Introduction

Giant clams are important fishery resources specific to the tropical Indo West Pacific area (Heslinga et al. 1984, Murakoshi 1991, Mingoa-Licuanan & Gomez 2007). They are utilized as ornamentation, food, tourism resources and pets (Knop 1996). To enhance the stock of giant clams depleted owing to overfishing and environmental destruction (e.g. Heslinga et al. 1984, Iwai et al. 2006), seed production has been developed in many countries (Murakoshi 1991, the term “seed” denotes both eggs and larvae in the present study).

Previous studies on seed production have recommended “cleaning”, i.e. removing contaminants from the seeds of giant clam, to increase their survival and growth rates (Jameson 1976, Braley et al. 1988, Ellis 1998, Mingoa-Licuanan & Gomez 2007). A typical cleaning technique is to run seawater containing the seeds through a coarse mesh and then through a fine mesh. The coarse and fine meshes have larger and smaller openings, respectively, in comparison with the body size of the seed. The coarse mesh is assumed to catch large contaminants (e.g. fouling materials detached from adult clams), whereas the fine mesh is assumed to catch the seeds and allow small contaminants (e.g. bacteria and embryos showing maldevelopment) to be washed away. The seeds caught on the fine mesh are moved into tanks filled with clean seawater. The cleaning of giant clam seeds is performed in various hatcheries (e.g. Jameson 1976, Braley et al. 1988).

The cleaning of giant clam seeds requires considerable labor (e.g. 10 min per seed in a 100-L tank, Kurihara, personal observations). Therefore, to determine whether the cleaning of giant clam seeds is worth the considerable labor, the efficacy of the technique should be clarified. There have been, however, no rigorous experiments on the efficacy of cleaning. Although Braley et al. (1988) suggested increased survival rate through the technique, the study lacked replication. Further, although cleaning of seeds is applicable to many shellfishes in tropical aquaculture (e.g. black-lip pearl oyster Pinctada margaritifera (Linnaeus), top shell Tectus niloticus (Linnaeus), green snail Turbo marmoratus Linnaeus, turban snail Turbo argyrostomus Linnaeus, Shokita et al. 1991), the efficacy has not been studied.

We investigated 2 aspects regarding the cleaning of larvae of giant clam Tridacna crocea during the planktonic stage.

Larval-cleaning technique for increasing the survival rate of giant clam *Tridacna crocea* during the planktonic stage

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**Abstract:** Giant clams are important species in tropical aquaculture. The larvae artificially hatched are often cleaned by running seawater containing the larvae through a coarse mesh, designed to catch contaminants larger than the larvae (e.g. fouling materials detached from adult clams); and then through a fine mesh, designed to catch the larvae and wash away smaller contaminants (e.g. bacteria). Such larval cleaning is assumed to improve the larval survival rate. We conducted experiments on how the cleaning improves the survival rate of larvae of the giant clam *Tridacna crocea* during the planktonic stage. The experiments revealed that larval cleaning improves the survival rate for larval densities of 0.3 to 9.8 indiv. mL⁻¹. The experiments suggested that the survival rate at 0.5 indiv. mL⁻¹, a typical larval density in hatcheries, would be 16.6% for the cleaned larvae, much higher than 1.4% for those not cleaned. Through larval cleaning, both bacteria and small *T. crocea* embryos showing maldevelopment were found to decrease in the water containing *T. crocea* larvae, which can explain in part the improved survival rate.

**Key words:** aquaculture, boring clam, cleaning of planktonic larvae, improved survival

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larvae cleaned and not cleaned show different survival rates during the planktonic stage for various larval densities. From the experimental data we also estimated survival rates for the larvae cleaned and not cleaned at a low larval density that is often used in giant clam hatcheries (Murakoshi 1991). The second aspect investigated was how the condition of larvae and environment improve through larval cleaning. For this purpose we tested whether larval cleaning reduces the percentage of embryos showing maldevelopment and the density of bacteria in seawater. Artificially-fertilized eggs in hatcheries occasionally show maldevelopment owing to procedural mistakes such as the excessive addition of sperm to eggs (Ellis 1998), which can worsen the seawater quality. Bacteria are likely to be released from adult giant clams together with gametes and may adversely affect the larvae (Fitt et al. 1992).

**Methods**

To compare survival rates between larvae cleaned and not cleaned, two runs of larval-cleaning experiments were conducted (Fig. 1). Run 1 was conducted at the larval densities $<5$ indiv. mL$^{-1}$, whereas Run 2 was at 5 to 10 indiv. mL$^{-1}$. The density range covered by the two runs, $<10$ indiv. mL$^{-1}$, is reported to be an acceptable range for seed production of giant clams (Fitt et al. 1984).

In Run 1, adult *Tridacna crocea* (shell length: 89.4 to 114.2 mm, n=12) were stimulated to spawn sperm and eggs in tanks on 18 October 2008, following the method in Kurihara et al. (2010). Of these clams, two specimens spawned eggs and sperm, which were crossed. The artificially-fertilized eggs were kept in a 100-L tank containing FSW (i.e. filtered sea water through sand and anthracite). One d after fertilization, D-shaped larvae (“D-larvae”, hereafter), $\approx 160 \mu m$ in the longest part of the body (Fig. 2a), were collected and divided into the “control group” and “clean group”. For the control group D-larvae in seawater were counted under a microscope to estimate the density. The D-larvae in seawater were then mixed with 7 different amounts of FSW, and the D-larvae of the resultant 7 different densities (0.3 to 3.4 indiv. mL$^{-1}$) were moved into 7 bottles (internal diameter: 47 mm, internal height: 53 mm, total volume of seawater: 60 mL). For the clean group, D-larvae and seawater were poured onto a mesh with 200-μm opening (Sefar Inc. “7XX-200”), which was designed to catch large contaminants and allow the D-larvae and small contaminants to pass through. The seawater with the D-larvae and small contaminants was then poured onto a mesh with 80-μm opening (Sefar Inc. “17XXX-80”), which was designed to catch the D-larvae and wash away small contaminants to pass through. The seawater with the D-larvae and small contaminants was then poured onto a mesh with 80-μm opening (Sefar Inc. “17XXX-80”), which was designed to catch the D-larvae and wash away small contaminants. The 80-μm mesh with the D-larvae was folded to wrap the D-larvae and was gently shaken in FSW for $\approx 30$ s to remove small contaminants as much as possible. The D-larvae cleaned in this way were then moved into 7 bottles at different densities (0.4 to 4.8 indiv. mL$^{-1}$), as for the control group. Both control and clean groups were kept in a 12D:12L condition ($\approx 28^\circ C$, $\approx 60 \mu mol^{-1} m^{-2} s^{-1}$) with no aeration, and the seawater in each bottle was agitated every day. The D-larvae were given cultured zooxanthella *Symbiodinium* sp. on 3 and 6 d after fertilization (the density of Zooxanthella in each bottle: 20 and 10 cells mL$^{-1}$, respectively). Seven d after fertilization on which most larvae settled on the bottom of the bottle (Murakoshi 1991), the dead and living larvae were counted under a microscope. The
dead larvae counted excluded the embryos showing maldevelopment (Fig. 2b). In a similar manner to Run 1, Run 2 was conducted: artificially-fertilized eggs taken from T. crocea on 18 September 2009 were kept in 5 bottles at different densities for each of the control group (5.4 to 8.3 indiv. mL\(^{-1}\)) and clean group (5.2 to 9.8 indiv. mL\(^{-1}\)). The dead and living larvae were counted 7 d after fertilization.

The survival rate of T. crocea larvae was calculated as (the number of living larvae)/(the total number of dead and living larvae). How the survival rates were affected by larval cleaning, larval density, and the interaction was analyzed for each run with a mixed-effects logistic regression (Crawley 2002), using R 2.4.1 (R Development Core Team 2006) and “glmmML” package (Broström 2008). The analyses used the following equation:

\[
S_i = \frac{1}{1 + \exp\{-k_1 - k_2 \cdot C_i - (k_3 + k_4 \cdot C_i) \cdot D_i - \varepsilon_i\}}
\]

where \(S_i\) denotes the survival rate for the i-th experimental bottle; \(i=1\) to 14 in Run 1 and 1 to 10 in Run 2; \(C_i\) is 0 or 1, corresponding to the control and clean groups, respectively, to which the i-th bottle belongs; \(D_i\) denotes the larval density in the i-th bottle; \(k_1\) denotes an intercept; \(k_2\) denotes the effect of cleaning; \(k_3\) denotes the effect of larval density; \(k_4\) denotes the interaction between cleaning and larval-density effects; and \(\varepsilon_i\) denotes a random variation for the i-th bottle.

Model 1 included all the parameters, namely, \(k_1\), \(k_2\), \(k_3\), \(k_4\), and \(\varepsilon_i\). Model 2 excluded \(k_4\) from Model 1. Models 3 and 4 further excluded \(k_2\) and \(k_3\), respectively, from Model 2. Of the 4 models, the one minimizing the Akaike Information Criterion (AIC; Sakamoto et al. 1983) was selected as the model that predicts larval survival rates at the highest likelihood. This model minimizing AIC was used to predict a survival rate at a low larval density, 0.5 indiv. mL\(^{-1}\), for each of the control and clean groups. This density is recommended by Murakoshi (1991) and is applied in many hatcheries.

We examined whether larval cleaning reduces (a) the percentage of embryos showing maldevelopment and (b) the density of bacteria in seawater. (a) In the foregoing manner, fertilized eggs were taken from adult T. crocea (shell length: 80.8 to 118.7 mm) on 2 December 2009. One d later the fertilized eggs were divided into 2 groups, only one of which was cleaned as in the larval-cleaning experiments. These seeds cleaned (n=59 specimens) and not cleaned (n=362) were categorized under a microscope into embryos showing maldevelopment (<120 μm in general; Fig. 2b) and D-larvae (~140 μm, Fig. 2a). Note that the 80-μm mesh was skewed and thus had actually the opening >80 μm, washing away contaminants >80 μm (possibly including embryos abnormally developed). (b) Fertilized eggs were taken from adult Tridacna derasa (shell length: ~40 cm) on 5 March 2010. One d later the fertilized eggs were divided into 2 groups, only one of which was cleaned as in the larval-cleaning experiments. From each group the seawater surrounding larvae was sampled through a 20-μm mesh. After 40- and 1,600-times dilution, 1 mL of the water samples was spread over agarose (Marine Agar 2216, Difco™) in petri dishes (3 replications) with a diameter of 82 mm. In total 12 petri dishes were used (2 groups×2 dilution×3 replications). The dishes were kept in a 12D:12L condition (≈10 μmol m\(^{-2}\) s\(^{-1}\)) at 28°C for 2 d, and colonies thereon were counted to calculate the density of bacteria in the original sample (The Pharmaceutical Society of Japan 2005).

Results

In the larval-cleaning experiments the clean group showed higher survival rates than the control group for a wide range of densities. In Run 1 (Fig. 3), the clean group presented higher survival rates than the control group for each density range of 0–1, 1–2, 2–3, and 3–5 indiv. mL\(^{-1}\). In Run 2 (Fig. 3), this trend was rather unclear for the density range of 7–10 indiv. mL\(^{-1}\) but was clear for 5–7 indiv. mL\(^{-1}\). These results were reflected in AIC (Table 1): it was lowest in each run for Model 2, which includes the effects of cleaning and larval density. Model 2 in Run 1 predicted the following relationship between survival rate (S) and larval density (D) for the larval density range <5 indiv. mL\(^{-1}\):

\[
S = \frac{1}{1 + \exp(1.34 + 0.55D)} \quad \text{clean group}
\]

\[
S = \frac{1}{1 + \exp(4.01 + 0.55D)} \quad \text{control group}
\]

Note that the survival rate varied markedly between bottles with similar larval densities for each group and run. Also note that Model 2 indicated some overdispersion, as the residual deviance exceeded the degrees of freedom of residuals. From Model 2, survival rate at 0.5 indiv. mL\(^{-1}\) was estimated to be 16.6% for the clean group, higher by 1 order than the estimate for the control group, 1.4%.

The condition of larvae and environment changed before and after larval cleaning. The percentage of embryos showing maldevelopment was lower by 1 order for the cleaned
larvae (1.7%) than for the non-cleaned larvae (23.2%). The density of bacteria in the seawater (colony forming unit mL\(^{-1}\); Fig. 4) was also lower by \(\geq 1\) order for the cleaned larvae (mean \(\pm\) S.D., 440 \(\pm\) 174 for 40-times dilution and 0 \(\pm\) 0 for 1,600-times dilution) than for the non-cleaned larvae (7,933 \(\pm\) 1,688 for 40-times dilution and 9,600 \(\pm\) 9,600 for 1,600-times dilution).

### Discussion

Previous studies on the seed production of giant clams have recommended larval cleaning (e.g. Ellis 1998, Mingoa-Licuanan & Gomez 2007). They did not, however, demonstrate whether or how the cleaning improves the survival rates of larvae. The larval-cleaning experiments found that survival rates were higher for the planktonic larvae cleaned than for those not cleaned over a wide range of densities (\(< 10\) indiv. mL\(^{-1}\)). Especially at low larval densities (\(< = 0.5\) indiv. mL\(^{-1}\), the density recommended by Murakoshi 1991 and applied in many hatcheries), the survival rate is expected to rise markedly through cleaning. Therefore, larval cleaning is concluded to be useful in the seed production of giant clams especially for low larval densities.

It should be noted that, for each of the clean and control groups, the survival rate differed even between experimental bottles with similar larval densities. The bottle-to-bottle differences partly explain the overdispersion in the mixed-effects logistic regression. Such differences somewhat blurred the effect of larval cleaning at high larval densities. The bottle-to-bottle differences in larval survival rate are due possibly to factors including light, temperature, and health condition of larvae (e.g. Iwai & Matsuoka 2005) which would differ between bottles. The effects of these factors on the survival rate of giant clam larvae should be examined in the future.

The efficacy of larval cleaning is attributable to at least two factors. First, larval cleaning removes the embryos that

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**Table 1.** Mixed-effects logistic regression examining how larval cleaning, larval density, and the interaction affect the survival rates of *Tridacna crocea* larvae. Model 1 includes 5 parameters: the intercept, cleaning effect, density effect, cleaning-density interaction, and random effect dependent on replication (expected value \(\pm\) standard error). Model 2 excludes cleaning-density interaction from Model 1. Models 3 and 4 further excludes density effect and cleaning effect, respectively, from Model 2. “df” denotes degrees of freedom, “SD” standard deviation, and “AIC” Akaike Information Criterion. The lowest AIC for each run is asterisked.

| Run   | Model 1 Residual deviance | Model 2 Residual deviance | Model 3 Residual deviance | Model 4 Residual deviance |
|-------|---------------------------|---------------------------|---------------------------|---------------------------|
|       | Residual df               |                           |                           |                           |
|       | The number of parameters included |                       |                           |                           |
|       | Intercept                 | -3.37 \(\pm\) 1.09         | -4.01 \(\pm\) 0.63         | -5.46 \(\pm\) 0.70         | -2.98 \(\pm\) 0.83         |
|       | Cleaning effect           | 1.94 \(\pm\) 1.22         | 2.67 \(\pm\) 0.62         | 2.61 \(\pm\) 0.83         |                           |
|       | Density effect            | -0.88 \(\pm\) 0.52         | -0.55 \(\pm\) 0.16         |                           | -0.38 \(\pm\) 0.29         |
|       | Cleaning-density interaction | 0.36 \(\pm\) 0.55       |                           |                           |                           |
|       | SD of random effect       | 0.64 \(\pm\) 0.23         | 0.64 \(\pm\) 0.23         | 1.11 \(\pm\) 0.30         | 1.42 \(\pm\) 0.39         |
|       | AIC                       | 34.45                     | 32.89*                    | 38.87                     | 46.68                     |

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**Fig. 4.** Bacterial density in seawater around *Tridacna derasa* D-larvae cleaned (○) and not cleaned (×). Seawater sample was diluted at 40 and 1,600 factors, and 3 replications were prepared.
show maldevelopment. Some artificially-fertilized eggs develop abnormally owing to various procedural mistakes (e.g. addition of excessive amounts of sperm to eggs; Ellis 1998), which may worsen the seawater quality. Removing such larvae is likely to improve the quality of the seawater. Although the non-cleaned larvae in the present study contained eggs showing maldevelopment more than larvae in many hatcheries (Kurihara, personal observations), so that the effect of removing such eggs might be overestimated, the present study is still considered to demonstrate that larval cleaning would decrease the number of abnormal eggs to some extent. The second merit of larval cleaning is the decrease of bacteria in the seawater, which possibly reduces harmful effects of bacteria (e.g. hypoxia, Malakoff 1998). Harmful effects of bacteria to giant clam larvae are suggested by Fitt et al. (1992); they significantly improved the survival rate of giant clam larvae through administering antibiotics, which eliminated the bacteria.

In tropical seed production, shellfishes other than giant clam (e.g. P. margaritifera, T. niloticus, T. marmoratus, T. argyrostomus, Shokita et al. 1991) are also likely to suffer from maldevelopment of larvae and propagation of bacteria (Chew & King 2000, Hawke 2000). It is possible that the larval-cleaning technique will alleviate the problems and increases the survival rate of larvae.

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