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Growth and survival of endangered angelwing clam, *Pholas orientalis* fed different algal diets

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Abstract. Mature angelwing clam, *Pholas orientalis* were collected from the wild and transported to the laboratory for broodstock development. They were fed with mixed algal cultures of *Isochrysis galbana*, *Chaetoceros* sp., and *Tetraselmis* sp. for a period of two months and a half to determine the effects of these algal cultures on growth and survival of the broodstock. Growth in terms of length was not significantly different among the treatment groups, whereas, there was significant reduction in the weight of the clams in the group fed with a mixture of *Isochrysis galbana*, *Chaetoceros* sp., and *Tetraselmis* sp. Survival of the clams decreased after two months of feeding with algal diets, with significant mortality observed in groups fed with a mixture of *Isochrysis galbana* and *Tetraselmis* sp. as well as with the mixture of the three microalgae. All the water quality parameters in the rearing tanks were within the optimum levels required for optimum growth of the clams. These results showed that mixed algal diets for broodstock development in angelwing clam have no direct effect on growth but moderate effect on survival. Future studies are aimed towards determining other factors that will contribute to better growth and survival of angelwing clam broodstock in captivity as well as for the search of alternative algal diets for angelwing broodstock, particularly by exploring the microalgae that are found in their natural habitat.
Key Words: *Pholas orientalis*, angelwing clam, broodstock, microalgae.

Introduction. Marine bivalves belonging to the family Pholadidae inhabit the tidal flats in Southeast Asian countries. Highly valued for their meat in Taiwan and Hongkong, *Pholas orientalis* (Gmelin, 1791) is known as “Angelwing clam” in English, “Diwal” in Filipino, “Mentarang” in Malay and “Pim” in Thai. This shellfish has the habit of extending its long siphon out of the muddy substrate during filter feeding. However, due to indiscriminate harvesting of these bivalve resources and the destruction of their natural habitat (Ronquillo & McKinley 2006), the indigenous population of angelwing clam is fast vanishing (Laureta & Marasigan 2000; Marasigan & Laureta 2001; Ronquillo & McKinley 2006; Ng et al 2009). Hence, research efforts are underway to develop hatchery techniques for this species in order to rehabilitate the depleted stocks in the wild (Ronquillo & McKinley 2006).

There have been few studies that detailed the reproductive biology of angelwing clam. One of the earliest reports on this bivalve species was undertaken by Ablan (1938), and it detailed the status of its fishery resource in Negros Occidental, Philippines. This was followed by assessment studies of the bivalve resource in Thailand (Saraya 1982; Amornjaruchit 1988) and in the Philippines (Young & Serna 1982). Initial attempts to spawn angelwing in the laboratory were reported by Sahavacharin et al (1988) but were not successful. It was the studies done by Laureta & Marasigan (2000) who reported the reproductive biology of this species and provided a brief description of the reproductive stages of this bivalves that were reared in the laboratory. They also observed that the peak spawning of *P. orientalis* took place in the months of June and October, which coincide with the rainy months in the Philippines. Marasigan & Laureta (2001) determined the effects of using mixed algal diets to improve gonadal development and maturation of angelwing clam under laboratory conditions, and found that a combination of *Chaetoceros calcitrans* and *Tetraselmis suecica* in the diet resulted in faster gonadal maturation and early spawning of the broodstock. Recently, Ronquillo & McKinley (2006) described the induced spawning techniques and the developmental stages of angelwing clam and discussed the potential for rehabilitation of its resources through restocking in coastal waters, whereas a similar study was done by Ng et al (2009) in Malaysia. Inspite of these studies done on the reproductive biology and hatchery techniques for angelwing, there is still a lack of information of the different aspects of its reproductive physiology, particularly on the different factors that affect gonadal maturation of this species in captivity. Hence, this study explored the use of different combination of algal species diets as a factor in influencing the growth and survival of angelwing broodstock reared in the laboratory.

Materials and Methods

Acclimation of the broodstock. Angelwing clam broodstock collected from a shoreline in Central Philippines (Punta Cogon, Roxas City, Capiz) were transported to the Hatchery Complex of the Institute of Aquaculture, College of Fisheries and Ocean Sciences, University of the Philippines Visayas. The broodstock were cleaned thoroughly to remove fouling organisms and debris, then placed in circular tanks. The clams were placed in the mud substrate obliquely inclined with the foot and protoplax buried approximately 5 cm below the substrate (Ronquillo & McKinley 2006). The tanks were provided with flow-through seawater and mild aeration. They were fed with *Tetraselmis* sp. for one week and starved for 24 hours prior to the experiment. Water quality parameters in the acclimation tanks were maintained almost similar to the natural habitat of the animals.

Experimental treatment. The experiment tested the effects of different combination of algal diets on growth and survival on angelwings broodstock. There were four treatments, namely, A – (*Chaetoceros* sp. and *Isochrysis galbana*); B- (*Tetraselmis* sp and *Chaetoceros* sp); C- (*Isochrysis galbana* and *Tetraselmis* sp) and D- (*Chaetoceros* sp + *Isochrysis galbana* + *Tetraselmis* sp.). Algal cultures were grown in their appropriate media following the procedures described by Ronquillo & McKinley (2006). All treatments were replicated three (3) times and each replicate was assigned randomly among the 12 fiberglass tanks (100L). Each tank was provided with a mud substrate and supplied with aerated seawater in a flow-through system. Five individuals were stocked in each tank. Algal food was given twice daily (in the morning at 8-9 AM and in the afternoon at 3-4 PM) at a concentration of 150,000 cells ml^{-1} for at least 30 min. During feeding, the flow-through system in the tank was stopped and restored at 30 min after feeding. Water quality parameters such as temperature, salinity and pH were monitored daily whereas ammonia was done once a week.

Growth and survival. Growth was measured using the length and weight data. Length and weight were measured using digital balance and caliper, respectively every two weeks for two months and a half, and were expressed in millimeters (mm) and grams (g), respectively. Survival rate was determined by the equation:

number of individuals at T_o ÷ number of individuals at T_f x 100,

where, To – time at initial sampling and Tf – time at final sampling.

Results and Discussion. Two months and a half after rearing the angelwing clam broodstock in tanks and fed with mixed algal diets, their growth in terms of length was not significantly different among the treatment groups (Figure 1). The average length of the broodstock in all treatments at the start of the experiment and during the final sampling was at least 100 mm. On the other hand, the growth of broodstock in terms of weight is shown in Figure 2. There was no significant difference in the growth before the start of the experiment and during the final sampling in all the treatment groups fed with the mixed algal diets. However, slight reduction in the growth was observed in treatments fed with algal diets of both *Isochrysis galbana* and *Tetraselmis* sp. and those fed with the mixed diet composed of the three microalgae.

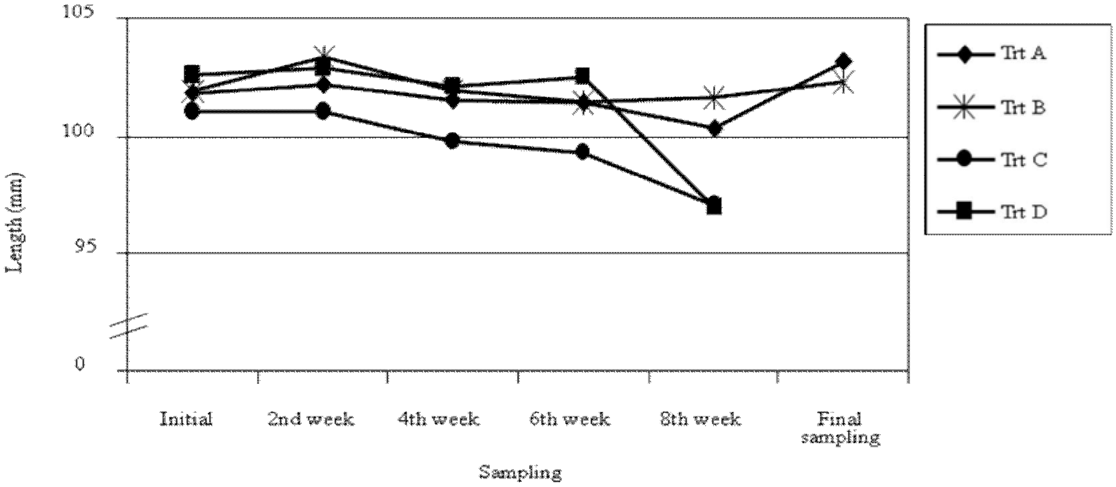

Figure 1. Length (mm) of angelwing clam broodstock fed with mixed algal species diets. Trt A: *Chaetoceros* sp. + *Isochrysis galbana*, Trt B: *Tetraselmis* sp. + *Chaetoceros* sp., Trt C: *Isochrysis galbana* + *Tetraselmis* sp. and Trt D: combination of three algal species.

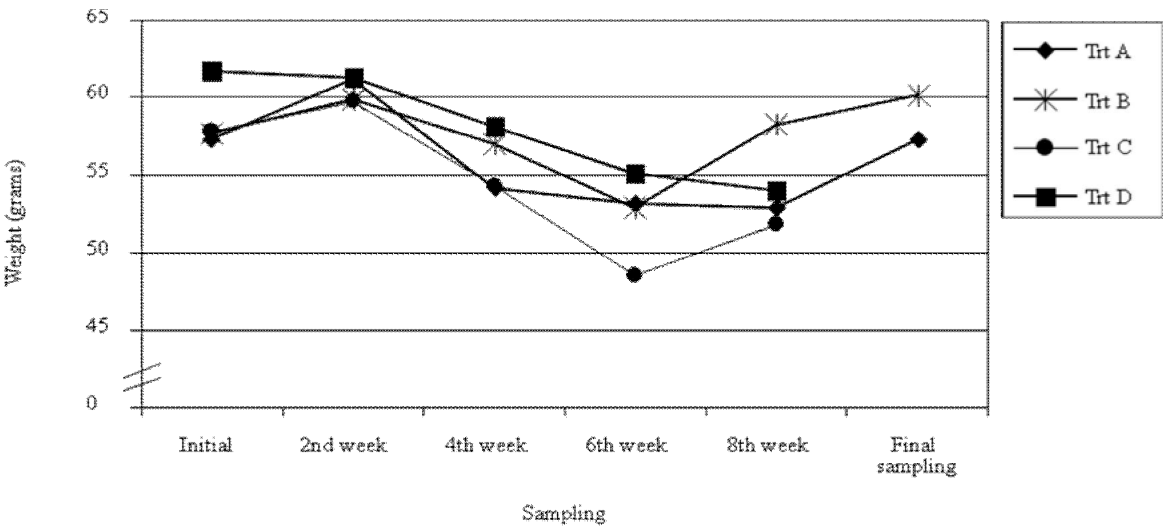

Figure 2. Weight (grams) of angelwing clam broodstock fed with mixed algal species diets. Trt A: *Chaetoceros* sp. + *Isochrysis galbana*, Trt B: *Tetraselmis* sp. + *Chaetoceros* sp., Trt C: *Isochrysis galbana* + *Tetraselmis* sp. And Trt D: combination of three algal species.

Figure 3 shows the survival of the angelwing clam broodstock fed with the mixed algal diets over a period of two months and a half. Treatment A (fed with mixed diet *Chaetoceros* sp. and *Isochrysis galbana*) had the highest survival rate (53.3%), while lowest survival was obtained in Treatments C (fed with *Isochrysis galbana* and *Tetraselmis* sp.) and D (fed with mixed diets of three microalgae) at 6.7%. Further test showed that survival in Treatments C and D was significantly lower in comparison with Treatments A and B.

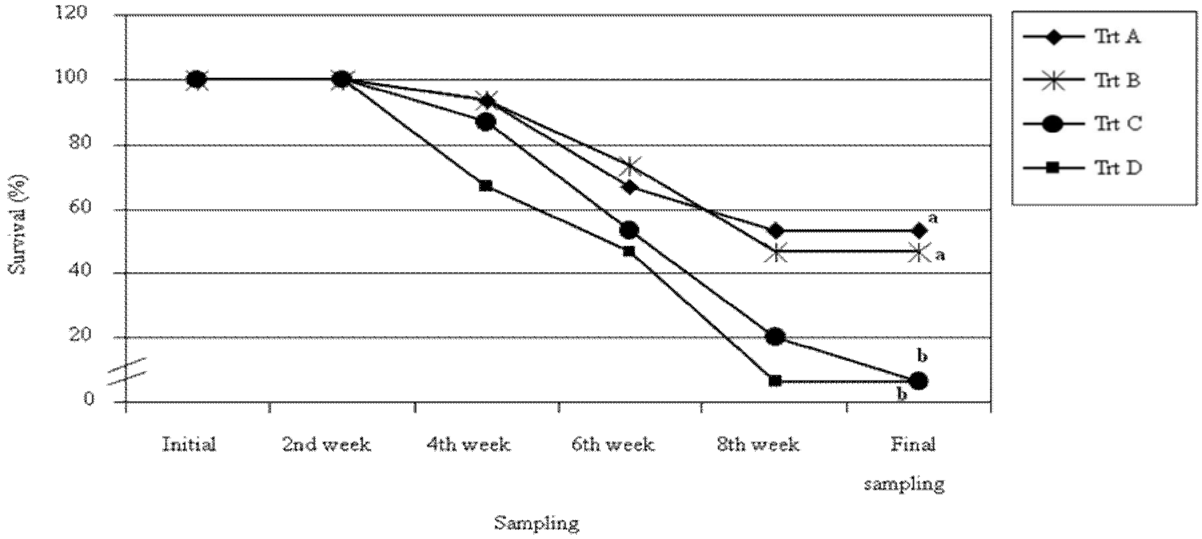

Figure 3. Survival (%) angelwing clam broodstock fed with mixed algal species diets. Trt A: *Chaetoceros* sp. + *Isochrysis galbana*, Trt B: *Tetraselmis* sp. + *Chaetoceros* sp., Trt C: *Isochrysis galbana* + *Tetraselmis* sp. And Trt D: combination of three algal species.

Water quality parameters such as dissolved oxygen, water temperature, salinity, pH and ammonia-N were monitored in all treatments during the experimental run. The ranges of these parameters were all within the optimum levels required for growth of the angelwing clam broodstock (Table 1).

Table 1

Water quality parameters in the broodstock tanks

Parameter	Range
Dissolved oxygen (D.O.), ppm	4.05 – 6.42
Water salinity, ppt	36 – 38
Water temperature, °C	27 – 29
pH	6.60 – 8.20
Ammonia-N, ppm	0.25 – 0.60

There have been few studies on broodstock development of angelwing clam, inspite of the huge popularity of this shellfish as a seafood. In the present study, we have determined the effects of feeding mixed algal diets on the growth and survival of angelwing clam broodstock. Marasigan & Laureta (2001) have demonstrated that the reduction of stressors and a combination of algal diets led to faster gonadal development and maturation of angelwing clam in indoor tanks compared to those found in the natural habitat. Using a combination of *Chaetoceros calcitrans* and *Tetrasemis suecica*, they found that angelwing clam broodstock spawned two months earlier than those fed with one algal species. Corda (1998) showed that highest filtration rate in angelwing clam was observed in those fed with *Isochrysis galbana*, and this is related to algal cell size. However, the preceding study was a transient experiment and survival rate was not determined. We have shown that the mixed algal diets did not affect the growth of the angelwing clam broodstock but these diets contributed to differences in the survival of the broodstock. A mixed algal diet consisting of *Chaetoceros* sp and *Isochrysis galbana* resulted in highest survival of the broodstock among the treatments, although the survival rate was just above 50%. Lowest survival of the broodstock was observed in the treatment receiving a combination of three microalgae, and the possible explanation for this could be related to food preference of this shellfish species. It would be interesting to determine other algal combinations, particularly those species of microalgae that are found in the natural habitat of the clam, which will contribute to better growth and survival of the broodstock. This is one of the major studies that could be done in the future.

In general, the survival of the broodstock fed with the mixed algal diets was quite low (the highest survival was 53.3%). Aside from the manipulation of the algal diets for this species of shellfish, there could be other factors that should be considered when monitoring survival of the broodstock in captivity. Alojepan (1999) observed that mature anglewing clam followed biorhythms when feeding, and one of the factors that affect feeding was the amount of light present. Angelwing clam had higher feeding activity at total darkness (Corda 1998). Hence, future studies should explore other factors that will contribute to better survival of the broodstock and multi-factorial experiments should be designed for this purpose.

In the course of the experiment, the water quality parameters that we monitored were within the optimum levels that are required for good growth and survival. In laboratory conditions, Alojepan (1999) showed that there was optimum feeding activity of mature angelwing clam when the water temperature was 27^{o}C and salinity at 35 ppt, whereas, Corda (1998) observed better rate of filtration when water temperature was 28^{o}C. In the natural habitat, the water quality parameters from the sampling sites of angelwing clam that were monitored in Malaysia over a period of two years were in the range of: 3.30 – 8.70 ppm for dissolved oxygen, 28 – 31 ppt for salinity and 27 – 32^{o}C for water temperature (Ng 2009). The values of the water quality parameters that we measured in the present study were similar to the values obtained by the aforementioned studies.

Conclusions. In summary, our study has shown that mixed algal diets have no significant effect on the growth of angelwing clam broodstock in tanks. The broodstock responded differently to the mixed algal diets as demonstrated by the differences in the survival rates when fed by the algal combinations. The survival of the broodstock when fed with these algal combinations was low, hence, future studies are aimed at determining other factors that will result in better growth and survival of the broodstock using multifactorial experiments as well as utilizing microalgae that are found in the natural habitat of angelwing clam as food source for broodstock development.

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