, 1996); 2) bacterial infection—resulting in poor health and eventual death (Acosta-Salmón & Southgate, 2005); and 3) slower regeneration which allowed fouling organisms to colonise the shells, impeding recovery and further stressing the clams (Acosta-Salmón & Southgate, 2005; Mamangkey & Southgate, 2009). Overall, however, our preliminary results demonstrate that the fluted giant clam, *Tridacna squamosa* can generally survive and regenerate its injured tissues within three months of cropping. This has implications for mariculture operations with respect to the zooxanthellar extraction methods applied to adult clams (e.g., the frequency of extraction to prevent excessive wounding), as well as the survivability of juveniles after restocking onto coral reefs where fish predators are present.

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