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Larval ecology of the fluted giant clam, *Tridacna squamosa*, and its potential effects on dispersal models

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ABSTRACT

Biophysical models are increasingly being used to visualise and predict dispersal and connectivity of marine larvae, providing the opportunity to examine spatial and temporal patterns that are difficult to assess in the field. The quality of the biology component of these models, however, often lags behind that of the physical data. Hence, quantifying and parameterising species-specific autecological information are critical steps in advancing the reliability of larval transport simulations. Here we quantify three hitherto untested aspects of the larval ecology of the fluted giant clam, *Tridacna squamosa*: 1) fertilisation success in relation to gamete age, 2) larval swimming speeds, and 3) settlement competency, and discuss how these factors may affect model predictions of giant clam larval dispersal and recruitment. Through laboratory experiments, we found that *T. squamosa* gametes were fertilisable for up to 8 h, but viability reduced with age. As giant clams are synchronistic spawners, this reduced gamete viability can impede fertilisation success if individuals are too distant from spawning neighbours. We also determined that ciliated *T. squamosa* larvae are not strong swimmers (104.0 to 1010.6 μm s^{-1}), but appear able to alter their depth distribution and actively select settlement sites. Average swimming speed peaked 6 days post-spawning, and subsequent reduction in speeds suggests 'readiness' for settlement. Trials indicated that the settlement competency period in *T. squamosa* is 14 days. No gregarious settlement was observed amongst conspecifics but settlement-competent larvae responded positively to associative cues from fresh crustose coralline algae. All three of these *T. squamosa* larval traits have the potential to influence the outcomes and interpretations of model simulations.

1. Introduction

The link between larval dispersal and marine population connectivity has been a focus of marine research for decades (as reviewed by Levin, 2006; Cowen and Sponaugle, 2009). Previous studies highlighted the importance of larval supply, transport, and survival in determining adult population dynamics (e.g., Roughgarden et al., 1987; Young, 1987). Sessile marine invertebrates, in particular, produce planktonic larvae that can remain pelagic for minutes to months, sometimes facilitating dispersal over great distances (Hadfield and Paul, 2001). Field observations of larval dispersal are inherently difficult, hence biophysical models (in either two or three dimensions) are increasingly being used to visualise and quantify larval transport and population connectivity (Cowen and Sponaugle, 2009; Levin, 2006; Metaxas and Saunders, 2009). Researchers have also inferred "source" and "sink" sites from their models (e.g., Lipcius et al., 2008; Neo et al., 2013; Tay et al., 2012), where sources may become optimal restocking areas and sinks targeted for conservation (Pulliam, 1988). Understanding source-sink dynamics in restocking and restoration of marine populations can also assist in preventing or mitigating potential anthropogenic impacts such as near-shore development (Lipcius et al., 2008).

Modelling the dispersal of marine invertebrates has advanced rapidly in recent years (Gallego, 2011; Levin, 2006; Metaxas and Saunders, 2009). Regardless of the biophysical model type (e.g., Eulerian or Lagrangian), they all typically incorporate a suite of physical and biological parameters (see Erftemeijer et al., 2009; Tay et al., 2012). While the physical parameters largely determine the hydrodynamic transport functions (Huret et al., 2010; van Maren and Gerritsen, 2012), including biological data is also important (as reviewed by Metaxas and Saunders, 2009). Critical factors such as planktonic larval duration (PLD: Shanks, 2009; Gilbert et al., 2010; Wolanski and Kingsford, 2014), timing of gamete or larval release (Edwards et al., 2007; Reitzel et al., 2004), swimming speeds (Bolle et al., 2009; Dekshenieks et al., 1996), diel vertical migration (Dickey-Collas et al., 2009; Edwards et al., 2007), mortality rates (Neo et al., 2013), and behaviour (Bolle et al., 2009; Neo et al., 2013) have been included into previous modelling studies for sessile

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marine invertebrates and fish. Duration of larval cycle, mortality rates, and larval behaviour, in particular, are known to significantly affect the success of larval dispersal, settlement and recruitment (Metaxas and Saunders, 2009). For example, shorter duration of larval stages in marine invertebrates usually results in lower predation and thus higher recruitment success (Reitzel et al., 2004). The selective incorporation of biological traits into biophysical models for larval transport can substantially enhance their predictive power (Gallego, 2011; Metaxas and Saunders, 2009).

Due to overfishing and coral reef degradation, giant clams are amongst the most highly threatened tropical Indo-Pacific invertebrates (Mingoa-Licuanan and Gomez, 2002; Neo et al., 2015). Stock depletion is detrimental to the reproductive strategy of giant clams, leading to lowered (or zero) fertilisation and recruitment, and eventual population collapse (Munro, 1992). Biophysical modelling can help examine the 'future prospects' of the remaining clam populations, as well as identify fine- and broad-scale (physical or biological) factors that could influence natural recovery of wild giant clam stocks. The aim of this paper is to quantify three hitherto untested aspects of *Tridacna squamosa* larval ecology: 1) fertilisation success in relation to gamete age, 2) larval swimming speeds, and 3) settlement competency. The potential effects of these components and their parameterisation on larval transport predictions are also discussed. These three parameters are critical components of the pre- and post-settlement processes that determine the recruitment success of many marine invertebrates. Improved understanding and availability of quantitative data for the characterisation of these processes will aid in better management of *T. squamosa* (Dickey-Collas et al., 2009; Lamare and Baker, 2001), which continues to face threats of overfishing (Mingoa-Licuanan and Gomez, 2002) and habitat degradation (Neo and Todd, 2012).

2. Materials and methods

2.1. Spawning, fertilisation, and rearing of larvae

Accessible mature *T. squamosa* broodstock (n = 9; size range = 23.6 to 37.5 cm) were collected from the reefs of the Southern Islands, Singapore. As local populations are low in abundance, this broodstock was mixed with imported individuals of Indonesian origins (n = 18; size range = 19.0 to 23.2 cm). Prior to their participation in spawning, imported clams were conditioned in local waters for more than six months. We expected little variation in spawning periodicity between the local and imported broodstock due to close geographic proximity and similar seasonality. Spawning was conducted at the Tropical Marine Science Institute on St John's Island (1°13′N, 103°50′E), Singapore. Spawning protocols closely followed Mingoa-Licuanan and Gomez (2007), and clams were induced to spawn on the following new moon phases in 2012: 26 Jan, 27 Mar, 14 and 15 Aug. Within the first 5 min of induction, sperm were released from individual clams and collected into 1 l containers. Upon egg release, the 'mother' clam was transferred to a new container filled with 1 μm UV-filtered seawater (UV-FSW) to separate the eggs from the sperm water. For every litre of egg suspension (egg density ~50 eggs ml^{-1}), 5 ml of sperm water (from at least three 'father' clams) was added for fertilisation (Mingoa-Licuanan and Gomez, 2007). Larval cultures were maintained in a large well-ventilated shed, with 1 μm UV-FSW exchanges every other day. Larvae were fed on Days 2, 4, 6, and 8 with 10,000 cells ml^{-1} cultured *Isochrysis* sp. (CS-177 T.ISO Tahiti) and infected with 10,000 cells ml^{-1} parental zooxanthellae on Days 5, 7, and 9. The final phase of rearing was the introduction of larvae into settlement tanks.

2.2. Combined egg and sperm longevity and viability

Experiments were performed on 26 Jan, 27 Mar, and 15 Aug. Eggs from a single 'mother' clam were used for the testing of combined gamete viability from each induced spawning. Only round and mature eggs (Braley, 1988) were chosen for the experiment (mean egg size = 100.27 ± S.E. 0.88 μm). For the trial in 26 Jan, nine time points (t = 5, 10, 15, 20, 30, 45, 60, 90, 180 min) were tested but for the two subsequent trials this was extended to 12 time points (t = 0, 5, 10, 15, 20, 30, 45, 60, 120, 240, 360, 480 min). At every time point, each container (n = 3) held 500 ml UV-FSW and ~15,000 eggs, which were fertilised with sperm water at a fertilisation ratio of 1:50 to prevent polyspermy (Neo et al., 2011). Experimental cultures were maintained at a mean temperature of 28.3 °C (data from a 32 K StowAway TidbiT temperature logger placed in a separate water-filled container). Salinity of experimental cultures ranged from 29 to 32 ppt. Each time trial was stopped after 12 h, and the eggs were filtered and stored in 96% ethanol. Fertilisation success was assessed by examining 500 eggs from each replicate. Using an inverted dissecting microscope, eggs were categorised either as fertilised with normal development, or as unfertilised. For all three trials, the proportion of fertilised eggs at each time point was calculated.

2.3. Swimming speeds and behaviour of pelagic larvae

Swimming rate here was defined as the distance travelled by a larva within the water column per unit time while actively swimming with its velum. To minimise wall effects (see Chia et al., 1984; Vogel, 1981), preliminary experiments were conducted for the selection of an appropriately sized experimental chamber. Wall effects can be computed using $Y > 20v/u$, where Y is the distance to the wall, v is the kinematic viscosity of seawater, and u is the swimming velocity of larva (Vogel, 1981). Based on ten observations of larval motion in chambers of 1.5, 3.5, and 8.5 cm internal diameter, wall effects were minimised when using the 8.5 cm chambers (Petri dishes), hence these were used for the experiment. Larval swimming speeds were measured on alternate days (Days 2, 4, 6, 8, 10), using larvae spawned on 14 Aug. Experimental setup consisted of three replicate Petri dishes filled with 25 ml UV-FSW and ~100 larvae. Ten swimming larvae per dish were then haphazardly selected for video tracking (Eikona Microscope Camera MVV5000) for at least 20 s under a dissecting microscope. Larva length and distance travelled in time t were calculated using video processing software (GIMP 2.8.2).

2.4. Protocol for settlement assays

Settlement competency of *T. squamosa* veligers here is defined as the time period before larvae are capable of settling and metamorphosing. We examined the settlement competency and behaviour of *T. squamosa* larvae in three separate experiments. Settlement competency protocols followed closely to those for broadcast spawning corals (see Edwards, 2010). Six-day old larvae were used at the start of all experiments that each ran for 12 days. Cultures were maintained at 27.4 °C (data from a 32 K StowAway TidbiT temperature logger placed in a separate water-filled container). Salinity of well cultures ranged from 29 to 32 ppt. In all experiments, settlement was considered to have occurred when 1) larvae had metamorphosed, i.e., lost the ability to swim and/or 2) larvae had attached with byssus to the substrate and could only be dislodged when pressure was exerted using forceps.

2.4.1. Settlement competency periods

Twelve *T. squamosa* larvae were introduced into each well of a 12-well plate (NUNC™). Each well was filled with 4 ml of 1 μm UV-FSW and a 9 mm^2 fresh crustose coralline algae (CCA) chip (Courtois de Vicose, 2000; Neo et al., 2009). This procedure was repeated every 24 h for 12 days, each time using a new plate. Each well was considered an independent replicate (i.e., 12 replicates per time point). Proportions of settled, swimming, and dead larvae in each well were recorded under a dissecting microscope at 24 h periods.