

Physiological performance of giant clams (*Tridacna spec.*) in a recirculation system

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Abstract. The importance of auto- and heterotrophy for growth, survival, zooxanthellae development and nutrient uptake of juvenile giant clams *Tridacna maxima* and *T. squamosa* was investigated. In addition experiments on the photosynthesis, metabolism and calcification were performed with specimens of *T. maxima* and *T. derasa* in an intermittent flow-through respiration system. Animals were reared in a recirculation system under different light conditions and different concentrations of nutrients. Both particulate organic (*Tetraselmis* algae) and dissolved inorganic (ammonia and phosphate) food was given. Results show that light intensity and spectra have a significant effect on survival and length or weight increase. Nutrient concentrations only seem to play a role when clams are kept in optimum light conditions. In this case fertilization with NH_4^+ and PO_4^{3-} is more effective than feeding with algae. However, the concentration of zooxanthellae is only moderately increasing with increasing nutrient concentration. Moreover photosynthesis, respiration rates and calcification seem to be linked to light conditions but not to increasing nutrient concentrations. It is concluded that juvenile clams depend more on autotrophy to satisfy nutritional requirements. The uptake of nutrients is limited and needs to be tested with larger clams and a variety of nutrient combinations.

Key words: giant clam, nutrient uptake, growth, photosynthesis, respiration

Introduction

Giant clams are the largest bivalved animals in evolutionary history (Yonge 1975). They are effective filter feeders and at the same time autotrophic due to their symbiosis with zooxanthellae. This gives them a nutritional and growth advantage over normal heterotrophic bivalves. The filter feeding still remains a significant component of their procurement of nutrition (Klumpp et al. 1992). Although Klumpp and Lucas (1994) showed that photosynthates can contribute more daily carbon than actually needed, filter feeding seems necessary to satisfy the requirements for essential amino acids, phosphorous and trace metals (Hawkins and Klumpp 1995).

Although recent research into ecological and physiological background of different stages of the life cycle of clams made commercial aquaculture possible, there is still a high mortality with early stages. While juveniles rely on both heterotrophic and autotrophic feeding, adult clams depend more on available light. Particularly in aquaria the quality of the light plays an important role (Knop 1994) both with regard to light intensity and wavelengths, but negative effects of insufficient light sources are not known in detail.

Clams are raised commercially for human consumption in the Pacific region, sometimes also to replenish natural stocks (Lucas 1994). In addition

they are a highly valuable resource for the international aquarium trade. However, losses during transport or during acclimation to aquaria are still high. Even after acclimation periods of several months, spontaneous mass mortalities in aquaria are reported (Knop 1994), most likely linked with water quality in closed recirculation systems. The availability of selected nutrients, particularly for the shell production, plays a key role.

Giant clams are known to prefer locations with gentle water movement, both for the supply of oxygen and nutrients (Knop 1994). When kept in high densities in small aquaria they are able to deplete nitrate and phosphate values considerably. This is why clams can play a potential role for bio-cleaning as additional water treatment module in a closed recirculation system.

Therefore, the importance of auto- and heterotrophy for growth, survival, zooxanthellae development and nutrient uptake of juvenile giant clams was investigated. In addition experiments on photosynthesis, metabolism and calcification under different light and nutrient conditions were performed.

The aims of this study are to:

1) estimate nutrient uptake rates for NH_4^+ , PO_4^{3-} and food algae, 2) find optimal light conditions for growth, survival, uptake rates, 3) get an estimation of oxygen

consumption and photosynthetic rates and finally 4) get a first impression of zooxanthellae development and ETR efficiencies.

Material and Methods

From September 2006 to July 2008, 120 juvenile giant clams (*Tridacna maxima*, *T. squamosa* and *T. derasa*; Fig. 1) were reared in a closed recirculation system in ZMT Bremen (500 l volume, 32 PSU salinity, 27 °C temperature, 12 hours light vs. 12 hours dark regime, use of protein skimmer, calcium reactor, nitrate reactor, biofilter, UV units for water treatment). Maintenance conditions and species identification were performed after Lucas (1994) and Knop (1994).

Uptake rate and growth experiments with 30 (5-10 cm) specimens of *T. maxima* and *T. squamosa* were carried out for 21 days under different light conditions (use of HQI 150 W, HQL 125 W, fluorescent lamps 54 W, brand: Aqualine, actinic light) and different concentrations of particulate organic and dissolved inorganic nutrients (microalgae, NH_4^+ and PO_4^{3-}). For experiments (up to four hours exposure) elevated concentrations through spiking were used (5000 cells ml^{-1} *Tetraselmis subcordiformis*; 0-150 μM NH_4^+ , using ammonium sulphate; 0-10 μM PO_4^{3-} using potassium phosphate). Nutrients were measured with photometer after Koroleff and Grasshoff (1983); cells were counted with a Neubauer chamber. Length (mm) and weight (g) measurements were repeated every 7 days with calliper and electronic scale (Sartorius Germany).

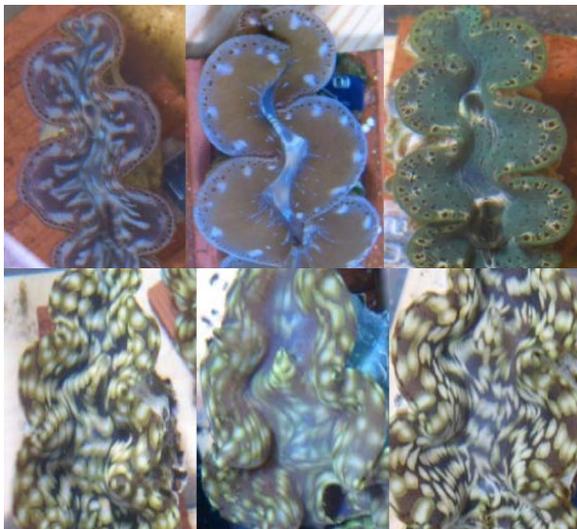


Figure 1: Juveniles of *Tridacna maxima* and *T. squamosa* in recirculation system in ZMT.

Experiments on photosynthesis (PS) and oxygen consumption (OC) were performed with 80 (5-8 cm) specimens of *T. maxima* and ten (10-12 cm) specimens of *T. derasa*.

A separate, PC-controlled intermittent flow respirometer with circular, flat-bottom acrylic respiration chambers of different volumes, according to size of animal, was used (detailed method see Kunzmann et al. 2007). 20 individual clams were measured for 2 hours in light and 2 hours in darkness. WTW 340i oxygen, pH and conductivity sensors were used for measurements of oxygen, salinity and temperature.

For PS measurements 20 individual clams and a PAM 2100 of Waltz (Germany) were used. Light measurements were performed with a Ramses ACC sensor from Trios (Germany), and a LI-250A light meter plus Hamamatsu Photonic multi channel analyser.

Results

Giant clams are capable of fast and efficient NH_4^+ (50 μM) and PO_4^{3-} (2 μM) uptake (Fig. 2, Fig. 3). Within less than 2-3 hours, nutrient concentrations elevated through spiking, are depleted down to normal levels. Only in the case of phosphate uptake in *T. squamosa* a slight delay was observed.

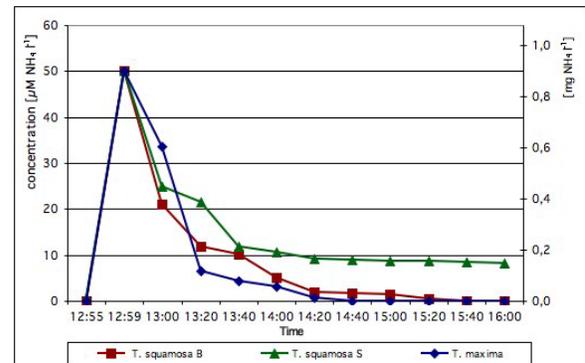


Figure 2: NH_4^+ uptake of individual giant clams in aquaria after spiking with 50 μM NH_4^+ , using ammonium sulphate (n=1).

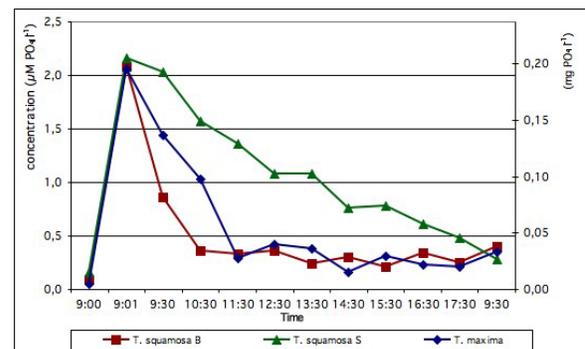


Figure 3: PO_4^{3-} uptake of individual giant clams in aquaria after spiking, using potassium phosphate (n=1).

Consumption of food algae cells reaches up to 7000 $\text{g}^{-1}\text{h}^{-1}$. The uptake rate was constant for the trial period of 10 hours ($r^2 = 0.9919$, $n=10$). Light intensity ($50 - 350 \mu\text{E s}^{-1} \text{m}^{-2}$) and spectra have a significant effect on survival and length and weight increase.

With fluorescent light the survival rate went down to 70%, whereas with HQL and HQI lights, survival rates were 90% and 100%, respectively. The condition factor, relating to the overall growth condition of the clams (wet weight/shell length), developed best under HQI light conditions. Fastest length and weight increase of *T. maxima* was achieved with HQI lights (Tab. 1).

Table 1: Growth of *T. maxima* ($n=10$) with different light sources, FL= fluorescent lamp.

	HQI	HQL	FL
Length (mm day^{-1})	0.0188 ± 0.007	0.007 ± 0.005	0 ± 0.003
Weight (g day^{-1})	0.08 ± 0.04	0.01 ± 0.02	0 ± 0.02
Survival rate (%)	100	90	70

Nutrient concentrations only play a role when clams are kept in optimum light conditions. In this case fertilization with NH_4^+ and PO_4^{3-} is more effective for zooxanthellae development or growth than feeding with food algae. However, the concentration of zooxanthellae is only moderately and not significantly increasing with increasing nutrient concentration (from $6 \times 10^8 \text{g}^{-1}$ wet weight at $0 \mu\text{M NH}_4^+$ to $9.5 \times 10^8 \text{g}^{-1}$ wet weight at $100 \mu\text{M NH}_4^+$ under HQI light).

The six HQI light sources provided the same spectrum but differed slightly in intensity (Fig. 4). Resulting ETR rates of different clams were almost identical and display a saturation level of $250 \mu\text{E m}^{-2}\text{s}^{-1}$ at PAR levels of $2500 \mu\text{q m}^{-2}\text{s}^{-1}$ (Fig. 5).

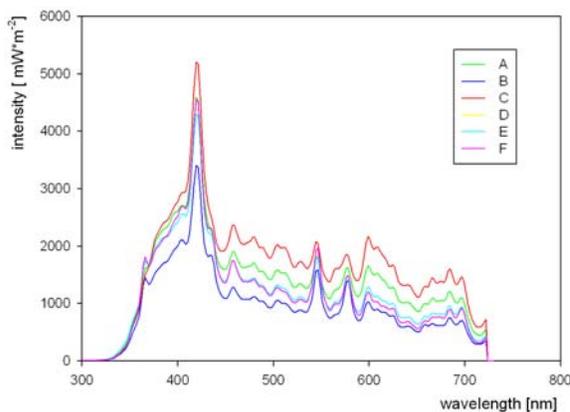


Figure 4: Spectrum comparison of HQI light in six different aquaria.

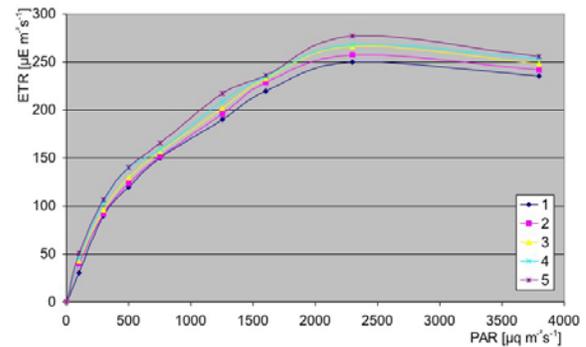


Figure 5: ETR of five different giant clams (*T. maxima*) as a function of PAR.

The oxygen consumption rates (OC) of the same individuals increased slightly over five months, from $0.011 \text{ml O}_2 \text{g}^{-1}\text{h}^{-1}$ to $0.017 \text{ml O}_2 \text{g}^{-1}\text{h}^{-1}$. Smaller sized specimens had a higher OC ($0.024 \text{ml O}_2 \text{g}^{-1}\text{h}^{-1}$ to $0.027 \text{ml O}_2 \text{g}^{-1}\text{h}^{-1}$, Fig. 6).

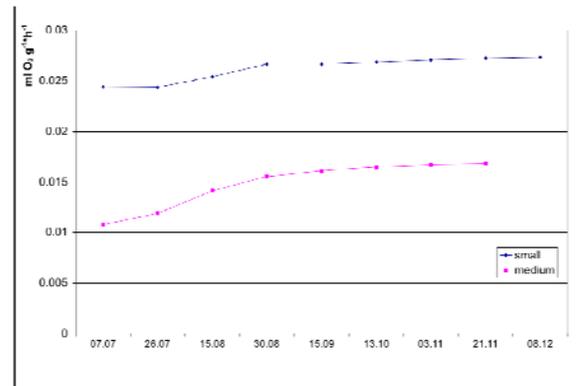


Figure 6: Oxygen consumption rates of the same individuals of *T. maxima* over five months.

Discussion

In this study clam survival is mainly dependent on the lighting conditions; nutrients play a secondary role, at least under experimental conditions and at that age. Only when the clam is kept under optimum conditions clam growth responds positively to the nutrient enrichment. This is in line with other studies (Griffiths and Streamer 1988; Klumpp et al. 1992; Klumpp and Griffiths 1994; Klumpp and Lucas 1994), which have shown that high light intensities may be able to satisfy the basic nutritional needs of clams. But according to Fitt et al. (1993) this strong reliance to zooxanthellae as a source of carbon makes light a limiting factor to growth and survival.

The positive response to nutrients also applies to its symbionts, shown in this study by increased zooxanthellae number, also detected by Braley et al. (1992). The fact that the concentration of zooxanthellae is only moderately and not significantly increasing with increasing nutrient concentration could be due to the short experimental period of 21 days. Ambariyanto and Hoegh-Guldberg (1997) detected a significant effect on zooxanthellae only after three months of nutrient enrichment. The uptake mechanism of zooxanthellae in closed systems might be a problem. The results also show that the symbionts do not have direct access to the nutrients from the water, as proposed by Belda and Yellowlees (1995).

It is not clear yet, why the nutrient uptake, which was also tested under unfavourable light conditions by Hernandez (2006), seems to remain constant, although it is not translated into growth, better survival rates or increase in number of zooxanthellae. Nutrients seem to play a role in the synthesis of the shell, as Schlüter (2005) found changes in shell stability. Marubini and Atkinson (1999) suggest that inorganic nutrients may slow down the calcification.

Similar observations on little effect of nutrient concentrations on clam growth were also made by Ambariyanto and Hoegh-Guldberg (1997) and Sparsis et al. (2001), which is in contrast to results from Hastie et al. (1992), Braley et al. (1992) and Fitt et al. (1993), where a significant contribution of nutrient enrichment to the biomass of the clams was seen. This might be due to the fact that the authors had maintained a continuous flow of nutrients into the water for more than 3 months, while in this study and the above-mentioned studies by Ambariyanto and Hoegh-Guldberg (1997) and Sparsis et al. (2001), spiking only lasted up to 2 to 3 hours a day and this only for a short period of two months. To get more details, additional investigations also on chlorophyll concentrations are planned.

This study has shown that effluents from aquaculture tanks can be supplied to these animals as a source of nutrients, offering possibilities for polyculture. The fact that clams tolerate high concentrations of NO_3^- (pers. observation) would even allow for joint culture with ornamental fish. However, the use of clams for removal of large quantities of nutrients has limits and needs to be investigated with larger specimens and different species. Due to the fact that effluents are always a mixture of several nutrients, the most suitable ratios of N:P need to be tested and adjusted accordingly.

From previous investigations (Lucas 1994) it is known that several specimen being kept in one aquaria, are able to deplete nutrient values considerably. This also applies to calcium, as both

calcium and phosphate are needed for the shell production. Growth is slowed down considerably, when either Ca^{2+} or PO_4^{3-} is not available (Schlüter 2005). This needs to be considered when relying entirely on effluents for nutrient supply; aquaculture effluents do not necessarily contain Ca^{2+} in sufficient concentrations.

It is **concluded** that: clams need optimal light conditions, significant nutrient removal is possible but limited, OC rates are size dependent. In future experiments also adult specimens need to be tested.

The aquaculture of giant clams should be increased, both for ornamental and for bio-cleaning purposes. Because of dwindling populations of natural stocks, future work of ZMT will concentrate on improving aquaculture conditions and exploring physiological performance of giant clams under varying environmental conditions, including stress situations and climate change scenarios. Some experiments conducted only with *T. maxima* and *T. derasa* need to be repeated with *T. gigas*, because of its exceptional fast growth.

Future experiments will concentrate on different size ranges and on the reaction to physical, chemical and biological stress, also by modelling effects induced by climate change.

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