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Early ontogeny of the pipi, *Donax* (*Plebidonax*) *deltoides* (Donacidae; Bivalvia)

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Abstract

Reduction in commercial catches of the pipi, *Donax* (*Plebidonax*) *deltoides*, has prompted interest in the potential for hatchery production of pipi juveniles as the basis for reseeding programs and assessments of culture potential. Adult pipis were collected from Port Stephens, New South Wales, Australia, in October/November 2008 and held in baskets in a recirculating holding system. Two consecutive batches of larvae were produced by stripping gametes from the gonads. Fertilised eggs averaged 75 µm in diameter. When incubated at 23ºC, first and second polar bodies were evident after 45-50 min and 95-100 min, respectively, while first cleavage occurred within 155 min of fertilisation. Embryos reached trochophore stage within 24 h and developed to D-veliger stage (112 ± 0.5 µm antero-posterior measurement (APM)) within 48 h. The first pediveligers (318 µm, APM) were observed after 14 days and settled spat (320 µm APM) were present one day later (Day 15). This study confirmed that the production of pipi spat is possible, but highlighted the need for further research into spawning, fertilization and larval nursery techniques for this species.

Key words: larvae, hatchery, New South Wales, Australia.

Introduction

The 'pipi', *Donax (Plebidonax) deltoides*, Lamarck, 1818 is a common bivalve found on open beaches throughout south eastern Australia (Dakin 1980). Despite this, there is a paucity of information available on the biology of the species. In New South Wales, pipis are thought to 'dribble' spawn throughout the year (Murray-Jones 1999). Their life cycle includes a brief larval stage, suggested to be 6-8 weeks (King 1976), before they recruit to the subtidal and intertidal zones of surf beaches. Pipis can achieve sexual maturity at a size of 3.6 cm (King 1976) within six months (Murray-Jones 1999) and can ultimately reach a length of 8 cm. Also known as 'Goolwa cockles' in South Australia and 'eugari' in southern Queensland, pipis have historically been an important part of the coastal aboriginal diet and are popular with recreational fishers as bait. More recently, pipis have been harvested and sold for human consumption, particularly to local Asian markets, where significantly reduced supply has seen prices exceed US$45 kg^{-1}.

Major pipi fisheries in Australia are found in New South Wales (NSW) and South Australia (Kialola *et al.* 1993). For over a decade in NSW, the pipi fishery has harvested between 200 and 400 t $annum^{-1}$, valued in excess of US$1.5 million. The bulk of this catch, approximately 200 t $annum^{-1}$, has come from the Central, Hunter and Mid-North Coasts regions. Recently, harvests across the State have fallen, particularly in the Hunter Zone, from an average of approximately 120 t $annum^{-1}$ to less than 15 t in the 2005-2006 and 2006-2007 seasons. Similar reductions in catches have occurred in South Australia, where in an effort to protect the fishery, a total allowable commercial catch of 1150 t in 2008 has subsequently been reduced to 300 t in 2010.

Although pipi populations are prone to large temporal variations in distribution and abundance, and show high recruitment variability (Murray-Jones and Steffe 2000), the recent reductions in commercial harvest have raised interest in reseeding juveniles into affected areas to promote the recovery of wild stocks (Phelps *et al.* 2009) and in the potential for pipi mariculture. Both these activities rely on hatchery propagation of pipi juveniles. But, while attention has been paid to the early ontogeny of members of the genus *Donax* overseas (Wade 1968; Chanley 1969; Frenkeil and Moueza 1979; Ruiz-Azcona *et al*. 1996), there is little information on the genus in Australia, and none on the early ontogeny of *D. deltoides*. This study confirmed that hatchery production of *D. deltoides* juveniles is possible using current techniques and infrastructure normally used to culture other molluscs, and provides the first record of larval development in the species.

Materials and Methods

Mature *D. deltoides* (35–65mm shell length) were collected by hand from the intertidal zone of Stockton Beach and One Mile Beach, NSW, Australia in October/November 2008. They were transported to the Port Stephens Fisheries Institute Mollusc Hatchery and held in baskets, without sand substrate, in a recirculating holding system for up to 4 weeks. Water temperatures were held at 23ºC and adults were fed a mixture of microalgae (all from the CSIRO culture collection; accession number provided)—*Isochrysis sp.* (unidentified species referred to in industry as Tahitian *Isochrysis* or T *Iso*, CS-177), *Pavlova lutheri* (Droop, 1953; CS-182) and *Chaetoceros muelleri* (Lemmermann, 1898; CS-176) at a rate of approximately 2 x 10^9 cells $adult^{-1}$ day^{-1}. Attempts to initiate spawning using temperature shocks (4-5ºC above ambient) failed. Gametes were obtained through incisions in the surface of the gonad from which the eggs and sperm were collected using a pipette. The eggs were then suspended in seawater with 0.05 N ammonium hydroxide for approximately 30 min before sperm was added to achieve a

concentration of approximately 10^5 sperm mL^{-1}. Due to marked variability in egg size and apparent maturity, the mean size of eggs was not determined until fertilization had occurred, and only those eggs with polar bodies were measured. Two larval batches were produced, the first combining gametes from two females and two males, the second combining gametes from eight females and four males.

Techniques for rearing *D. deltoides* were similar to those described for other bivalves (Chanley 1975). Zygotes were stocked at a density ≈ 5 ml^{-1} into a 1000-L, polyethylene tank of aerated seawater held at 23 ± 1ºC. All seawater (34 g kg^{-1} salinity) used was filtered with 1 µm (nominal) cartridge filters. After 48 h, the tank was drained and D-veliger larvae were collected on a 35 µm sieve. A total of 1.0 x 10^5 larvae were then stocked into fresh seawater in a second 1000-L tank. Larvae were sampled daily, from which 30 larvae were chosen at random for size determinations. Seawater was changed every 48 h at which time larvae were placed in a clean tank of freshly filtered seawater (23 ± 1ºC). Larvae were fed a mixture of T. *Iso*, *P. lutheri* and *Chaetoceros calcitrans* (Takano, 1968; CS-178) on an equal dry weight basis in accordance with the feed curve described by O'Connor *et al*. (2008). The daily algal ration was divided equally over a morning and afternoon feeding.

Results

Adult *D. deltoides* initially appeared to adapt well to recirculating systems, despite the absence of a substrate in which to bury. The majority of adults extended the foot and siphons and readily filtered the algal diet (faeces apparent within 12 h). Very little (<2%) mortality was observed prior to gamete collection, although shortly thereafter, losses began to occur among the remaining stock and escalated to the point at which approximately half the stock died over the ensuing three weeks (weeks 4–7 after capture).

The gonads of male and female *D. deltoides* are creamy white to pale yellow. The total number of stripped oocytes collected from each female varied and it was apparent that oocytes of varying stages of maturity were present in each sample. On average, only 35,000 'mature' oocytes, capable of fertilisation under the conditions applied, were obtained from each female. After 30 mins incubation in seawater the oocytes were 75 ± 0.5 µm (mean ± SD, n= 30) with a prominent germinal vesicle and surrounded by a 15 µm thick chorion (Fig. 1A). Following fertilisation, first and second polar body extrusion occurred 45–50 and 95–105 min post-fertilisation respectively (Fig. 1B). First and second cleavage occurred between 150–155 and 175–190 min post-fertilisation respectively.

Trochophores were observed after 24 h (Fig. 1C) and the first D-veligers (Fig. 1D) were seen at 30 h post fertilization. After 48 h approximately 90% of embryos had developed to D-veliger stage (n>1000), having a prodissoconch I shell with antero-posterior measurements (APM) from 111 to 112.5 µm and a dorso-ventral measurement (DVM) from 75 to 76.5 µm. Little variation in size was observed in the early stages of larval development between the two larval cultures. Umbonate larvae were first observed on Day 5 and by Day 10, larvae had reached a mean APM of 211.8 ± 13 µm and 223 ± 31 µm, and a mean DVM of 158 ± 15.3 µm and 188 ± 30 µm, first and second larval cultures respectively. From Day 12 onwards prominent statocysts were observed (Fig. 1E).

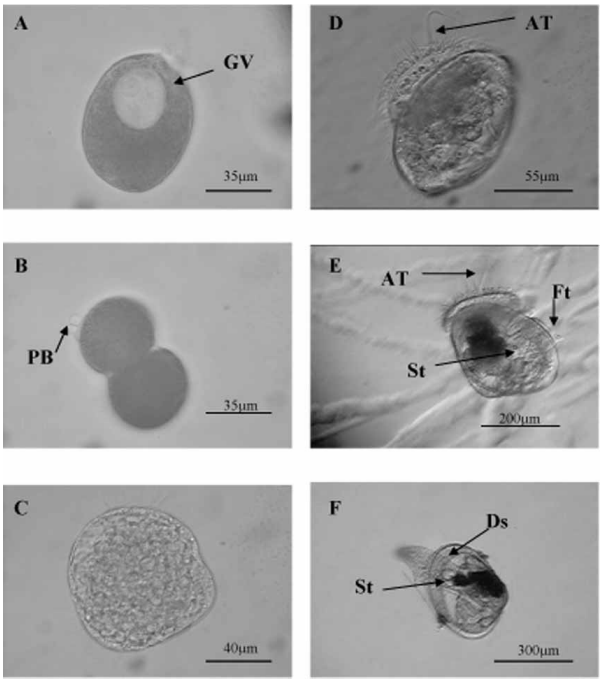

FIGURE 1, Early developmental stages of *Donax* (*Plebidonax*) *deltoides*: **A,** egg (GV—germinal vesicle), **B,** first cleavage (PB - polar body), **C,** trochophore , **D,** D-veliger larvae (AT—apical tuft), **E,** umbonate larvae (St—statocyst, Ft—foot), **F,** settled larvae (Ds—dissoconch shell).

By Day 13 the foot had developed, although no crawling behavior was evident (Fig.1E). The first pediveligers to exhibit crawling behavior were observed on Day 14 (318–328 µm APM) and by Day 15 the first metamorphosed spat were present (Fig. 1F). By Day 16, between 4 to 10% of larvae had metamorphosed and by Day 17, 10 to 15% had successfully metamorphosed. No eye spot was observed during larval rearing.

Growth rates for *D. deltoides* larvae were linear (Fig. 2) with little difference in growth rates observed between the two larval cultures (r=0.975, df 1, 11 P<0.001). Survival to Day 15, the time at which metamorphosing larvae were first observed in both larval cultures, was 87.5 and 92% in larval cultures 1 and 2 respectively. Larvae continued to metamorphose over the following week until approximately 35% (n>100) had entered the spat stage. The remaining larvae died progressively over the following week, apparently incapable of settling. The mean size of prodissoconch II shells at metamorphosis was 323 ± 2.5 µm (APM). The delineation between the darker prodissoconch II and the subsequent dissoconch shell was clearly evident (Fig. 1F).

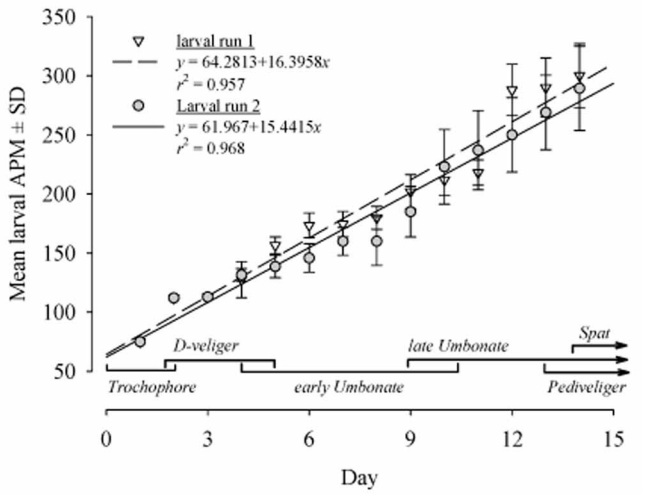

FIGURE 2, Mean daily larval antero-posterior measurement (APM) increase for two independent *Donax* (*Plebidonax*) *deltoides* cultures.

Discussion

Although preliminary in nature, this study has confirmed that the early ontogeny of *D. deltoides* is similar to that of other members of the genus and that existing hatchery techniques and equipment are capable of producing pipi seed. Further, it has highlighted several areas of research that warrant attention before large scale production of *D. deltoides* is undertaken.

In all measures used in this study, the early ontogeny in *D. deltoides* is similar to that reported for other donacids (Chanley 1969; Frenkiel and Moueza 1979; Ruiz-Azcona *et al*. 1996). Morphologically, *D. deltoides* developed through trochophore and veliger stages before metamorphosing from the plankton into the adult form. The dimensions of the various ontogenetic stages are broadly similar to those reported previously, especially during the planktonic veliger stages. Like *Donax trunculus* Linnaeus, 1758, D veligers were initially a little over 100 µm in shell length (Ruiz-Azcona *et al*. 1996) and in common with *Donax variabilis* Say, 1822 (Chanley 1969), when larvae had reached 300 µm in size the majority had developed into pediveligers. An apical flagellum, observed in other donacids (Chanley 1969), was present at all stages of larval development and was only lost at metamorphosis with the loss of the velum. Consistent with observations by Chanley (1969) in *D. variabilis*, no eye spot was observed during larval development of *D. deltoides* and a prominent statocyst began to develop at the base of the foot in 12-day-old larvae. By Day 13, the foot could be seen protruding past the shell margins in *D. deltoides* but no active crawling was observed. The first crawling pediveligers were observed on Day 14 and metamorphosed spat, denoted by the presence of dissoconch shell growth, were first observed on Day 15. The time taken to reach metamorphosis by *D. deltoides* was several days less that that observed for *D. variabilis* (Chanley 1969); however, this could be due to culture conditions. For example, multispecies diets such as used in this study have been observed to significantly improve larval growth and survival in comparison to monoalgal diets such as that used by Chanley (1969).

Adult *D. deltoides* were readily held in substrate free containers for short periods of time, although in the longer term (4–7 weeks) losses began to increase suggesting alternate holding methods should be investigated. The presence of fecal strings suggested that losses were not the result of food shortage *per se*, but ingestion does not imply the nutritional quality of the diet used. As the losses began shortly after initial spawning attempts, it is possible that they may have resulted from additional stress imposed through increased handling or serial temperature shocks (down to 5°C), used in attempts to initiate spawning.

In previous studies, techniques used to spawn donacids have varied. Chanley (1969) attempted temperature induction but was forced to strip eggs from the gonad and activate with ammonium solutions, while Ruiz-Azcona *et al.* (1996) were able to initiate spawning in *D. trunculus* with temperature manipulation when used in conjunction with the addition of sperm suspensions. Temperature induction, with and without the addition of sperm suspensions, failed to induce spawning in *D. deltoides* and we too resorted to strip spawning and ammonia activation, although it was thought that this contributed to the variability in size (and assumed quality) of the eggs and the low fecundities recorded. The success of strip spawning would appear to be species specific with reports of its utility ranging from poor (Loosanoff and Davis 1963, Chanley 1975, Hooker 1995) to good (Debrosse and Allen 1991). Importantly in the context of this study, which sought to look mainly at production potential of the species, *D. deltoides* gametes can be stripped, viable gametes can be obtained and thus the uncertainties surrounding the timing and control of natural spawnings can be reduced.

In general, observations made here correspond with life history predictions for an outcrossing planktotrophic species in a variable environment. Sexually mature pipis were collected at an early age, produced comparatively large numbers of small eggs and had low, but variable reproductive effort. The protracted spawning season noted by Murray-Jones (1999) and evident in the collections made during this study, may serve to mitigate the effects of a stochastic environment, particularly if spawning in *D. deltoides* is in response to oceanographic triggers. The variance in egg size and maturity was indicative of prolonged partial spawning, which may ameliorate the potential pitfalls of life in exposed environments with fluctuating environmental conditions.

From an economic perspective, ontogenetic similarities are of some assistance to those who may be interested in donacid culture. The similarities allow the use of common equipment such as sieves and setting screens without modification and timing schedules used for the propagation of other donacids can be applied; however, some care should be taken in the interpretation of this and similar ontogenetic comparisons. Improvements in spawning and fertilisation success, and increases in settlement success may be

dependent on the application of techniques that are either new or have not been used in association with this or closely related genera.

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