PHYSIOLOGICAL RESPONSES OF THE FLUTED GIANT CLAM, *TRIDACNA SQUAMOSA*, EXPOSED TO DECREASED IRRADIANCE AND REDUCED SALINITY

Eva Blidberg, Tina Elfwing & Michael Tedengren.
Department of Systems Ecology, Stockholm University. S-106 91 Stockholm, Sweden

ABSTRACT

The primary production and respiration of the giant clam *Tridacna squamosa* were measured at different irradiance of natural sunlight by studying changes in dissolved oxygen. Moreover, the clearance rate (i.e. the filtration capacity) and absorption efficiency were studied in 100 % light and in complete darkness. The physiological responses in these metabolic rates to reduced salinity was also examined, since production, respiration, clearance rate and absorption efficiency were studied at two different salinities, ambient (~32 ‰) and 60% (~20 ‰ S) seawater. From the gross production (P_g) and respiration (R) the ratio (P_g/R) was calculated in order to estimate the relative importance of autotrophic production and heterotrophic feeding under different environmental conditions. At light intensities above compensation (P_g/R=1), the clams had higher P_g/R-ratios in ambient salinity than in lower salinity, due to higher production but also slightly lower respiration. The clams had significantly reduced clearance rates at the lower salinity, but clearance rates was generally higher in darkness, although significant only at 20 ‰ S, which indicates some ability to compensate reduced light through increased heterotrophic feeding. The light intensity at which production exceeds respiration on a 24 hours basis in our laboratory experiments corresponds to the measured light penetration at about six meters in the field throughout the experimental period (November-January). This suggests that the maximum depth distribution of the giant clam *T. squamosa* could be largely restricted by reduced light availability in areas subjected to increased sediment output. To some extent these results can explain why *T. squamosa* is rarely found deeper than six meters in the inner Gulf of Thailand, whereas literature data from areas less affected by siltation and fresh water suggest a depth distribution of 5-15 m.

INTRODUCTION

The giant clam family, Tridacnidae, comprising nine living species, two of which in the genus *Hippopus* (Lewis and Ledua 1988, Rosewater 1992) and seven *Tridacna* species (Lucas *et al.* 1991). The giant clams commonly inhabit coral reef environments throughout the indo-pacific region, where they have been traditionally harvested for their meat and shells (Lucas 1994). Over the past two decades the giant clams have been in focus for a lot of scientific interest (Svane 1996). One of the reasons for this is their intriguing symbiotic relationship with photosynthesising algae, the zooxanthellae *Symbiodinium microadriaticum* (Freudenthal 1962). The symbiotic relationship with algae gives these clams a nutritional and growth advantage over normal heterotrophic bivalves (Johnson *et al.* 1995), since their food intake is largely supplemented by energy gained from the photosynthesis of the zooxanthellae (Klumpp & Griffiths 1994). Therefore they can be very large and are also very fast growing. Photosynthesis dependence also affects the depth distribution of the giant clams and they are only found in clear shallow waters and their depth distribution are known to decrease in turbid waters (Lucas 1988; Klumpp & Lucas 1994). The

distribution and abundance of most giant clam species is now decreasing in many areas and there is evidence of widespread overfishing in recent times, not only by modern shellfish industry but also by traditional consumers (Lucas 1994). This is most likely partly due to increased population pressure but also industrial- and agricultural development, leading to increased siltation and pollution in coastal areas thus promoting the general deterioration of coral reef environments in many regions that can contribute to the recorded decline of giant clam populations.

The Gulf of Thailand is no exception in this respect, and the rapid conversion of the mangrove forest to other land-uses, sewage from densely populated coastal areas, industrial waste, agriculture run-off as well as fisheries are all sources of marine pollution in Thailand (Suvapepun 1991). Eutrophic water and high amounts of sediment reduce the light penetration necessary for autotrophic organisms including organisms with symbiotic zooxanthellae. Furthermore, four large rivers drain into the inner Gulf of Thailand, which periodically cause reduced salinities in the area.

The purpose of this laboratory study was to investigate the physiological responses of the giant clam, *Tridacna squamosa*, in different light intensities at normal and reduced salinities.

MATERIAL AND METHODS

This study was performed in Thailand from November 1997 through January 1998, at Sichang Marine Research and Training Station, Sichang Island in the inner Gulf of Thailand.

Giant clams, *Tridacna squamosa*, were provided by the hatchery of Prachuab Khiri Khan Coastal Aquaculture Development Centre, Department of Fisheries, Thailand. Between the experiments the clams were kept in a 225 litres tank with continuous flow of natural seawater (0.1 litre per minute). The clams were acclimated for one week prior to the laboratory experiment. The shells of the clams were carefully cleared from epibionts and were kept clean throughout the experimental period. The clams were exposed to two different salinities, the ambient (average 32 ‰ S) and 20 ‰ S and the time of exposure to reduced salinity before measurements was 12-16 hours in all the experiments. The physiological measurements were made in 1.3 litre transparent chambers, exceeding the recommended water:organism volume ratio (Bayne *et al.* 1985). There were 16 replicate clams (7.8-9.9 cm) in each treatment and their wet weight ranged from 65 to 125 g (average ~100 g). All physiological rates were thus related to a 100 g (wet weight including shell) "standard clam".

The chambers were placed in a tank with continuous seawater flow to prevent temperature changes that would influence the measurements. The different irradiances (origin from natural sunlight) were measured with a light meter (LiCor LI 1000 Data logger). Measurements of changes in dissolved oxygen concentration were made every 15 minutes. This was repeated at different light intensities, including complete darkness, using a polarographic oxygen electrode (Microprocessor Oximeter OXI 196). The oxygen concentration was not allowed to exceed 130 % saturation or drop below 70 % at any time during measurements, since these levels are known to affect physiological rates in marine molluscs (Bayne *et al.* 1985). Two control chambers without mussels were also included to compensate for any activity in the water. The results were used to calculate the oxygen production vs. irradiance. Platt & Jassby (1976) found that the hyperbolic tangent equation was a consistently successful description in their experiments with phytoplankton:

$$P\ (mg\ O_2/h \cdot 100g\ ww) = P_{max} \cdot \tan h \cdot (aI / P_{max})$$

where P = production (mg O_2/h · 100g ww), P_{max} = maximal production at light saturation (mg O_2/h · 100g ww), a=the slope of the light-saturation curve at low light levels, i.e. in linear range, I=irradiance (µE/m^2 ·s). With this equation and the data analyse program CurveExpert 3.1, maximal production (P_{max}) and daily production rate was calculated.

The ratio between production (P_g) and respiration (R) was used to evaluate the bioenergetic effects of reduced light and salinity. The P_g:R ratio was calculated on a 24 hours basis according to the equation:

$$P_g : R_{(24)} = P_{gDt} \cdot I_{24} \; / \; (R_{Dt} \cdot 24 \cdot I_{Dt})$$

where P_g = daily gross production (mg O_2/24 h · 100g ww), P_{gDt} = gross production during the incubation (mg O_2/h · 100g ww), R_{Dt} = respiration during incubation (mg O_2/h · 100g ww), I_{Dt}=insolation during the incubation (µE/m^2 · s), I_{24}=the daily solar radiation (µE/m^2 · s). The equation is based on the assumption that the gross production is directly proportional to the amount of solar radiation (Sorokin 1993) and that the respiration rate is constant during the 24 hours cycle (McCloskey *et al.* 1978).

Giant clams utilise both autotrophic production and heterotrophic feeding. To assess the different ways to maintain the energy needs, the heterotrophic energy uptake was also investigated by measuring the clearance rate. The chambers were filled with one litre of water. The water contained phytoplankton (*Chlorella* sp.) in a concentration of about 40 000 cells per ml, and the clams were allowed to filtrate for a period of 15 minutes when a second sample was taken. Two chambers with algae but without clam were used as controls in each experiment. The feeding experiment was carried out at midday, in light and in darkness at both ambient and reduced salinity. The particle concentration was determined through visual counting in a microscope using a counting chamber. Clearance rate is defined as the volume of water cleared from particles per time unit calculated according to Tedengren *et al.* (1990):

$$\text{Clearance rate (l / h)} = ((\ln C_0 - \ln C_1) - (\ln C_0 - \ln C_g)) \cdot V / T$$

where C_0 = cell concentration at time T_0, C_1 = cell concentration at time T_1, C_g = cell concentration at time T_1 in the control chambers and T = time elapsed between the two readings.

The absorption efficiency (AE) i.e. the ability of the organism digestive system to absorb the food available is of importance when investigating the heterotrophic part of the energy budget. The absorption efficiency was estimated according to the ratio-method of Conover (1966):

$$AE = ((f - e) / (1 - e) \cdot f)) \cdot 100$$

where f and e are the organic fractions of food and faeces respectively. Faeces were collected from the chambers after each filtration experiment. Some of the samples within treatments were pooled since the amounts of faeces were too small. The faeces were filtered onto pre-weighed, pre-ashed GFC filters. The filters were then dried at 70 °C to constant weight and ashed at 450 °C for four hours and weighed again. Food samples were treated in the same way.

The rate of nitrogen excretion is generally closely coupled to respiration rate and energy expenditure. Only ammonia was measured in the present study, since it is the energetically most important nitrogen containing excretory product in bivalves (Bayne and Scullard 1977). Clams were put in the measuring chambers (including two controls without animals) and ten ml water samples were taken instantly and after 30 minutes. The samples were analysed according to the phenol hypochlorite method described by Strickland and Parson (1972). Ammonia excretion rates were calculated for

clams under light and dark conditions at both salinities. The results from the physiological measurements and calculated P_g:R-ratios were statistically analysed in Excel 97 program using Student t-test, two-samples test with unequal variance.

RESULTS

The production capacity of *Tridacna squamosa* at different light intensities is shown in the P- I response curves (Fig. 1). The clams had the highest maximum photosynthesis in ambient salinity and it decreased with 24 ‰ from 23.7 to 17.9 mg O_2/ h for a 100 g (ww) animal if the salinity was reduced to 20 ‰ (Table 1). The respiration rate appears to be somewhat higher when exposed to 20 ‰ salinity compared to ambient, since it increased by 15 % from 2.3 to 2.7 mg O_2/h*100g ww (Table 1). Production exceeded respiration on a 24 hour basis (P_g/R–ratio > 1) at light saturation in both salinities (Fig. 2). However, the P_g/R-ratios decreased significantly (by 20 %) when clams were exposed to the lower salinity (Table 1). The lowest irradiance value giving a P/R-ratio of at least one for a 24 h period (the "compensation irradiance") in this study was

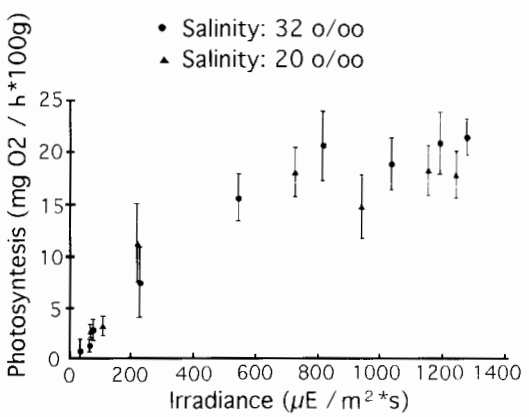

Figure 1. Production-irradiance for *Tridacna squamosa* clams (7,8-9,9 cm) in 32 ‰ and 20 ‰ salinity. The values are calculated for 100 g wet weight of clam, which is close to the actual mean weight. Error bars represent Standard error (n=16).

Table 1. Energy requirements in *Tridacna squamosa*. Where P_{max}=maximal production (mg O_2/h), R=respiration (mg O_2/h), P_g/R=ratio between total daily production and respiration. CR=clearance rate (l/h), AE=absorption efficiency. The values are calculated for a 100 g (wet weight including shell) clam, which is close to actual mean weight, (n=16, mean ± SE).

	Salinity 32 ‰	Salinity 20 ‰
Pmax (mg O_2/h)	23.7±0.92	17.9±0.62
R (mg O_2/h)	2.3±0.08	2.7±0.06
Pg/R-ratio	3.3±0.05	2.6±0.08
CR_{light} (l/h)	3.06±0.24	0.47±0.12
$CR_{darkness}$ (l/h)	3.45±0.24	2.41±0.16
AE	0.61±0.04	0.73±0.02

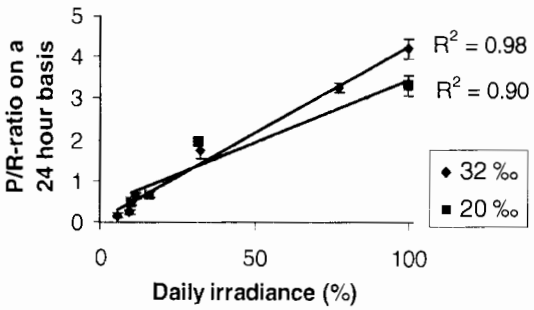

Figure 2. Production-respiration ratios for *Tridacna squamosa* clams (7,8-9,9 cm) at 32 ‰ and 20 ‰ salinity. Error bars represent Standard error (n=16).

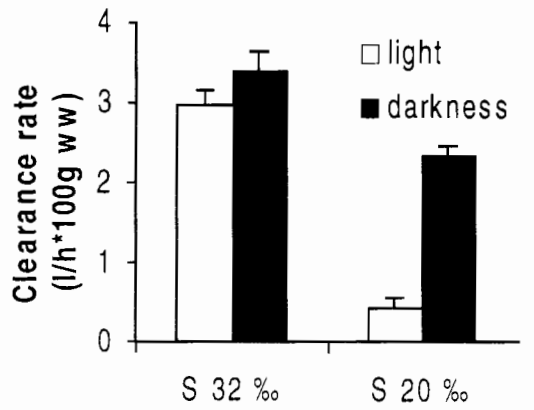

Figure 3. Clearance rate in light and darkness for *Tridacna squamosa* clams (7,8-9,9 cm) in 32 ‰ and 20 ‰ salinity. The values are calculated for 100 g wet weight of clam, which is close to the actual mean weight. Error bars represent Standard error (n=16).

between 200-300 $\mu E/m^2 \cdot s$, which corresponds to about 20 % of the intensity measured in the study area during the experimental period (Figs. 1 and 2). The light saturation value, i.e. when no further increase in production was observed at further increase light intensities, was similar in the two salinities (~700 $\mu E/m^2 \cdot s$) (Fig. 1), and the daily P/R values were thus calculated to 4.2 at ambient and 3.3 in reduced salinity respectively (Fig. 2).

Clearance rates seem to be highly salinity dependent with significantly lower rates at reduced salinity. The clearance rates was, however, generally higher in darkness compared to light (Fig. 3). At ambient salinity (32 ‰) *T. squamosa* increased clearance rates with 13 % from 3.06 to 3.45 l/h for a 100 g clam, which is not a significant increase (Table 1). When exposed to the lower (20 ‰) salinity the measured clearance rate was only 0.47 l/h in light compared to 2.41 l/h in darkness, which represent a highly significant (512 %) increase (Fig. 3). The absorption efficiency is 61 % in ambient salinity and increases with 20 % to 73 % in the lower salinity. This is however not significant and is probably explained by the fact that assimilation efficiencies generally increases when feeding activity decreases in bivalve molluscs (Bayne *et al.* 1985). The ammonia excretion was not possible to estimate, even in complete darkness, suggesting a very effective nutrient retention by the zooxanthellae.

DISCUSSION

The giant clam, *Tridacna squamosa*, showed as expected a reduced production in reduced light intensities. The irradiance values for light saturation (P_{max}) and compensation (P=R) was not affected by salinity changes, although production maximum was significantly reduced. The respiration rates increased by 15 % during exposure to 20 ‰ S, although filtration activity was significantly reduced in the same treatment, indicating osmotic disturbance. This is a general stress response in marine bivalve molluscs when exposed to reduced salinities (Tedengren *et al.* 1990). The P/R-ratio decreased in lower salinity, but at light saturation (>~700 $\mu E/m^2 \cdot s$) the clams had P/R-ratios over one, indicating autotrophy also at reduced salinities. The estimated light needed for self-maintenance was 20 % of the daily surface light.

The giant clam, *T squamosa*, seems to be suffering from the lowered salinity in this study, and increased respiration rates is not the only indication of this. Responses to salinity changes show that the autotrophic energy intake is reduced by approximately 25 % in maximal production (Table 1, Fig. 1). However, we did find P/R-ratios (on a 24 hours basis) higher than one during periods with sufficient light intensities, also in 20 ‰, but this implies other environmental conditions to be optimal.

Field surveys on the abundance of giant clams *T. squamosa* and *T. crocea* in the inner Gulf of Thailand shows that they are not very frequent in this area, and that *T. squamosa* is rarely found deeper than six meters in the waters surrounding Sichang Island (Plantman *et al.* In press). When comparing those field observations with the data on photosynthetic activity reported in the present study, it is interesting how well they correspond. The clams in our laboratory study needed approximately 20 % of daily surface irradiance in ambient salinity to maintain a P/R-ratio above one (self-maintenance) on a 24 hour basis. The light penetration in the water outside Sichang Island was found to be about 20 % at a depth of about six meters, indicating that irradiance is a limiting factor for depth distribution of the giant clam *T. squamosa* in the Inner Gulf of Thailand.

Klumpp & Lucas (1994) could not detect increased heterotrophic feeding activity in *T. tevoroa* or *T. derasa* when light intensities where decreased in order to simulate a depth gradient. However, our studies reported here showed that the giant clam, *T. squamosa*,

increased clearance rate in darkness. This indicates some ability to compensate a photo synthetic energy loss due to reduced light with increased heterotrophic energy intake e.g. filter-feeding (Fig. 3 and Table 1), although other factors (e.g. variable predatory pressure) can also induce "behavioural" changes in bivalves at different light regimes (Nielsen & Stromberg 1985; Reimer *et al.* 1994)

In conclusion, all species are to some extent capable to adapt physiologically to environmental changes. The limits of this capacity of adaptation must be appreciated if we wish to achieve an understanding of how different species respond to human induced environmental stress, superimposed on natural environmental fluctuations (Van Straalen 1994). The knowledge about the sensitivity of the giant clams to sedimentation and turbidity causing decreased light availability, as well as to other types of environmental disturbances including chemical pollution, is thus important if restocking and conservation of giant clams stocks are to be successful.

ACKNOWLEDGEMENT

We would like to thank Professor Padermsak Jarayabhand at Marine Sciences and Aquatic Resource Research Institute, Chulalongkorn University and all staff at Sichang Marine Research and Training Station. Also very special thanks to Ms Jintana Nugranad and Mr Tanate Poomtong at Prachuab Khiri Khan Coastal Aquaculture Development Centre.

REFERENCES

Bayne, B. L., Brown, D. A., Burns, K., Dixon, D. R., Ivanovici, A., Livingstone, D. R., Lowe, D. M., Moore, M. N., Stebbin, A. R. D. and Widdows, J. 1985. The effect of Stress and Pollution on Marine Animals. Praeger Publishers CBS Education and Professional Publishing, New York, pp. 348.

Bayne, B. L. and Scullard, C. 1977. Rates of nitrogen excretion by species of *Mytilus* (Bivalvia:Mollusca). - J. mar. biol. Ass. U.K., **57**: 355-369.

Conover, R. J. 1966. Assimilation of organic matter by zooplankton. - Limnol. Oceanogr. **18**: 338-385.

Freudenthal, H. D. 1962. *Symbiodinium* gen. nov. and *Symbiodinium microadriaticum* sp. nov., a zooxantheallae: Taxonomy, life cycle and morphology. Journal of Protozoology, pp. 45-52.

Johnson, M., Yellowlees, D., and Gilmore, I. 1995. Carbon isotopic analysis of free fatty acids in a tridacnid-algae symbiosis: Interpretation and implications for the symbiotic association. - Biological sciences **260**: 293-297.

Klumpp, D. W. and Lukas, J. S. 1994. Nutritional ecology of the giant clams *Tridacna tevoroa* and *T. derasa* from Tonga: influence of light on filter-feeding and photosynthesis. - Mar. Ecol. Prog. Ser. **107**: 147-156.

Klumpp, D. W. and Griffiths, C. L. 1994. Contributions of phototrophic and heterotrophic nutrition to the metabolic and growth requirements of four species of giant clam (Tridacnidae). - Mar. Ecol. Prog. Ser. **115**: 103-115.

Lewis, A. D. and Ledua, E. 1988. A possible New Species of Tridacna (Tridacnidae: Mollusca) From Fiji. *In*: Giant Clams in Asia and the Pacific. Eds. Copeland, J. W. and Lucas, J. S. (1988). Aciar Monograph no. 9, Australian Centre for International Agricultural Research. Canberra, pp. 82-84.

Lucas, J. S. 1988. Giant Clams: description, distribution and life history. *In*: Giant Clams in Asia and the Pacific. Eds. Copeland, J. W. and Lucas, J. S. (1988). Aciar Monograph no. 9, Australian Centre for International Agricultural Research. Canberra, pp. 21-32.

Lucas, J. S. 1994. The biology, exploitation, and mariculture of giant clams

(Tridacnidae).- Rev. Fish. Sci. **2**(3): 181-124.

Lucas, J. S., Ledua, E. and Bradly R. D. 1991. *Tridacna tevoroa* : a recently describedspecies of Giant Clam (Bivalvia: Tridacnidae) from Fiji and Tonga. - Nautilus **105**: 92-103.

McCloskey, L. R., Wethey D. S., Porter J. W. 1978. Measurement and interpretation of photosynthesis and respiration in reef corals. *In*: Stoddart D. R. and Johannes R. E. (eds) Coral Reefs: research methods. Unesco, Paris, pp. 379-396.

Nielsen, V. M. and Stromgren, T. 1985. The effect of light on the shell length growth and defecation rate of *Mytilus edulis* (L.). - Aquaculture. **47**: 205-211.

Plantman, P., Wijnbladh, E. and Tedengren, M. Distribution and Abundance of the Fluted Giant Clam *Tridacna squamosa* and *T. crocea*. Along a Pollution- and Salinity Gradient in the Gulf of Thailand. In Press, - The Fifth Asian Fishery Forum Proceeding.

Platt, T. and Jassby, A. D. 1976. Mathematical formulation of relationship between photosynthesis and light for phytoplankton. - Limnol. Oceanogr. **12**: 540-547.

Reimer, O., Olsson, B. and Tedengren, M. 1994. Growth, physiological rates and behavior of *Mytilus edulis*. - Mar. Fresh. Behav. Physiol., **25**: 233-244.

Rosewater, J. 1982. A new species of *Hippopus* (Bivalvia: Tridacnidae). - Nautilus **96**: 3-6.

Strickland, D.H. and Parson, T. R. 1972. A practical handbook of seawater analyses. Fisheries research board of Canada, Ottawa, Bulletin 167, pp 310.

Sorokin, Y. I. 1993. Coral Reef Ecology. Ecological studies 102. Springer Verlag, Berlin.

Suvapepun, S. 1991. Long term Ecological Changes in the Gulf of Thailand. - Marine Pollution Bulletin. **23**: 213-217.

Svane, I. 1996. Some recent advances in the studies on the biology of giant clams (Tridacnidae). - Phuket marine Biological Center Special Publication. **16**: 221-241.

Tedengren, M., André, C., Johannesson, K. and Kautsky, N. 1990. Genotypic and phenotypic differences between Baltic and North Sea populations of *Mytilus edulis* evaluated through reciprocal transplantation. III. Physiology. - Mar. Prog. Ser. **59**: 221-227.

Van Straalen, N. 1994. Biodiversity of ecotoxicological responses in animals. - Netherlands J. Zool. **44** (1-2): 112-129.