

Make it Count: Maximising the Stocking Density of Juvenile Fluted Giant Clams (*Tridacna squamosa* Lamarck) During Simulated Live Transportation

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Abstract

Juvenile giant clams are usually transported at stocking densities of 1-2 bag⁻¹ for up to 48 hr. In this study the effect of stocking juvenile fluted giant clams (*Tridacna squamosa*) at densities ranging from 2-19 bag⁻¹ were assessed. Juveniles ranging from 50-70 mm in shell length (SL) and 11.3- 39.9g in wet weight (WW) were packed in plastic bags filled with 1L of 5µm-filtered seawater (FSW). The bags were then packed in styrofoam boxes and kept at 22.0-25.0 °C for 48hr. There was a relationship between stocking density and ammonia concentration of FSW, and inverse relationships between stocking density and pH, and stocking density and dissolved oxygen (DO). Following a 21 day resting period, no mortalities were recorded at stocking densities up to 13 juveniles/bag⁻¹. Both the WW and SL of juveniles in all treatments were significantly higher after the resting period than prior to the transportation trial. However, there was no relationship between stocking density and growth rate in either WW or SL. The results of this study suggest that juvenile *T. squamosa* can be transported without mortalities and effects on growth at a density of up to 13 juveniles/bag⁻¹.

Introduction

Giant clams (Mollusca: Tridacnidae) are brightly coloured bivalves inhabiting the shallow coastal reefs across the Indo-Pacific region (Lindsay et al. 2004; Kinch and Teitelbaum, 2009). Giant clams are collected for their meat and shells (Cowan, 1988; Taniera, 1988), and live specimens are sourced by aquarium hobbyists (Munro, 1993; Fatherree, 2006). Market demand has historically been met by exploitation of wild stocks, which has led to severe depletion in many areas (Heslinga et al. 1988; Munro, 1993; Lindsay et al. 2004).

Consequently, giant clam aquaculture has been established since the mid 1980s to supply demand and replenish wild stocks (Munro, 1993; Lindsay et al. 2004; Kinch and Teitelbaum 2009). Giant clams are relatively easy to cultivate due to their ability to gain nutrients through filter-feeding, and via symbiosis with the dinoflagellate alga *Symbiodinium microadriaticum* (Munro, 1993; Wabnitz et al. 2003; Fatherree, 2006; Kinch and Teitelbaum, 2009). Apart from maintaining good water quality and adequate sunlight, giant clams need relatively little care (Ellis, 2000).

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Although thousands of juvenile giant clam specimens are traded globally every year (Wabnitz et al. 2003), information on transportation methods is limited. If the travel time is less than 12hr, giant clams can be transported dry without a supply of seawater (Ellis, 2000). However, similar to aquarium fish, the transportation of juvenile giant clams for more than 12hr requires seawater with an oxygen:seawater ratio of 3:1 (Braley, 1992; Knop, 1996; Ellis, 2000; Fatherree, 2006). Most exporters pack the juvenile giant clams in two highly durable plastic bags, with insulating material such as newspaper inserted between the bags (Ellis, 2000; Fatherree, 2006). Typically, exporters pack only one or two juvenile giant clams in a bag (Fatherree, 2006).

The Darwin Aquaculture Centre (DAC) in Australia's Northern Territory (NT) has successfully spawned and reared the fluted giant clam (*Tridacna squamosa* Lamarck, 1819) since 2008 (Handley, 2010). The DAC, in collaboration with an industry partner, has been supporting the development of a *T. squamosa* industry in the NT, aimed at supplying international markets in the USA, Asia and Europe. Given the distances to the major markets, this will involve live packaging and transportation of juvenile *T. squamosa* for between 12 and 48 hr.

Transportation costs can significantly add to the overall operational costs for exporters of marine organisms (Wood, 1985; Ellis, 2000). However, transport costs can be reduced by sending large shipments at high stocking densities (Ellis, 2000; Cole et al. 1999). As exporters typically pack only one or two juvenile giant clams in a bag (Fatherree, 2006), significant reductions in transport costs could be made by increasing the stocking density. Therefore, it was the aim of this study to assess the effects of a range of stocking densities on growth and survival of juvenile *T. squamosa* in a simulated 48hr transportation trial.

Materials and Methods

This experiment was conducted at DAC from 27-29 July 2011 and used a total of 415 juvenile *T. squamosa* of age 18 months. Spawning, larval rearing and nursery culture of the juveniles followed the methods of Braley (1992). The initial shell length (SL) of the juveniles ranged from 50-70mm, and their initial wet weight (WW) ranged from 11.3-39.9 g.

The juveniles were detached with a metal scraper from the substratum within a grow-out tank on 25 July 2011. The juveniles were placed into a small container, and the external surfaces of the valves were lightly scrubbed with a hard bristled brush to remove excess growth and algae. The juveniles were then rinsed with 5 µm-filtered seawater (FSW) and evenly and randomly distributed amongst five partly shaded 9 tonne holding tanks. Each tank was sectioned into eight replicate groups (one for each of the seven treatments and one for the control group) using PVC piping, and supplied with flow-through FSW and light aeration.

The following day, the juveniles were removed from the holding tanks and placed onto a dry tray. The right valve of each juvenile was dried with paper towel and a small plastic identification

tag glued between the second and third vertical folds, adjacent to the byssal orifice. The SL was then measured and the juveniles returned to their allocated section within the holding tank.

The experiment consisted of seven treatments of varying stocking densities of juveniles set up as a randomised complete block ($n=5$). An additional control group of 10 juveniles was kept in each 9 tonne holding tank for the duration of the experimentation. The stocking densities tested during the simulated transport were 2, 5, 8, 10, 13, 16 and 19 juveniles bag^{-1} , plus bags filled only with FSW. The bags used to retain the juveniles during the trial were in a triple bag configuration. The triple bag configuration consisted of a smaller, 100 μm thick plastic bag (200 x 245 mm) placed inside a larger, 50 μm plastic bag (405 x 245 mm). A third 50 μm plastic bag (405 x 245 mm) was then placed inside the double-bag arrangement. The inner bags were filled with 1 L of FSW taken from a single 50 L container filled just before the experiment ($\text{pH } 8.0 \pm 0.0$, dissolved oxygen (DO) $6.3 \pm 0.1 \text{ mg L}^{-1}$, ammonia $0.1 \pm 0.0 \text{ mg L}^{-1}$, salinity $34.0 \pm 0.0 \text{ ppt}$ and temperature $24.9 \pm 0.0 \text{ }^{\circ}\text{C}$ ($n=3$)).

Following that, the juveniles were removed from the holding tank in replicate groups, individually weighed and placed into the inner bag, taking particular care not to puncture the bag or invert the juveniles. The bags were then gently pressed to remove air and filled with pure oxygen (ratio oxygen: seawater 3:1 (v:v), (Braley, 1992)). The bags were tightly sealed using rubber bands, checked for leakages and the total bag weight measured. The sealed triple bags were then randomly placed into one of five styrofoam boxes (570x380x330 mm). Each of the styrofoam boxes was sectioned into eight compartments using waxed cardboard, and housed one bag from every treatment. Finally, the styrofoam boxes were lidded, taped with packing tape and kept undisturbed in a temperature controlled room ($22.0\text{-}25.0 \text{ }^{\circ}\text{C}$) for 48 hr. After 48 hr the boxes were opened and the bags weighed and assessed for leakages. The bags were then opened and the juveniles checked for mortality. Subsequently, the juveniles were removed from the bags and returned to their designated compartments within their respective 9 tonne holding tanks. The pH, DO, salinity and temperature was then recorded for the FSW in the bags, and a 50mL sub-sample was collected for ammonia testing. The sub-samples were refrigerated overnight and the ammonia tests conducted the next morning using a commercially available test kit (Nutrafin Ammonia Test, Hagen, Canada). For 21 days post-experimentation, the juveniles were monitored for mortality or ill health. At the termination of the 21-day resting period the SL and WW of each juvenile was measured.

Bag weight, water quality parameters, SL and WW data were analysed by ANOVA. The assumptions for ANOVA were checked by visual inspection of residual values for each model. Before and after measurements within a treatment were compared with two-tailed dependent samples t-tests (paired). Differences in measurements between treatments were compared using unweighted means ANOVA (Type III) to allow for the unequal numbers per treatment and, where appropriate, pairwise comparisons for means were done using the Tukey method. Assessment of the final proportion of individuals surviving between treatments was done using a generalised linear model with binomial errors and comparing scaled deviance for terms using an F-test to correct for

overdispersion. Results are expressed as the mean $\pm$ SE and differences between treatments were considered significant at $P\leq 0.05$.

Results

There was no significant difference in total bag weight (juveniles plus 1L of seawater) of any treatment after the 48 hr transportation trial as compared to the initial weight (Table 1).

Table 1. Bag weights (BW) of plastic bags containing 1 L of seawater and juvenile *T. squamosa*, before and after a 48 hr transport period. Values are the mean $\pm$ SE (n=5).

Stocking density (juveniles/bag ⁻¹)	Initial BW (kg)	Final BW (kg)
0	1.04 $\pm$ 0.02	1.04 $\pm$ 0.02
2	1.10 $\pm$ 0.01	1.11 $\pm$ 0.01
5	1.17 $\pm$ 0.02	1.17 $\pm$ 0.03
8	1.21 $\pm$ 0.02	1.21 $\pm$ 0.02
10	1.26 $\pm$ 0.02	1.26 $\pm$ 0.02
13	1.32 $\pm$ 0.02	1.32 $\pm$ 0.02
16	1.38 $\pm$ 0.03	1.36 $\pm$ 0.03
19	1.47 $\pm$ 0.02	1.47 $\pm$ 0.02

The water quality parameters of the FSW in the bags used to transport juveniles for 48hr are listed in Table 2. There were no significant differences in salinity and temperature of the FSW among the treatments after 48hr. The pH (8.1 ± 0.0) and DO (13.2 ± 0.6 mgL⁻¹) of the FSW in the bags with no juveniles were significantly higher than that of the FSW used to transport juveniles. Moreover, the pH (7.2 ± 0.0) and DO (11.1 ± 0.7 mgL⁻¹) of FSW stocked with two juveniles were significantly higher than that of FSW stocked with a greater number of juveniles. The inverse relationship between stocking density of juveniles and pH ($r^2=0.51988$, $P\leq 0.05$) and stocking density of juveniles and DO ($r^2=0.89811$, $P\leq 0.01$) of FSW after 48hr was significant. The ammonia concentration (0.00 ± 0.00 mgL⁻¹) of the FSW in the bags with no juveniles was significantly lower than that of FSW stocked with juveniles. Filtered seawater stocked with two juveniles had a significantly lower ammonia concentration than FSW stocked with 8, 10, 13 and 19 juveniles. However, the ammonia concentration of FSW stocked with 2, 5 and 16 juveniles was not significantly different. The relationship between stocking density of juveniles and ammonia concentration was significant ($r^2=0.62876$, $P\leq 0.02$).

Table 2. Water quality parameters of seawater used to transport juvenile *T. squamosa* for 48 hr*. Values are the mean $\pm$ SE (n=5). Significant differences within a column are indicated by different superscripts ($P \leq 0.05$).

Stocking density (juveniles bag ⁻¹)	Water quality parameter				
	pH	DO (mg L ⁻¹)	ammonia (mg L ⁻¹)	salinity (ppt)	temperature (°C)
0	8.1 $\pm$ 0.0 ^a	13.2 $\pm$ 0.6 ^a	0.00 $\pm$ 0.0 ^a	34.0 $\pm$ 0.0 ^a	25.5 $\pm$ 0.3 ^a
2	7.2 $\pm$ 0.0 ^b	11.1 $\pm$ 0.7 ^b	0.11 $\pm$ 0.0 ^b	34.0 $\pm$ 0.0 ^a	25.5 $\pm$ 0.1 ^a
5	7.1 $\pm$ 0.0 ^c	8.6 $\pm$ 0.4 ^c	0.18 $\pm$ 0.0 ^{b, c}	34.0 $\pm$ 0.0 ^a	25.9 $\pm$ 0.5 ^a
8	7.1 $\pm$ 0.0 ^c	6.9 $\pm$ 0.6 ^c	0.20 $\pm$ 0.0 ^c	34.0 $\pm$ 0.0 ^a	25.4 $\pm$ 0.0 ^a
10	7.0 $\pm$ 0.0 ^d	4.3 $\pm$ 0.0 ^d	0.19 $\pm$ 0.0 ^c	34.0 $\pm$ 0.0 ^a	25.4 $\pm$ 0.0 ^a
13	7.0 $\pm$ 0.0 ^d	4.3 $\pm$ 0.0 ^d	0.19 $\pm$ 0.0 ^c	34.0 $\pm$ 0.0 ^a	25.4 $\pm$ 0.0 ^a
16	7.0 $\pm$ 0.0 ^d	3.7 $\pm$ 0.0 ^d	0.18 $\pm$ 0.0 ^{b, c}	34.0 $\pm$ 0.0 ^a	25.5 $\pm$ 0.1 ^a
19	6.9 $\pm$ 0.0 ^e	3.1 $\pm$ 0.0 ^d	0.24 $\pm$ 0.0 ^c	34.0 $\pm$ 0.0 ^a	25.5 $\pm$ 0.1 ^a

*Parameters at stocking were pH 8.0 $\pm$ 0.0, DO 6.3 $\pm$ 0.1 mg L⁻¹, ammonia 0.1 $\pm$ 0.0 mg L⁻¹, salinity 34.0 $\pm$ 0.0 ppt and temperature 24.9 $\pm$ 0.0 °C (n=3).

Survival in the control treatment and at stocking densities of 2 to 16 juveniles ranged from 100.0 $\pm$ 0.0 to 97.5 $\pm$ 2.5%, and was not significantly different (Table 3). However, survival of juveniles stocked at a density of 19 bag⁻¹ was significantly lower compared to all other treatments (Table 3).

Table 3. Survival of juvenile *T. squamosa* transported in plastic bags for 48 hr and held for a resting period of 21 days. Values are the mean $\pm$ SE (n=5). Significant differences are indicated by different superscripts ($P \leq 0.05$).

Stocking density (juveniles bag ⁻¹)	Survival (%)
Control*	100.0 $\pm$ 0.0 ^a
2	100.0 $\pm$ 0.0 ^a
5	100.0 $\pm$ 0.0 ^a
8	100.0 $\pm$ 0.0 ^a
10	100.0 $\pm$ 0.0 ^a
13	100.0 $\pm$ 0.0 ^a
16	97.5 $\pm$ 2.5 ^a
19	84.2 $\pm$ 11.9 ^b

*Juveniles in the control treatment were kept in 9 tonne holding tanks.

The WW of juveniles in all treatments was significantly higher after the 21 day resting period than prior to the transportation trial (Table 4). The growth rate in WW varied from 47.3 $\pm$ 8.6 μ g day⁻¹ in juveniles stocked at a density of 5 bag⁻¹, to 114.0 $\pm$ 19.8 μ g day⁻¹ in the control treatment (Fig. 1). The growth rate of the juveniles in the control group, and that of juveniles stocked at 10 and 19 bag⁻¹ was significantly higher compared to the growth rate of juveniles stocked at 5, 8 and 16 bag⁻¹. However, there was no significant relationship between stocking density of juveniles and their growth rate in WW ($r^2=0.11569$, $P \geq 0.45$).

Table 4. Wet weight (WW) of juvenile *T. squamosa* transported in plastic bags for 48 hr and held for a resting period of 21 days. Values are the mean $\pm$ SE (n=5). Significant differences within a row are indicated by the letter a ($P \leq 0.05$).

Stocking density (juveniles bag ⁻¹)	Initial WW (g)	Final WW (g)
Control*	21.2 $\pm$ 1.0	23.9 $\pm$ 1.4 ^a
2	20.2 $\pm$ 1.5	22.0 $\pm$ 1.6 ^a
5	21.1 $\pm$ 1.5	22.2 $\pm$ 1.6 ^a
8	20.3 $\pm$ 1.4	21.6 $\pm$ 1.3 ^a
10	23.1 $\pm$ 0.3	25.5 $\pm$ 0.4 ^a
13	20.8 $\pm$ 0.4	22.7 $\pm$ 0.3 ^a
16	21.2 $\pm$ 0.8	22.5 $\pm$ 0.5 ^a
19	22.6 $\pm$ 0.7	25.4 $\pm$ 0.6 ^a

*Juveniles in the control treatment were kept in 9 tonne holding tanks

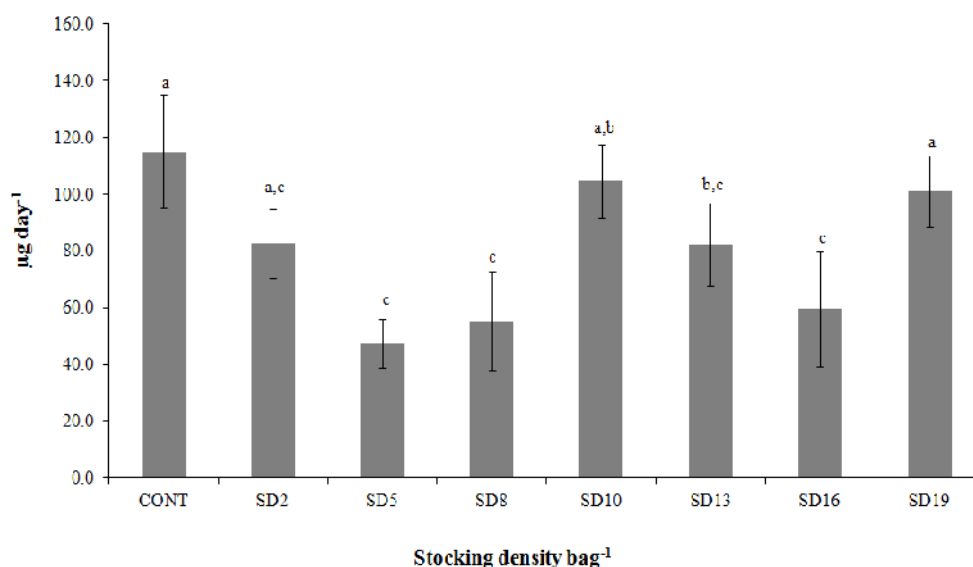


Fig. 1. Growth ($\mu\text{g day}^{-1}$) in wet weight (WW) of juvenile *T. squamosa*, transported in plastic bags for 48 hr and held for a resting period of 21 days. Values are the mean $\pm$ SE (n=5). Significant differences between treatments are indicated by different letters ($P \leq 0.05$).

Similarly to WW, the SL of juveniles in all treatments was significantly higher after the 21 day resting period compared to the initial SL (Table 5). The growth rate in SL varied from $53.6 \pm 15.6 \mu\text{m day}^{-1}$ in juveniles stocked at a density of 16, to $123.6 \pm 17.2 \mu\text{m day}^{-1}$ for 19 juveniles bag⁻¹ (Fig. 2). The growth rate in SL of juveniles in the control group and juveniles stocked at a density of 19 bag⁻¹ was significantly higher than that of juveniles stocked at a density of 5, 13 and 16 bag⁻¹. However, the relationship between stocking density of juveniles and their growth rate in SL was non-significant ($r^2=0.21442$, $P \geq 0.25$).

Table 5. Shell length (SL) of juvenile *T. squamosa* transported in plastic bags for 48hr and held for a resting period of 21 days. Values are the mean $\pm$ SE (n=5). Significant differences within a row are indicated by the letter a ($P \leq 0.05$).

Stocking density (juveniles'bag ⁻¹)	Initial SL (mm)	Final SL (mm)
Control*	58.3 $\pm$ 0.7	61.1 $\pm$ 1.2 ^a
2	58.2 $\pm$ 1.5	59.7 $\pm$ 1.5 ^a
5	59.2 $\pm$ 1.2	60.5 $\pm$ 1.4 ^a
8	57.5 $\pm$ 1.2	59.6 $\pm$ 1.0 ^a
10	60.3 $\pm$ 0.2	62.8 $\pm$ 0.4 ^a
13	58.2 $\pm$ 0.5	60.0 $\pm$ 0.4 ^a
16	58.0 $\pm$ 0.6	59.3 $\pm$ 0.6 ^a
19	59.9 $\pm$ 0.5	62.9 $\pm$ 0.7 ^a

*Juveniles in the control treatment were kept in 9 tonne holding tanks.

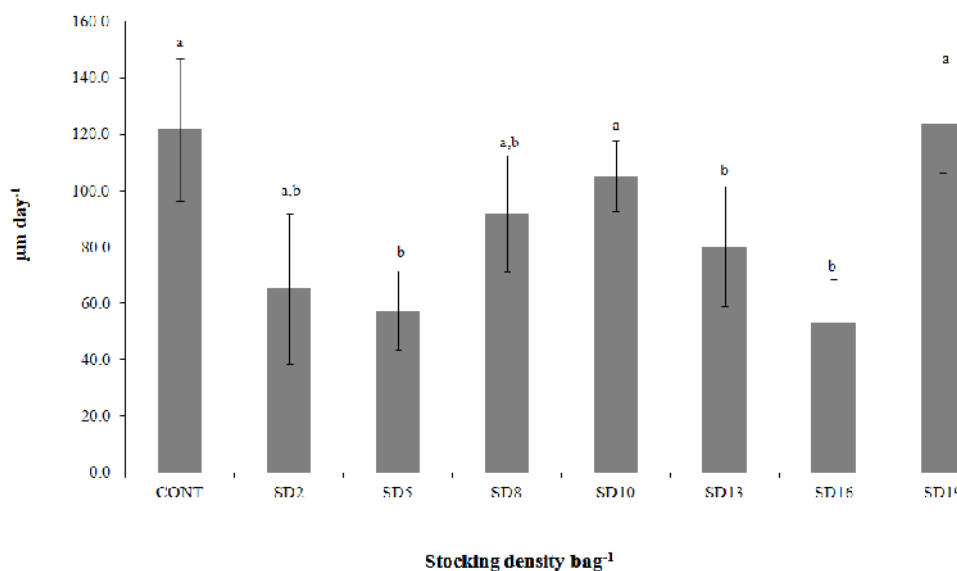


Fig. 2. Growth ($\mu\text{m day}^{-1}$) in shell length (SL) of juveniles *T. squamosa*, transported in plastic bags for 48 hr and held for a resting period of 21 days. Values are the mean $\pm$ SE (n=5). Significant differences between treatments are indicated by different letters ($P \leq 0.05$).

Discussion

The present study showed that juvenile *T. squamosa* can be transported at densities of up to 13 juveniles'bag⁻¹ for 48 hr, without incurring mortalities. This presents a six to ten-fold increase to the usual commercial stocking density of one or two juveniles'bag⁻¹ (Fatherree, 2006), and has potentially significant implications with regards to shipping costs. While the 84.2 % survival rate of juveniles stocked at 19 bag⁻¹ falls well below the 5-10% mortality rate recorded for ornamental fish transport (Wood, 1985; Pyle, 1993), the survival rate of 97.5% recorded for a stocking density of 16 juveniles'bag⁻¹ falls well within these parameters. However, exporters of live specimens for the

ornamental aquarium trade always aim at minimising mortalities as these are both economically and socially undesirable (Ellis, 2000; Wood, 2001; Pyle, 1993). Assuming a farm gate value of approximately AUS\$100 juvenile⁻¹ (Anonymous, 2011), even a 2% mortality rate in a shipment of 300 juvenile *T. squamosa* would still amount to a loss of AUS\$600.

At all stocking densities the pH of the FSW after the 48 hr transportation period was below the recommended level of 7.9 (Braley, 1992; Knop, 1996). Similarly, the DO level of FSW for stocking densities higher than eight juveniles/bag⁻¹ was below the recommended level of 5.0 mg·L⁻¹ for transportation of warm water fish species (Berka, 1986). The ammonia levels in FSW stocked with juveniles were also above the recommended level of 0.10 mg·L⁻¹ (Fatherree, 2006). Importantly, even at stocking densities that did not result in mortalities, the pH, DO and ammonia levels of the FSW in the bags were sub-optimal to recommended levels. These results show that juvenile *T. squamosa* can survive in FSW with sub-optimal pH, DO and ammonia levels for at least 48 hr.

Similarly, the 48 hr transportation period did not appear to affect the growth of juveniles. Both the SL and WW of juveniles at all stocking densities were found to have increased significantly after the 21 day resting period. There were significant differences in growth rates of both SL and WW among treatments; however, there was no relationship between stocking density for either SL or WW. Therefore, the survival rate of juveniles is probably the main criterion to evaluate when deciding on an optimal stocking density for juvenile *T. squamosa*.

Loss of water from breakages is an issue affecting the health of live aquatic organisms when transported in bags (Cole et al. 1999). Excessive loss of FSW from bag breakages during preliminary packing trials (A.J. Gould, unpubl. data) highlighted the need for more durable bags when transporting juvenile giant clams. Plastic bags with a thickness of 50 and 100 µm were used in this experiment, resulting in a maximum loss of 20 mL FSW at a stocking density of 16 juveniles/bag⁻¹. However, the loss of FSW after 48 hr was not significant in any treatment and proved both the bags and packing method in individual compartments to be suitable for commercial transport.

Over-crowding is an important factor when transporting live aquarium species in plastic bags (Cole et al. 1999; Ellis, 2000). High stocking densities can inhibit natural movement, reduce water quality and increase the chances of mortality (Cole et al. 1999). In the present study over-crowding was defined as the need to stack juveniles on top of each other in a bag, and occurred at stocking densities higher than 10 juveniles/bag⁻¹. When the bags were opened after the simulated 48 hr transportation, the scutes on the shells of some juveniles were found to interfere with the ability of other juveniles to close their shells completely. In contrast, at all stocking densities some juveniles were found to have inverted during transportation, despite initially being placed upright in the bags. Although the health of juvenile *T. squamosa* can deteriorate when they are either inverted or are

unable to close due to obstructions (E. W. Needham, pers. observation), this appeared to have no effect on the juveniles at stocking densities of up to 13 juveniles'bag⁻¹.

Conclusion

Transporting *T. squamosa* at stocking densities of up to 13 juveniles'bag⁻¹ did not affect their growth and did not result in mortalities. A stocking density of 13 juveniles'bag⁻¹, rather than the usual commercial stocking density of up to two juveniles'bag⁻¹ (Fatherree, 2006), would significantly reduce the cost of transportation. It has to be emphasised, however, that the commercial feasibility of transporting juvenile *T. squamosa* at high densities has to be assessed in actual 48 hr transportation trials in airplanes.

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