Korean J. Malacol. 25 (1): 35-40, 2009

Effects of Three Microalgae, *Tetraselmis suecica*, *Chaetoceros calcitrans*, and *Phaeodactylum tricornutum* on Larvae and Spat Growth of the Trumpet Shell *Charonia sauliae*

Kyoung Ho Kang, Seung Cheon Seon, Jae Min Kim, Liang Liang Zhuo and Sang Min Lim

Division of Marine Technology, Chonnam National University, Yeosu 550-749, Korea

ABSTRACT

The trumpet shell *Charonia sauliae* is an endangered and valuable species with potential for aquaculture. For artificial propagation of *C. sauliae*, the effects of three different food microalgae on the development, growth, and survival rate of the larvae and spat were investigated. For the larval feeding experiments, we utilized six microalgae species as food sources, namely *Pavlova lutheri*, *Tetraselmis suecica*, *Nannochloris oculata*, *Isochrysis galbana*, *Chaetoceros calcitrans*, and *Phaeodactylum tricornutum*; for the larval and spat growth and survival experiments, we utilized *T. suecica*, *C. calcitrans*, and *P. tricornutum*. The results showed that the temporal digestion index (TDI) for the veliger larvae was significantly different for *C. sauliae* fed the different microalgae species ($p < 0.05$), that the *T. suecica*, *C. calcitrans*, and *P. tricornutum* cultivars were better suited for larval consumption ($p < 0.05$), and that the growth and survival of the larvae and spat were significantly influenced by food type, specifically *P. tricornutum* ($p < 0.05$). Further research is needed to evaluate the effects of other microalgae species, different algal concentrations, and biochemical composition on the growth and survival of *C. sauliae*.

Key words: food, microalgae, *Charonia sauliae*, larvae, spat.

INTRODUCTION

The trumpet shell, *Charonia sauliae* is an endangered and valuable species, mainly distributed in the tropical and semi-tropical areas of the Atlantic, Indian, and Pacific Oceans. The trumpet shell is an important species contributing to research of marine toxins (Narita *et al.*, 1981; Noguchi *et al.*, 2006). Because its food preference is starfish, a predatory species that threatens many economically important aquatic animals, trumpet shell propagation may provide a means of biological control of starfish populations (Kang and Kim, 2004).

Establishing an adequate feeding regime is a key issue required for maintaining healthy animals and achieving enhanced aquaculture production. Diet influences many aspects of marine organism metabolism, such as gonadal development in females, performance of the produced seed (Santiago *et al.*, 1983; Gunasekera *et al.*, 1997), activity of digestive enzymes (Garcia-Esquivel and Felbeck, 2006), and tissue composition (Durazo-Beltrán *et al.*, 2003).

In this study, we investigated the effects of different food organisms on the growth of the trumpet shell, *C. sauliae*. Growth rate is a principle biological criterion that must be considered for propagating a new aquaculture species (Webber and Riordan, 1976; Le Francois *et al.*, 2002). Additionally, previous studies have shown that diet significantly affects the growth rate of marine gastropods (Narvarte and Pascual, 2003; Najmudeen and Victor, 2004; Woodcock and Benkendorff, 2008).

To succeed in culturing *C. sauliae*, we must determine the optimum food type for this organism

Received March 23, 2009; Accepted May 5, 2009
Corresponding author: Kang, Kyoung Ho
Tel.:+82 (61) 659-3165; e-mail: mobidic@chonnam.ac.kr
1225-3480/24317

during its larval and spat stages, as related reports on this topic are rare. Thus, ours is the first study to investigate the effect of food type on the growth and survival rates of *C. sauliae* larvae and spat, the results of which will contribute directly to the artificial propagation of *C. sauliae*.

MATERIALS AND METHODS

1. Determination of temporal digestion index (TDI)

Experiments on the feeding behavior of *C. sauliae* larvae were conducted according to the methods of Aldana-Aranda *et al.* (1997). Hatched *C. sauliae* veliger larvae were cultured for 2 h with one of six food microalgae (*Pavlova lutheri*, *Tetraselmis suecica*, *Nannochloris oculata*, *Isochrysis galbana*, *Chaetoceros calcitrans*, and *Phaeodactylum tricornutum*). After the culture time had lapsed, the larvae were washed and transferred to clean containers filled with filtered seawater, where the digestion of algal cells was observed within the stomach using an epifluorescence microscope once every hour. Variations in fluorescence intensity observed among the algal cells within the larval gut were classified arbitrarily into four nutritional stages, the qualitative characteristics of which are summarized in Table 1. We used the TDI (Aldana-Aranda *et al.*, 1991) to study the feeding behavior of *C. sauliae* larvae.

The TDI was calculated according to the following equation:

$$TDI = (n_2 + n_3)/n_0$$

where n_2 is the number of larvae at stage 2, n_3 is the number of larvae at stage 3, and n_0 is the number of total larvae observed in the experiments.

2. Culture of larvae and spat

To determine the influence of the six different algal food types on *C. sauliae* larvae and spat growth, we cultured veliger-stage larvae at the same temperatures and salinities, altering only the algal food type. The temperature and salinity regime of this experiment is illustrated in Figure 1. Three food treatments (*T. suecica*, *C. calcitrans*, and *P. tricornutum*) were used in this study. Algae used in this experiment were reared in the laboratory in F/2 medium (insert manufacturer, location). Before feeding them to the larvae and spats, the algal cells were separated from their culture medium by centrifugation (3000 rpm for 5 min). In each food treatment, the algal concentration was maintained at approximately 1 x 10^5 cells ml^{-1} between water changes. Samples from each temperature treatment were obtained every 10 days to determine body size and survival rate. After measurements of the larvae and spat were determined, we re-cultivated the specimens in their cups or in tanks. The specific growth rates were calculated according to the following equation by Hopkins (1992):

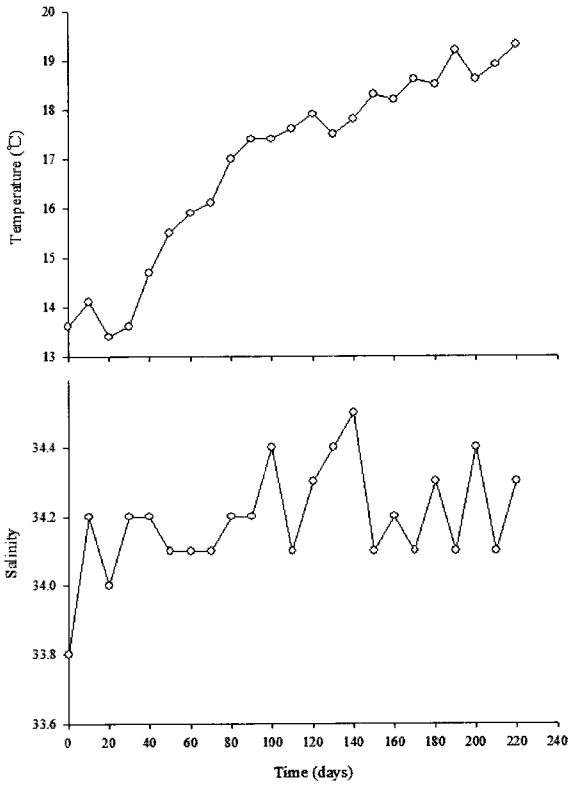

Fig. 1. Water temperature and salinity regime used in these experiments.

$$SGR = \frac{\mathrm{Ln}(L_f/L_i)}{t}$$

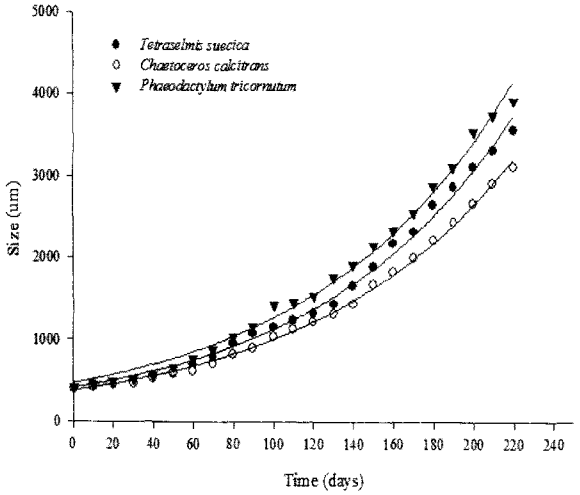

Fig. 4. Growth of trumpet shell *Charonia sauliae* larvae and spat fed with different food microalgae species (*Tetraselmis suecica*, *Chaetoceros calcitrans*, and *Phaeodactylum tricornutum*).

where L_t and L_i are the final and initial length (mm) of the larvae, and t is the time.

3. Statistical analysis

Data obtained from the different treatment groups were subjected to one-way analysis of variance (ANOVA); Tukey's HSD test was used to determine which treatments were significantly different. $P < 0.05$ indicated statistical significance. Statistics were performed using the statistical software SPSS for Windows (SPSS Inc., Chicago, IL).

RESULTS

The growth stages observed in this experiment are illustrated in Figure 2. The TDI values of the trumpet shell veliger larvae fed with different food organisms are presented in Figure 3. TDIs were recorded at 4 h, with values of 0.13, 0.42, 0.07, 0.33, 0.53, and 0.55 for trumpet shell larvae fed with *P. lutheri*, *T. suecica*, *N. oculata*, *I. galbana*, *C. calcitrans*, and *P. tricornutum*, respectively. At 4 h, the TDI was higher than 0.4 for larvae fed with *T. suecica*, *C. calcitrans*, and *P. tricornutum*. After 8 h, the highest TDI recorded was 0.65 for the larvae fed with *P. tricornutum*, whereas larvae fed with the other microalgae showed TDIs between 0.14 and 0.51. The growth curve of trumpet shell larvae and spat

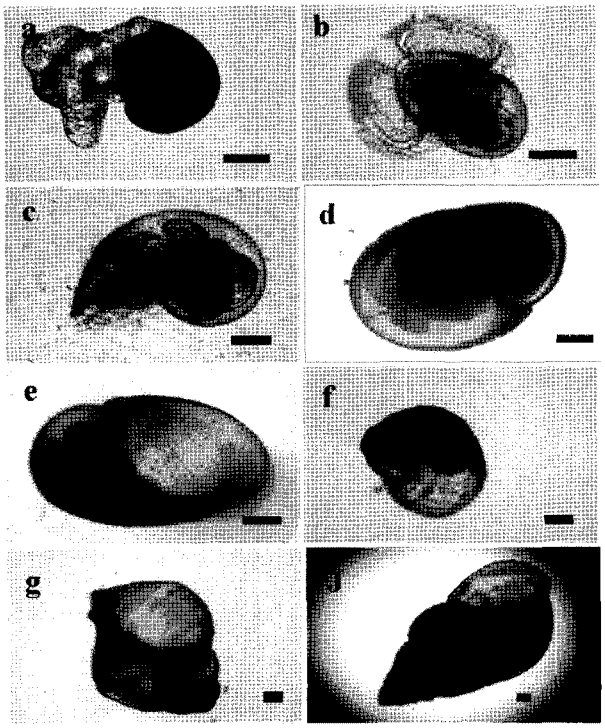

Fig. 2. Developmental stages of the trumpet shell *Charonia sauliae*: (a) trochophore; (b) veliger; (c) postlarvae; (dh) spat. Scare bar: 100 μm.

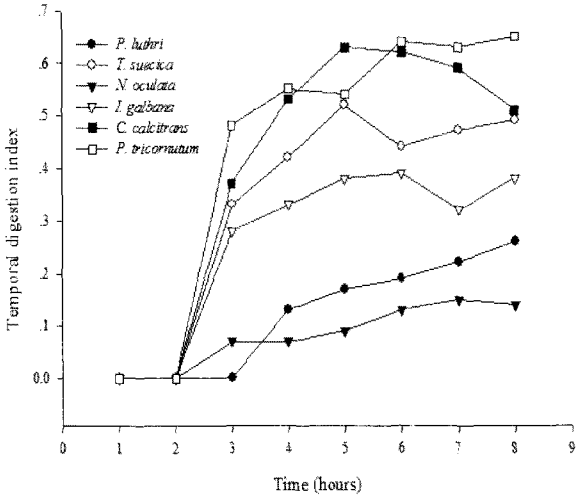

Fig. 3. Temporal digestion index (TDI) values for the trumpet shell *Charonia sauliae* fed with different food microalgae species (*Pavlova lutheri*, *Tetraselmis suecica*, *Nannochloris oculata*, *Isochrysis galbana*, *Chaetoceros calcitrans*, and *Phaeodactylum tricornutum*).

is presented in Figure 4. The growth of the larvae and spat was significantly influenced by food type ($p < 0.05$). In general, larvae and spat fed with *P. tricornutum* displayed markedly higher growth rates than larvae fed with *T. suecica* or *C. calcitrans*.

The specific growth rate of trumpet shell is presented in Figure 5, and was significantly influenced by food type ($p < 0.05$). The specific growth rate of trumpet shell fed with *P. tricornutum* was significantly higher than that in individuals fed with *T. suecica* or *C. calcitrans* ($p < 0.05$).

The final survival rate that we calculated for trumpet shell spat is shown in Figure 6, and was significantly influenced by food type ($p < 0.05$). The survival rates of the trumpet shell spat fed with *T. suecica* (55.2 ± 2.3%) and *P. tricornutum* (53.1 ± 2.1%) were significantly higher than that of trumpet shells fed with *C. calcitrans* (33.1 ± 2.5%; $p < 0.05$). However, no significant difference was observed between the survival rates of spat fed with *T. suecica* or *P. tricornutum* ($p > 0.05$).

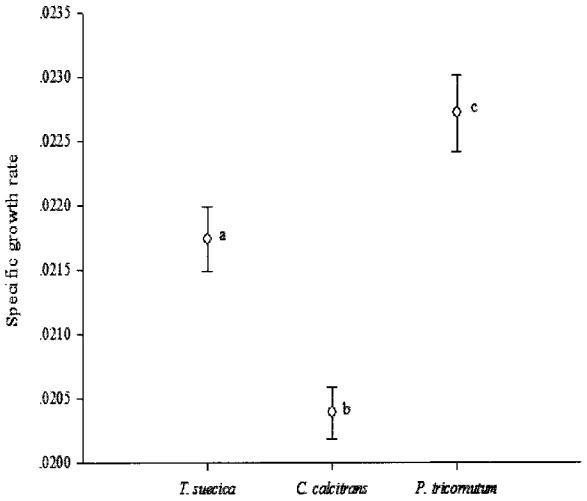

Fig. 5. Specific growth rates of trumpet shell *Charonia sauliae* fed with different food microalgae species (*Tetraselmis suecica*, *Chaetoceros calcitrans*, and *Phaeodactylum tricornutum*). Data and groups with different letters indicate statistical significance ($p < 0.05$).

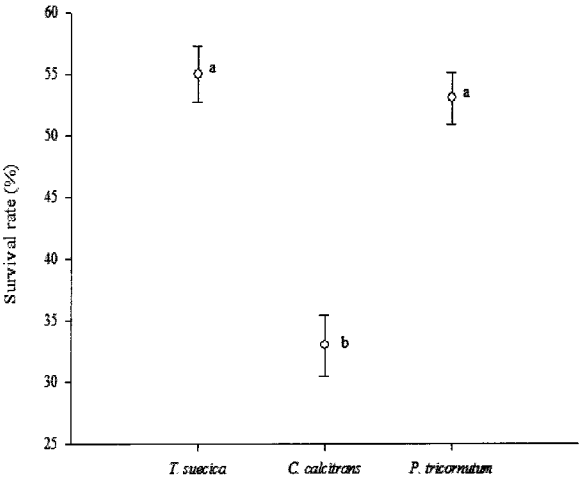

Fig. 6. Survival rates of trumpet shell *Charonia sauliae* fed with different food microalgae species (*Tetraselmis suecica*, *Chaetoceros calcitrans*, and *Phaeodactylum tricornutum*). Data and groups with different letters indicate statistical significance ($p < 0.05$).

DISCUSSION

Aldana-Aranda *et al.* (1997) demonstrated that the epifluorescence microscope is a useful tool for studying differences in larval feeding behavior. In the present study, we demonstrate that this method is also useful for evaluating the digestibility of *P. lutheri*, *T. suecica*, *N. oculata*, *I. galbana*, *C. calcitrans*, and *P. tricornutum* in *C. sauliae* veliger larvae. The use of an epifluorescence microscope permitted not only the observation of the digestive process, but also a quantitative estimation of the food particles digested. Our results showed that larvae fed with *P. tricornutum*, *C. calcitrans*, and *T. suecica* displayed higher TDI scores, whereas those fed with *N. oculata* exhibited the slowest digestion processes. *P. tricornutum*, *C. calcitrans*, and *T. suecica* proved to be better food sources for *C. sauliae* veliger larvae, so these species were investigated further as potential food sources for both larvae and spat and their suitability for *C. sauliae* culture. Notably, *C. sauliae* larvae and spat fed with *P. tricornutum* showed higher growth rates than those fed with *C. calcitrans* or *T. suecica*.

However, it must be noted that there are other microalgae or microalgal mixtures that have not been tested with *C. sauliae* larvae and spat. Bayne (1983), Webb and Chu (1983), and Lucas (1990) reported that

the nutritive value of algal diets as a food source for molluscan larvae is a function of several factors, such as cell size and shape, algal concentration, size of the ingesting larvae, algal cell digestibility, and the balance among the nutrient contents Aldana-Aranda *et al.* (1997) indicated that differences in biochemical composition (i.e., lipids, fatty acids, proteins, and carbohydrates) among algae species, and even among the different growth phases of the algae, may lead to observable differences in the growth and development of different algae species (Fabregas *et al.*, 1985; Pillsbury, 1985; Fernández-Reiriz *et al.*, 1989; Delaunay *et al.*, 1993). In addition, the nutritional requirements of the aquacultured larvae must be considered (Pechenick and Fisher, 1979). Further research must be conducted to evaluate the effect of the different algal species, different cell concentrations, and biochemical composition on the growth and survival rate of *C. sauliae*.

ACKNOWLEDGMENTS

This research was supported by funding from the Korea Institute of Marine Science & Technology Promotion.

REFERENCES

Aldana-Aranda, D., Lucas, A., Brulé, T., Andrade, M., García-Santaella, E., Maginot, N. and Le Pennec, M. (1991). Observations on ingestion and digestion of unicellular algae by *Strombus gigas* larvae (Mollusca Gastropoda) using epiflurescence microscopy. *Aquaculture*, **92**: 359-366.

Aldana-Aranda, D., Patiño-Suárez, V. and Brulé, T. (1997) Nutritional potentialities of *Chlamydomonas coccoides* and *Thalassiosira fluviatilis*, as measured by their ingestion and digestion rates by the Queen Conch larvae (*Strombus gigas*). *Aquaculture*, **156**: 9-20.

Babinchak, J. and Ukeles, R. (1979) Epifluorescence microscopy, a technique for the study of feeding in *Crassostrea virginica* veliger larvae. *Marine Biology*, **51**: 69-76.

Bayne, B.L. (1983) Physiological ecology of marine molluscan larvae. *The Mullusca*, **3**: 299-343.

Delaunay, F., Marty, Y., Moal, J. and Samain, J.F. (1993) The effect of monospecific algal diets on growth and fatty acid composition of *Pecten maximus* (L.) *Journal of Experimental Marine Biology and Ecology*, **173**: 163-179.

Durazo-Beltrán, E., D'Avramo, L.R., Toro-Vazquez, J.F., Vasquez-Peláez, C.V. and Viana, M.T. (2003) Effect of triacylgycerols in formulated diets on growth and fatty acid composition in tissue of green abalone (*Haliotis fulgens*). *Aquaculture*, **224**: 257-270.

Fabregas, J., Herrero, C., Cabezas, B. and Abalde, J. (1985) Mass culture and biochemical variability of the marine microalgae *Tetraselmis suecica* Kylin (Butch) with high nutrient concentrations. *Aquaculture*, **49**: 231-244.

Fernández-Reiriz, M.J., Pérez-C., A., Ferreiro, M.J., Blanco, J., Planas, M., Campos, M.J. and Labata, U. (1989). Biomass production and variation in the biochemical profile (total protein, carbohydrate, RNA, lipids and fatty acids) of seven species of marine microalgae. *Aquaculture*, **83**: 17-37.

Garcia-Esquivel, Z. and Felbeck, H. (2006) Activity of digestive enzymes along the gut of juvenile red abalone, *Haliotis rufescents*, fed natural and balanced diets. *Aquaculture*, **261**: 615-625.

Gunasekera, R.M., Shim, K.F. and Lam, T.J. (1997) Influence of dietary protein content on the distribution of amino acids in oocytes, serum and muscle of Nile tilapia, *Oreochromis niloticus* (L.) *Aquaculture*, **152**: 205-221.

Hopkins, K.D. (1992) Reporting fish growth: a review of the basics. *Journal of the World Aquaculture Society*, **23**: 173-179.

Kang, K.H. and Kim, J.M. (2004) The predation of trumpet shell, *Charonia* sp., on eight different marine invertebrate species. *Aquaculture Research*, **35**: 1202-1206.

Le Francois, N.R., Lemieus, H. and Blier, P.U. (2002) Biological and technical evaluation of the potential of marine and anadromous fish species for cold-water mariculture. *Aquaculture Research*, **33**: 95-108.

Lucas, A. (1990) Feeding and digestion in bivalve larvae. *In*: Proceedings of Memorial Symposium in Honor of Sir Charles Maurice Yonge. (ed. by Morton, B.) pp. 173-190. Hong Kong.

Mok, F., Thiyagarajan, V. and Qian, P. Y. (2008) Larval development and metamorphic behaviour of the subtropical spionid polychaete *Pseudopolydora vexillosa*. *Journal of Experimental Marine Biology and Ecology*, **357**: 99-108.

Najmudeen, T.M. and Victor, A.C.C. (2004) Seed production and juvenile rearing of the tropical abalone *Haliotis varia* Linnaeus 1758. *Aquaculture*, **234**: 277-292.

Narita, H., Noguchi, T., Maruyama, J., Ueda, Y., Hashimoto, K., Watanabe, Y. and Hida, K. (1981) Occurrence of tetrodotoxin in a trumpet shell, "boshubora" *Charania sauliae*. *Bulletin of the Japanese Society of Scientific Fisheries*, **47**: 935-941.

Narvarte, M.A. and Pascual, M.S. (2003) Fertilization, larval rearing and post-larval growth of the Tehuelche scallop *Aequipecten tehuelchus* D'Orb., 1846. *Aquaculture*, **217**: 259-274.

Noguchi, T., Arakawa, O. and Takatani, T. (2006) TTX accumulation in pufferfish. *Comparative Biochemistry and Physiology,* Part D, **1**: 145-152.

Pechenick, J.A. and Fisher, N.S. (1979) Feeding assimilation and growth of mud snail larvae, *Nassarius obsoletus* (Say), on three different algal diets. *Journal of Experimental Marine Biology and Ecology*, **38**: 57-80.

Pillsbury, K.S. (1985) The relative food value and biochemical composition of five phytoplankton diets for queen conch *Strombus gigas* (Linné) larvae. *Journal of Experimental Marine Biology and Ecology*, **90**: 221-231.

Santiago, C.B., Aldaba, M.B. and Laron, M.A. (1983) Effect of varying dietary crude protein levels on spawning frequency and growth of *Sarotherodon niloticus* breeders. *Fisheries Research Journal of Philippines*, **8**: 9-18.

Webb, K.L. and Chu, F.L.E. (1983) Phytoplankton as a food source for bivalve larvae. *In*: Proceedings of the 2nd International Conference on Aquaculture Nutrition: Biochemical and Physiological Approaches to Shellfish Nutrition. World Mariculture Society Special Publication 2. (ed. by Pruder, G.G., Langdon, C. and Conklin, D.) pp. 272-291.

Webber, H.H. and Riordan, P.F. (1976) Criteria for candidate species for aquaculture. *Aquaculture*, **7**: 107-123.

Woodcock, S.H. and Benkendorff, K. (2008) The impact of diet on the growth and proximate composition of juvenile whelks, *Dicathais orbita* (Gastropoda: Mollusca). *Aquaculture*, **276**: 1-4.