Spawning, settlement, and growth of the New Zealand venerid *Ruditapes largillierti* (Philippi 1849) in culture

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Abstract   Spawning, larval rearing, and growout of *Ruditapes largillierti* (Philippi 1849) were investigated in a series of trials conducted at the University of Tasmania (UTAS), Launceston and Georges Bay, St Helens, Tasmania, Australia. Intramuscular injection of serotonin (3×10^{-7} to 1.5×10^{-6} moles) failed to induce spawning in female *Ruditapes largillierti*, although some males did spawn. Fecundity of *R. largillierti* induced to spawn by thermal stimulus ranged from 0.5 to 0.9×10^{6} eggs female^{-1}. Fertilised eggs developed into trochophore larvae by 24 h at 20°C and D veligers with a mean shell length of 85.3 ± 4.7 μm within 48 h. Early larvae were frequently deformed and their mortality rates were very high. Development to pediveliger stage (mean shell length 200.3 ± 7.3 μm) took between 11 and 16 days at 20°C, and metamorphosis to spat (mean shell length 240 μm) occurred between Days 16 and 19. There was no significant difference in efficacy of epinephrine, norepinephrine, or untreated groups for inducing larval settlement. Average growth of juveniles held subtidally within trays or baskets (mesh size 1.7–12.0 mm) was 1.3 mm month^{-1}. The aquaculture potential of this subtidal venerid clam warrants further investigation.

Keywords   clam; venerid; *Venerupis*; *Ruditapes*; *largillierti*; spawning; larval rearing; survival; growth

INTRODUCTION

Clam aquaculture is a significant industry in many countries and accounted for 29% by value and 26% by weight of world molluscan aquaculture production in 1995 (FAO/FIDI 1997). In Australia, however, commercial interest in clams has been largely restricted to a relatively small fishery, although, the aquaculture potential of several venerid species is being evaluated (Nell et al. 1994, 1995; Patterson & Nell 1997; Kent et al. unpubl. data). The major venerid species of interest in Tasmania for fisheries and aquaculture research are the stepped venerid *Katelysia scalarina* (Lamark 1818) and the New Zealand venerid, *Ruditapes largillierti* (Philippi 1849) (Treadwell et al. 1992; Kent et al. unpubl. data). A lack of information on appropriate hatchery and growout techniques for local species has been identified as an impediment to establishment of commercial clam culture industries (Nell et al. 1994). A major purpose of this work was to assess the suitability of current bivalve hatchery protocols for aquaculture production of *R. largillierti* and ascertain the performance of hatchery-produced juveniles in the wild.

Ruditapes (= Venerupis) largillierti is endemic to New Zealand but its range has extended to Tasmania (although not mainland Australia) during this century where it remains indistinguishable from New Zealand populations, on the basis of allozyme analysis (Maguire & Ward unpubl. data). *R. largillierti* grows to a length of 70 mm and a height of 50 mm and is found subtidally in both muddy and sandy substrates in shallow estuarine waters with high current flow (Cook 1997). It occurs sympatrically in Tasmania with native *Katelysia scalarina*, the latter occurring in the more intertidal or shallow subtidal zone. In Georges Bay, the site of the only commercial fishery for this species in Tasmania, *R. largillierti* can be collected by superficial digging with a pitch fork in heavily reduced subtidal shell beds. It is not exploited commercially in New Zealand (R. Creese pers. comm.), although its potential is recognised (Gribben 1998). Adult *R. largillierti* can be found in an advanced state of gametogenesis through most of the year in Tasmania (Maguire & Kent unpubl. data), although the largest numbers of ripe clams have been observed to occur between late winter and early spring (Paturusi 1994). In response to the heterogeneous gonad maturation of individual *R. largillierti* and to difficulties encountered in spawning other clam species (Kent et al. unpubl. data), several methods to artificially induce spawning were examined as part of this study.

MATERIALS AND METHODS

Spawning

All spawning and larval rearing trials with the exception of one trial to produce clams for growout, were conducted at the Department of Aquaculture, University of Tasmania (UTAS) facilities at Launceston, Tasmania. One trial on the production of clam seed for growout was done at Geordy River Aquaculture, a commercial bivalve hatchery at Georges Bay, St. Helens, Tasmania (Fig. 1). Broodstock for all spawning trials were collected from Georges Bay. Before spawning, clams were held out of water for 12 h (Manzi & Castagna 1989) typically at c. 14°C. Spawnings were conducted in a dark flat fibreglass trough ($100 \times 600 \times 900$ mm) filled with recirculating filtered sea water. Clams were held corporately in the trough and ripe clams were encouraged to spawn by cycling water temperatures (Loosanoff & Davis 1963) and the addition of stripped gametes (Castagna & Kraeuter 1981; Utting & Spencer 1991). In some spawning events a UV light was also included in the recirculating system to help maintain low bacterial levels and stimulate spawning (Bourne et al. 1989). Spawning clams were removed from the tray and placed in individual 1 litre beakers with filtered sea water (FSW) (Utting & Spencer 1991) or allowed to spawn out collectively in the tray (Table 1).

Clams which did not spawn after temperature cycling (13–20°C, at 30 min intervals), had a small notch filed in the valve margin adjacent to the anterior adductor muscle and injected with various concentrations and volumes of serotonin (5-HT) (Table 2) as described by Gibbons & Castagna (1984, 1985), Heasman et al. (1994), and O'Connor & Heasman (1995), and placed in 1 μm FSW at 20°C.

Larval rearing (UTAS)

All sea water (FSW) for spawning and larval rearing was 1 μm filtered (cartridge). Disodium ethylenediaminetetra-acetic acid (Na_2EDTA) was added to water at 1 mg litre^{-1} during egg incubation (Day 0), as per Utting & Helm (1985). Fertilised eggs were stocked at between 10–36 eggs ml^{-1}, and larval densities ranged from 1 to 5 larvae ml^{-1}. Larvae were cultured in 300-litre conical bottom, and 1000-litre flat bottom fibreglass tanks. For all cultures, larvae were retained on submerged nylon mesh screens, of appropriate mesh size, before being transferred to a clean tank every second day. Rearing temperatures were maintained at 20 ± 2°C after preliminary trials at temperatures of c. 25°C (which is commonly used for rearing larvae of the Pacific oyster, *Crassostrea gigas*) resulted in high mortality and large numbers of deformed D larvae by 48 h. Larvae were fed a 1:1 (by cell number) mixture of *Pavlova lutheri* (Droop) and *Isochrysis galbana* (Tahitian strain) at c. 10 000 cells/larvae (Toba et al. 1992).

Induction of metamorphosis

In June 1996, the efficacy of the catecholamines, epinephrine and norepinephrine in inducing metamorphosis ("set") was assessed using Day 16 pediveligers (PVs) with a mean shell length of 178 μm. The experimental design consisted of triplicate 1-litre plastic beakers at 20°C for each treatment. FSW (1 μm) in the beakers contained $10^{-3}M$ epinephrine, or $10^{-3}M$ arterenol (norepinephrine), both predissolved in a small volume of $0.05N$ HCl (Coon et al. 1986), or no catecholamine (controls). 11×10^3 PVs were added to each of the nine beakers and gently aerated. Treatments and controls were screened, rinsed, and replaced with FSW 18 h after initiation, and thereafter daily. Counts were made of

competent larvae and metamorphosed animals, as defined by Coon et al. (1985) 3 and 6 days post-treatment (Day 19 and 22 respectively). Separate, single fixed factor ANOVAs were conducted on Day 19 and Day 22 percentage settlement data in relation to catecholamine treatments after arcsin square root transformation and confirmation of homogeneity of variance with Cochran's test (Sokal & Rohlf 1995).

Growout

In April 1995, 72 clams were spawned by elevating water temperature from ambient (13°C) to 16°C at a commercial bivalve hatchery at St Helens, resulting in 18×10^6 eggs. Eggs were stocked in 2000-litre flat bottom fibreglass tanks at 9 eggs ml^{-1}. Resultant larvae were reared at 19–20°C and fed a combination of *P. lutheri*, *I. galbana*, and *Chaetoceros calcitrans* (Paulsen) (equal cell numbers) at 25 000–30 000 cells ml^{-1} (equivalent to 5000 cells larvae^{-1} at Day 2 to 30 000 cells larvae^{-1} at Day 9 through to settlement). As a result of mortality, larval densities decreased from 2.5 to 0.45 larvae ml^{-1} between Day 2 and Day 18 at which time survivors were placed into a 2000-litre settlement tank as PVs at 190–220 μm shell length. The tank contained lengths of curved Poly Vinyl Chloride (PVC) "slats" (see Holliday 1996) previously used for oyster settlement. Slats were conditioned in sea water
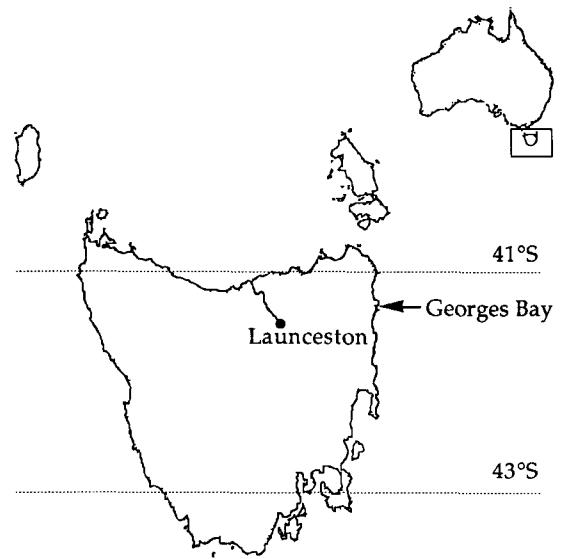

Fig. 1 Collection, larval rearing, and growout sites for *Ruditapes largillierti* in Tasmania, Australia.

Table 1 Mean fecundity, and numbers of male and female *Ruditapes largillierti* induced to spawn collectively by thermal stimulation. (Cy = 30 min cycling, E = single temperature elevation.)

Spawning date	No. of broodstock	Thermal stimulus (°C)	No. spawned		Total no. of eggs ($\times 10^6$)	Mean fecundity (eggs $\times 10^6$ clam^{-1})
			Male	Female		
19 May 1994	50	13–23 E	15	11	5.2	0.5
6 Feb 1996	54	19–25 Cy	5	9	3.6	0.4
3 Jun 1996	67	13–20 Cy	25	40	36	0.9
4 Jul 1996	86	14–19 Cy	31	55	43.2	0.8

Table 2 Numbers of male and female *Ruditapes largillierti* induced to spawn by injection of serotonin at different volumes and concentrations. Range of total amount of serotonin injected was (3×10^{-7} to 1.5×10^{-6} moles clam^{-1}).

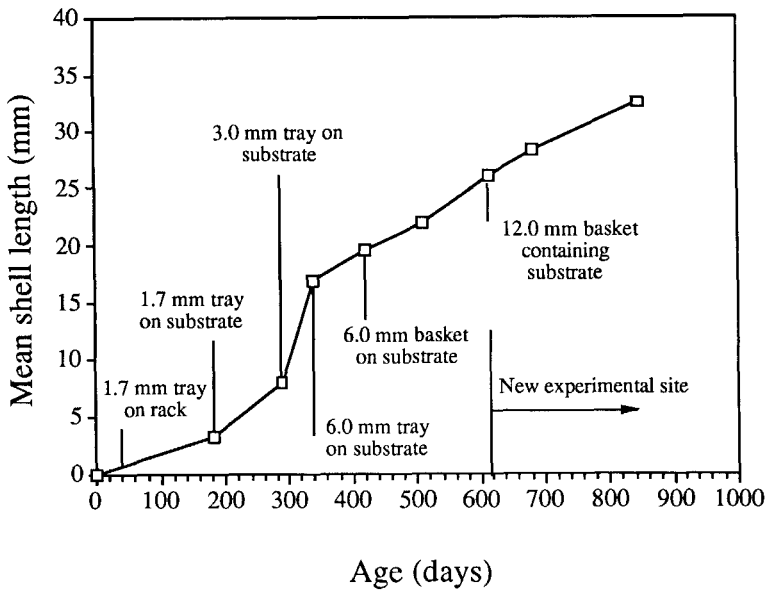| Number of broodstock	Injection volume (μl)	Concentration (mM)	Number spawned	
			Male	Female
38	0	0	0	0
10	100	15	0	0
25	40	20	2	0
20	100	10	2	0
20	30	10	3	0

Fig. 2 Growth (expressed as shell length) of *Ruditapes largillierti* in Georges Bay, Tasmania, Australia. Points are mean ± SE) (*n* = 10–40). Where not visible, SE range is 0.1–0.8 mm.

with clam broodstock for 1 week before settlement. Spat were subsequently placed into outdoor tanks at ambient temperature (13°C) and then reared in oyster trays with a 1.7 mm mesh, without slats, in an intertidal nursery pond. At Day 182, clams were placed in 1.7 mm plastic mesh trays and transported out into the bay. Trays were located subtidally and manually worked into the substrate to encourage some sediment to be maintained in the enclosures. Trays were held directly on the substrate by means of wooden stakes. Clams were progressively moved to 3 and 6 mm mesh trays and 6 mm baskets (same methods as for 1.7 mm trays) and were periodically sampled to assess growth and survivorship. At Day 618 the clams were moved to a more sheltered site and transferred to 12 mm baskets into which surrounding substrate was placed to a depth of c. 60 mm (Fig. 2).

RESULTS

Ripe *R. largillierti* were induced to spawn by thermal stimulus (6–10°C above ambient). Fertilised eggs were 63.8 ± 2.5 μm in diameter (mean ± SD), with mean fecundity ranging from 0.5 to 0.9 × 10^6 eggs female^{-1} (Table 1).

Intramuscular injections of serotonin, at various doses and concentrations, failed to induce spawning in female clams, although some males did spawn (Table 2). Male and female clams that were not injected did not spawn.

Fertilised eggs developed into trochophore larvae by 24 h at 20°C and D veligers with a mean shell length of 85.3 ± 4.7 μm within 48 h (Fig. 3). Deformed larvae with fouled velums or unusual shell shape were observed in nearly all larval trials. In the 1995 larval trial at Geordy River Aquaculture about half of 2-day-old larvae were identified as deformed. Development to pediveliger stage (mean shell length 200.3 ± 7.3 μm) took between 11 and 16 days at 20°C, and metamorphosis to spat (mean shell length 240 μm) occurred between Days 16 and 19 (Fig. 3).

Settlement in treatments with epinephrine, norepinephrine, or no catecholamines did not differ significantly after 19 or 22 days ($P > 0.05$). However, the percentage of settled *R. largillierti* significantly increased over time ($P < 0.0001$) and by Day 22 exceeded 75% (Fig. 4).

In all larval trials, survival to set was low, falling to <3% before metamorphosis (Fig. 5) with high mortality occurring during early D veliger development (Days 2–4). These results agree with observations of deformities and mutations in a large percentage of newly developing larvae. In larval trials conducted at UTAS, survival through settlement was also compromised by the presence of a marine ciliate (resembling *Uronema nigricans*, see Munday et al. 1997) which were observed invading larvae.

Growth of hatchery-produced clam seed raised in trays and baskets on the substrate was modest (32 mm shell length in 28 months from spawning, an average of 1.3 mm month^{-1} over the growout period) (Fig. 2). This was slower than for *R.*
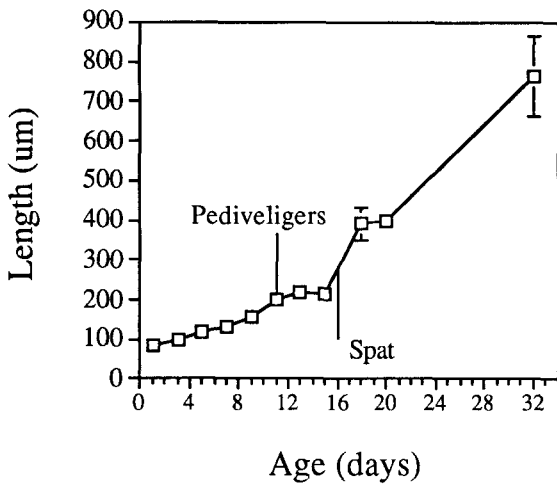
Fig. 3 Larval and early juvenile growth (expressed as shell length) of *Ruditapes largillierti*. Points are mean ± SE (*n* = 6–37).
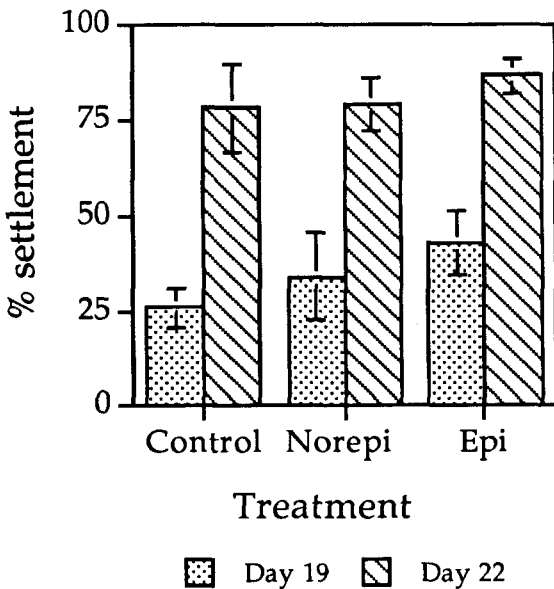

Fig. 4 Comparison of efficacy of the catecholamines, epinephrine, and norepinephrine in inducing metamorphosis in *Ruditapes largillierti* pediveliger larvae. Error bars ± SE (*n* = 3).

largillierti seed, observed following a major natural settlement event, on the adjacent seabed (A. Flintoff pers. comm.).

DISCUSSION

In a list of desirable biological factors to be considered when selecting suitable clam candidates for aquaculture, Kraeuter & Castagna (1989) identified ease of spawning over a substantial period, ease of larval culture, shallow burial depth, rapid growth to market size, and tolerance to wide variations in environmental conditions. We have demonstrated that ripe *R. largillierti* can be readily induced to spawn with thermal stimulation. However, mean fecundities of between 0.5×10^6 and 0.9×10^6 eggs female^{-1} were lower than that for the stepped venerid *Katelysia scalarina* (0.7×10^6–2.4×10^6 eggs female^{-1}) (Kent et al. unpubl. data) and the Manila clam *Ruditapes philippinarum* (0.9×10^6–2.7×10^6 eggs female^{-1}) (Laing & Lopez-Alvarado 1994) and 1.5×10^6 eggs female^{-1} (Utting et al. 1996). Further, the survival of larvae to Day 5 of between 2 and 10% (Fig. 5), was considerably lower than Day 5 survival of 30–50% for *K. scalarina* cultured under similar conditions (Kent et al. unpubl. data). In addition, whereas ripe *R. largillierti* could be spawned using thermal stimulation, moderately developed broodstock (++ as defined by Garland et al. 1993) failed to spawn after repeated exposure to temperature cycling or the addition of gametes. Intramuscular injection of serotonin at various doses into these clams also failed to stimulate spawning in any female stock, although some males did spawn. Many studies have reported ditypic spawning activity of male and female bivalves in response to serotonin injection (Gibbons & Castagna 1985; Ram & Nichols 1993; Monsalvo-Spencer et al. 1997). In contrast, an Australian venerid, *Tapes dorsatus*, can be induced to spawn when females are injected with serotonin (J. Nell pers. comm.). In addition, Martinez et al. (1996) have reported successful spawning induction of both male and female gametes in the scallop *Argopecten purpuratus* by gonadal injection of a combination of serotonin and prostaglandin E$_2$ (PGE$_2$) or dopamine and PGE$_2$. Given the poor survival of the larvae of other clam species produced from stripped eggs (Kent et al. unpubl. data), and the ineffectiveness of serotonin injections on female clam stock, combination injections of serotonin and PGE$_2$ or dopamine and PGE$_2$ should be investigated for this species. In addition, Martinez et al. (1996) also demonstrated significantly higher percent fertilisation and survival of D larvae using combination injection of dopamine and PGE$_2$ as against temperature induced spawning, possibly as a result of only optimum quality eggs being released. These potential benefits may be important for culture of *R.*
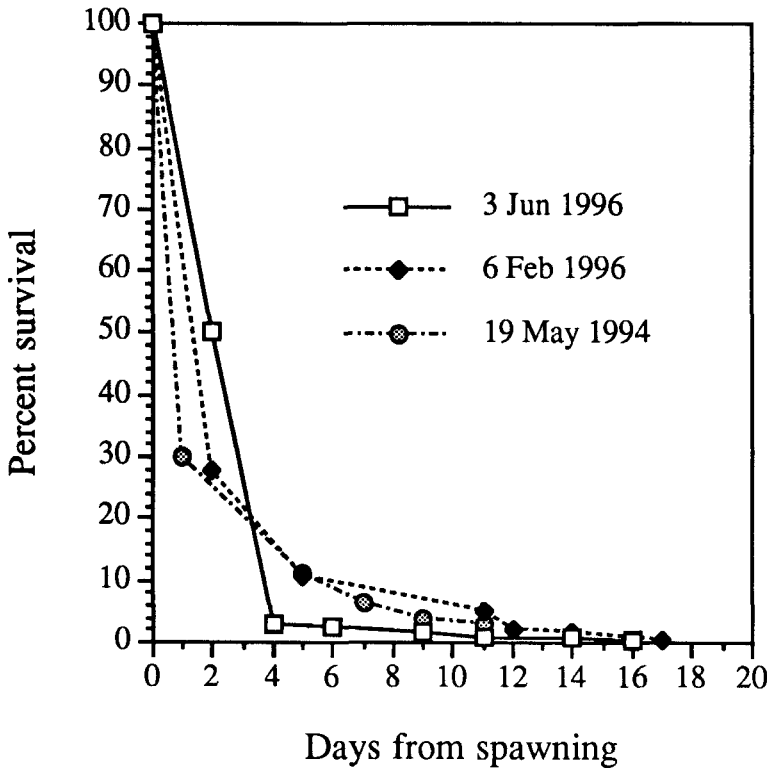
Fig. 5 Survival of three batches of *Ruditapes largillierti* larvae to metamorphosis, expressed as a percentage of total number of eggs obtained at spawning.

largillierti, given the heterogeneous gonad development of this species (Gribben 1998; Maguire & Kent unpubl. data) and the poor survival of D larvae we observed.

As a result of gametogenic variation in *R. largillierti*, development of appropriate broodstock conditioning protocols for this species may prove beneficial, as they have for many clam species (Castagna & Manzi 1989; Ruiz-Azcona et al. 1996; Numaguchi 1997) including other venerids (Kent et al. unpubl. data). Aquaculturalists may find broodstock conditioning particularly useful for *R. largillierti* which appears to only partially spawn in any given event (Paturusi 1994; Gribben 1998; Maguire et al. unpubl. data), thus allowing for year-round supply of hatchery seed.

Average growth of juvenile clams of 1.3 mm month^{-1} over the experimental growout period was comparable to juvenile (12 mm) *R. philippinarum* seeded in sand in Bouin, France (de Valence & Peyre 1990), but lower than that for *K. rhytiphora* (2.6 mm month^{-1}) cultured in 6 mm plastic mesh baskets buried in a medium sand substrate in Port Stephens, Australia (Paterson & Nell 1997). In this study

growth of clams from 20 mm shell length (1.0 mm month^{-1}) was also lower than that recorded by Paturusi (1994) for similar sized *R. largillierti* (27.4 mm initial length) of 1.4 mm month^{-1}. Observation of clams from the major natural settlement noted previously suggest that growth of *R. largillierti* in Tasmania can exceed 2 mm month^{-1} (A. Flintoff pers. comm.). In our growout trial, growth was probably compromised by difficulties with maintaining substrate in the trays and baskets plus reduced water flow over clam seed caused by the observed macroalgal fouling of the culture units. Peterson & Black (1993) also reported that growth in cages was significantly slower than that for a fenced stock of *Katelysia* spp., although fenced stock grew better than those in enclosure-free plots. Although growth of clams in this study was less than optimum we observed uniform growth rates within samples (Fig. 2). These observations are similar to other growth data for *R. largillierti* (Paturusi 1994), and *K. rhytiphora* and *Tapes dorsatus* (Paterson & Nell 1997) which are also characterised by low sample variance. Presence of shell debris in the spat transferred to 1.7 mm trays within Georges Bay precluded accurate

estimate of stock numbers and hence survival during growout. However little mortality has been evident while spat have been in 6–12 mm baskets.

The aquaculture potential of this species warrants further investigation as spat become available from commercial hatcheries. Unfortunately, farming subtidal clams is less convenient than intertidal clams which can be planted on beaches under protective netting (Toba et al. 1992). It would be useful to reduce initial larval mortality, and use of serotonin/PGE$_2$ and further investigation into optimum water temperature for larval rearing (Heasman et al. 1996) maybe worthwhile in this regard. Given the presence of the European shore crab *Carcinus maenus* in Tasmanian waterways (Gardner et al. 1994), some predator protection will probably be required. However alternatives to mesh cages and trays, for example shell beds, should be assessed.

ACKNOWLEDGMENTS

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