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OBSERVATIONS ON THE HYPOSALINITY TOLERANCE OF FLUTED GIANT CLAM (*TRIDACNA SQUAMOSA*, LAMARCK 1819) LARVAE

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ABSTRACT. **—** In conservation management, it is important to understand environmental tolerances at every stage of a species' life cycle. As salinity is considered one of the most significant ecological stressors for marine bivalves, this ex-situ study examined several larval stages of the fluted giant clam (*Tridacna squamosa* Lamark, 1819) exposed to hyposaline water. Late stage pediveligers or early stage juveniles survived in distilled fresh water for 10 min to 5 h, and showed no sign of injury during a 48 h follow-up period. Trochophores were able to survive for 10 min to 3 h in 9 ppt salinity water, and veligers were able to survive for 1 h to 42 h in 12 ppt salinity water. Results suggest that giant clam larvae in Singapore's waters are able to survive exposure to hyposaline water such as that associated with high rainfall or river outflows.

KEY WORDS. — marine bivalve larvae, pediveliger, salinity, Singapore, *Tridacna squamosa*, trochophore, veliger

INTRODUCTION

Giant clams are now extremely rare in Singapore (Guest et al., 2008; Neo & Todd, 2012). They face threats from harvesting, land reclamation, industrial pollutants, and anthropogenic turbidity, but conservation efforts, including a restocking programme, are underway (Neo et al., 2012). In order to determine appropriate locations for protecting and/or restocking giant clams, and to further understand their ecology, it is important to understand their environmental tolerances at every stage of their life cycle.

Salinity is considered one of the most consequential environmental stressors for marine bivalves (Tettelbach & Rhodes, 1981; Miller et al., 2007; de Albuquerque et al., 2012). When experiencing water with elevated temperatures and low salinity, many bivalve species have the ability to settle in, or migrate to, deeper water which is cooler and more saline. This is not an option for giant clams in turbid waters such as those around Singapore, as their algal symbionts require light for photosynthesis. Marine larvae in Singapore's waters are likely to encounter hyposaline water, either in tidepools or other shallow water during periods of heavy rain, or in the vicinity of river or reservoir outflows. Fresh water may also be used in giant clam mariculture facilities as a method of killing parasites or to remove algal mats.

There has been very little research into the salinity tolerances of giant clams at the larval or other life stages. Neo et al. (2013) exposed *Tridacna squamosa* larvae to water with reduced salinity, but only as low as 27 ppt, while Blidberg (2004) exposed *Tridacna gigas* larvae to salinities as low as 20 ppt. Although the older life stages are generally easier to study experimentally, we are aware of only one hyposalinity study on *Tridacna gigas* juveniles (Rachman & Anshary, 1997), and one on *Tridacna squamosa* adults (Blidberg, 1998).

There have been attempts to infer the salinity tolerances of bivalve larvae empirically by recording environmental variables at locations where larvae are present or absent (Thompson et al., 2012; Soria et al., 2013; Borges et al., 2014), but this approach carries the risk of unidentified confounding factors, and is unlikely to be successful with giant clams, the timing of whose reproductive cycle is not fully understood. Studies on gill tissues have been used to determine a theoretical maximum salinity tolerance for some species (Yaroslavtseva et al., 1990; Yaroslavtseva & Sergeeva, 2009), but there is no guarantee that larvae can survive up to this theoretical point. There are also reports of within-species differences in tolerance based on genetic variation (Innes & Haley, 1977; Newkirk, 1978; Deng et al., 2009; Eierman & Hare, 2013), and evidence that the timing of salinity changes can impact mortality (Davenport et al., 1975).

Much of the marine bivalve research into larval hyposalinity tolerance has little applicability to *Tridacna squamosa*'s ability to survive such conditions. Rather than directly record mortality, many marine bivalve studies instead investigate fertilisation success (Wang et al., 2012), trochophore motility (Suquet et al., 2013), larval growth rate (Thiyagarajan & Ko, 2012), vertical migration (Hidu & Haskin, 1978; Mann et al., 1991; Dekshenieks et al., 1996; Ishida et al., 2005), or settlement (Devakie & Ali, 2000; Tezuka et al., 2013). Studies that do examine salinity-induced mortality are often conducted on brackish-water species (Cain, 1973; Verween et al., 2007) or species which are euryhaline. Euryhaline oysters in the genus *Crassostrea* are the most frequently studied marine bivalves in larval salinity tolerance research (Lemos et al., 1994; Tan & Wong, 1996; Xu et al., 2011), although larvae of many other euryhaline species are also used, including pearl oysters (*Pinctada*; Doroudi et al., 1999; O'Connor & Lawler, 2004), other oysters (*Crassostrea*, *Ostrea* and *Placuna*; Davis, 1958; Davis & Ansell, 1962; Madrones-Ladja, 2002), mussels (*Mytilus*; Brenko & Calabrese, 1969; Qiu et al., 2002; Yaroslavtseva & Sergeeva, 2009; Vekhova et al., 2012), shipworms (*Teredo;* Hoagland, 1986) and sediment-dwelling clams (*Cyrtopleura*, *Donax*, *Mercenaria*, *Mulinia*, *Mya*, and *Ruditapes*; Davis, 1958; Stickney, 1964; Calabrese, 1969; Gustafson et al., 1991; Numaguchi, 1998; Carstensen et al., 2010). Low salinity responses of the larvae of more stenohaline marine bivalve species such as scallops (*Argopecten*, *Mimachlamys*, and *Pecten*; Tettelbach & Rhodes, 1981; O'Connor & Heasman, 1998; Christophersen & Strand, 2003) may be more comparable to those of giant clams, which are fully marine. There are also studies into the effects of hypersaline water on marine bivalve larvae (Iso et al., 1994; Arellano & Young, 2011; Voorhees et al., 2013), but hypersaline conditions only occur in Singapore in the immediate vicinity of desalination plant effluent, or in tidal pools during the dry season. Some previous experiments have produced limited results as only a narrow range of salinities was tested (Nell & Holliday, 1988; Robert et al., 1988; His et al., 1989), whereas others have used a broad range of salinities and established lethal limits for their target species (Davis, 1958; Davis & Ansell, 1962; Calabrese, 1969).

We conducted several ex-situ observational studies, exposing *Tridacna squamosa* larvae to extremely low salinities to observe any changes in behaviour and determine whether survival was possible under these conditions. If exposure to low salinities (such as those associated with high rainfall or river outflows) were to cause mortality during the larval dispersal phase it would have implications for the conservation management of *Tridacna squamosa*. Such information is also useful to determine whether it is safe to use fresh water as a parasite/algal control method in giant clam aquaculture.

MATERIAL AND METHODS

All three observational studies used larvae of *Tridacna squamosa* which were spawned and reared at the Tropical Marine Science Institute, on St. John's Island, Singapore.

Study 1. — In Nov.2012, six late stage *Tridacna squamosa* pediveligers or early stage juveniles of shell length from 2.3 to 3.0 mm were placed in zero-salinity distilled water for time periods ranging from 10 min to 5 h. The studies were carried out indoors in an air-conditioned room. Each clam was removed from the flow-through seawater aquaculture system, and placed directly into a well plate (one clam per well) which contained approximately 15 ml of distilled water. Each clam was left for a different time period (10 min, 20 min, 30 min, 1 h, 2 h, and 5 h) after which it was placed in a petri dish of seawater for immediate observation under a dissecting microscope and then moved outdoors to its own 1 L glass container which received flow-through seawater. The clams were then monitored for 48 h for any mortality; behavior was also observed.

Study 2. — In May 2014, 4 ml samples of seawater (salinity 32 ppt) containing *Tridacna squamosa* trochophores were added to five wells in a well plate containing 10 ml of distilled water, and one well containing seawater as a control. The salinity of the non-control wells (10 ml distilled water + 4 ml seawater) was verified to be 9 ppt using a hand-held refractometer. The study was carried out in a large outdoor shed which provided some shade, but was not air-conditioned. After varying periods of time (10 min, 30 min, 1 h, 2 h, and 3 h), the water and larvae were removed from the well plate and placed into a petri dish full of seawater. The petri dish was then observed under a dissecting microscope for actively swimming trochophores.

Study 3. — Also in May 2014, 6 ml samples of seawater (salinity 32 ppt) containing *Tridacna squamosa* veligers were added to 10 wells each containing 10 ml of distilled water, and two wells containing seawater as controls. The salinity of the non-control wells (10 ml distilled water + 6 ml seawater) was verified to be 12 ppt using a hand-held refractometer. The studies were carried out in the same shed as the trochophore experiment (Study 2). After varying periods of time (1 h, 2.5 h, 4 h, 6 h, two at 18 h, two at 24 h, and two at 42 h), the water and larvae were removed from the well plate and placed into a petri dish full of seawater. The petri dish was then observed under a dissecting microscope for actively swimming veligers, and observed a second time one hour later to determine if swimming patterns had changed.

RESULTS AND DISCUSSION

Upon being placed in distilled water (Study 1), the six late stage pediveligers or early stage juveniles withdrew their mantle tissue and siphons and closed their valves tightly. This is a short-term survival strategy as, if employed for extended time periods, it will induce hypoxia (Kim et al., 2001). When returned to seawater, the clams opened their valves and extended their mantle and siphon tissues within 30 minutes. The clams which had been kept in distilled water longer appeared to take longer to return to their normal state, however, this was possibly an effect of longer exposure to low water temperatures (due to the room's air conditioning). All of the clams survived during the 48 h follow-up observation period, and were able to climb up the sides of their glass containers to the air-water interface (a common behaviour among *Tridacna squamosa* of this size when kept in smooth-surfaced containers).

Upon being placed in water of 9 ppt salinity (Study 2), all trochophores ceased swimming and sank to the bottom of the well plates. This sinking behavior may benefit the organisms in the wild—when larvae near the ocean's surface are exposed to hyposaline water during rainstorms, they may sink into deeper, more saline water where it is safe to resume their normal activities. When the trochophores in this experiment were placed back into seawater, some in each group resumed swimming, although those in the 2 h and 3 h groups swam with a weak tumbling motion, rather than the distinctive vigorous circling pattern of healthy trochophores. The subsequent veliger experiment raises the possibility that this weaker swimming pattern may have been temporary.

Upon being placed in water of 12 ppt salinity (Study 3), the veligers also ceased all swimming activity and sank to the bottom of the well plates. When placed back into seawater, some veligers in each group resumed swimming, although they appeared to be pivoting around a point rather than swimming in large circles (their usual behavior). However, after remaining in seawater for one hour, the veligers had resumed their normal swimming activity. Shortly before the 42-h observation, veligers were seen actively swimming in one of the hyposaline (12 ppt) wells. As they were not under continuous observation, it is possible that they periodically engaged in short periods of swimming in order to obtain oxygen. It is also possible that evaporation from the well raised the salinity above a threshold where the veligers could conduct normal activities safely.

As *Tridacna* species have a short pelagic larval cycle, the long-term exposure of trochophores and veligers to hyposalinity cannot be studied. However, settlement rates of *Tridacna* larvae under hyposaline conditions could be measured, as has been done for other species (Devakie & Ali, 2000; Tezuka et al., 2013). Pediveligers and juveniles could be exposed to sublethal hyposalinity for longer time periods, which in other marine bivalves affects immune response (Gagnaire et al., 2006; Matozzo et al., 2007) and growth rate (Nell & Holliday, 1988; Navarro & Gonzalez, 1998). Chronic hyposalinity may even lead to a 'dwarf' bivalve population (Westerbom et al., 2002; Riisgard et al., 2013). A longer growth period or smaller ultimate size will impact survival, as smaller bivalves are less likely to survive adverse environmental conditions (Nell & Paterson, 1997), and may not reach an 'escape size' from some forms of predation. Bivalves living in hyposaline water may also be more vulnerable due to weaker shells, adductor muscles, and/or byssal threads (Wang et al., 2012).

Giant clams in Singapore which are exposed to hyposaline water are likely to be simultaneously exposed to additional stressors, particularly elevated water temperatures and turbidity-induced shading. Being exposed to these other stressors is likely to reduce the clams' ability to cope with hyposalinity (Chanley, 1958; Castagna & Chanley, 1973; La Peyre et al., 2013), therefore multi-stressor studies will be necessary to determine *Tridacna squamosa*'s tolerance to hyposaline waters under natural conditions.

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