Heterotrophic Nutrition of Giant Clams from the Jordanian Coast of the Gulf of Aqaba, Red Sea

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ABSTRACT

This study was carried out to investigate the Heterotrophic nutrition of the giant clams (Tridacnidae) in the Jordanian coast of the Gulf of Aqaba, Red Sea. In the present experimental work, a Tridacna maximum was used as a target species, because of its abundance in natural stocks as compared to the other species. Different species of the microalgae Isochrysis galbana, Nannochloropsis oculata, Tetraselmis suecica, and natural phytoplankton were used in the feeding of three size groups (3-5, 5-7, 7-9 cm's) of Juvenile Tridacna maxima. Within the different microalgae species Isochrysis galbana was found to be a good food for all growth stages of giant clams. The results showed a significant difference in the grazing rate of different microalgae species in both short and long-term experiments. The grazing rates of 1.64, 1.22, 0.96 and 0.72 µg l-1 d-1 chlorophyll a (chl a) for Isochrysis galbana, Tetraselmis suecica, Nannochloropsis and natural phytoplankton, respectively. Significant differences in the grazing rate of the different size classes of the juveniles were also observed. However, the grazing rate of the different shell-length classes was in the following order 3-5 > 5-7 > and 7-9 cm. Sampling time does not seem to have any significant effect on the grazing rate of T. maxima on the selected microalgae species. There were no significant differences in the grazing rate of T. maxima veliger larvae under two feeding conditions. The grazing rate was 0.13 µg l-1 d-1 chl a for feeding conditions of 600 cell. ml-1 and 0.07 µg l-1 d-1 chl a for feeding conditions of 300 cell.ml-1. The correlation between the grazing rate and veliger number was highly positive.

Keywords: Tridacna, Nutrition, Gulf of Aqaba, Jordan, Red Sea.

1. INTRODUCTION

The feeding of the giant clams in different stages is similar to that of other molluscan; they depend on the filtration of the fine particles, dispersed in the seawater, through the gills where food particles are filtered from water, trapped in mucus and transport to the mouth. The heterotrophic nutrition is particularly important during the early stages of the life cycle of giant clams. Filter feeding in surrounding water provide 65% of the total carbon needed for respiration and growth of small clams. By comparison, the large clams acquire only 34 % of their carbon from this source (Klumpp et al., 1992).

The giant clams feed daily or at least every second day, food particles are selected according to clams needs and the animal usually discriminate between the planktonic and non planktonic parts which are then expelled as clumps by contraction of the shell (Daniel 1996; Klumpp et al., 1992). Appropriate size for ingestion ranges between 1 and 15 µm for filter feeding and 10 to 100 µm for the grazers (Webb et al., 1983; Kawamara et al., 1998). Clams are well adapted to handling resuspended silt in high concentration and may sort algae cell from inorganic particles prior to ingestion (Jeffery et al., 1992). Clam of 0.3 g dry meat weights continue to filter even if seawater silt particles densities exceed 300 mg.l⁻¹ (Eaton 1981). Microalgae such as Nannochloropsis oculata, Isochrysis galbana and Tetraselmis suecica are microscopic organisms with plant like properties, and are an important component of aquatic ecosystems because they provide food for a large population of aquatic animals (Brown and Robert 2002; Tafi 2002). In particular, they are of great importance to the commercial culture of bivalve molluscs larvae, Juveniles, and adults (Kawamura and Nicholson 1998). They are utilized in aquaculture as live food for all

* Marine Science Station, Aqaba, Jordan(1); Yarmouk University, Irbid, Jordan(2). Received on 5/3/2009 and Accepted for Publication on 14/1/2010.
growth stages of oysters, scallops, clams, and mussels (Jeffrey and Brown, 1992; Horstmann 1965; Pruder 1986; Webb and Chu 1993). The giant clams *Tridacna maxima* is a very common bivalve mollusk in the coral reefs of the Jordan Gulf of Aqaba, Experiments are on-going to grow them in a specific raceway and aquaria set up at the Marine Science Station, Aqaba -Jordan. The present study is to examine the Heterotrophic nutrition by filter-feeding of the giant clams *Tridacna maxima* that is available at the reef flat and reef edge of the Jordanian coast of the Gulf of Aqaba, Red Sea in the aim of (1) Assessing the grazing rate of giant clam on cultured species of the microalgae *Nannochloropsis oculata*, *Isochrysis galbana*, *Tetraselmis suecica* and natural microalgae from the water of the Gulf of Aqaba. (2) Determine the effect of feeding of different size classes of the juvenile clams (3) Evaluate the effect of clams veliger density on the microalgae species.

2. MATERIALS AND METHODS

Clams Collection and Preparation

*Tridacna maxima* juveniles were collected by SCUBA diving from four different sampling sites along the Jordanian coast of the Gulf of Aqaba; Marine Science Station (MSS), National Tourist Camp (NTC), Al-Mamlah (Tala Bay) area and Jordan Fertilizer Industry (JFI), at a depth of 2 to 5 m (Figure 1). Specimens were transported immediately after collection from the site of collection to the raceway of the mariculture unit at the Marine Science Station for three weeks of acclimatization. Thereafter, clams were divided into three size classes 3-5, 5-7 and 7-9 cm by using a caliber. The shells of all clams were thoroughly cleaned during the acclimation period and before the beginning of the experiment using plastic brush for the removal of any algal growth.

![Fig. 1. Collection sites along the Jordanian coast of the Gulf of Aqaba](image)

**Fig. 1. Collection sites along the Jordanian coast of the Gulf of Aqaba**

**Juvenile Feeding Experiment (set-up)**

Twelve glass aquaria (30 x 30 x 60 cm) were used in the experiment. The aquaria were divided into three groups each of which contains four aquaria, randomly distributed in a 20 m long x 2m with concrete tank. Six juveniles of *Tridacna maxima* with shell length (SL) of the 3-5, 5-7 and 7-9 cm were put in three aquaria of each group, while the fourth aquarium was kept without specimens as a control. The twelve aquaria were put in a water bath in the raceway. The water bath was supplied with flow-through seawater system to maintain temperature of the aquaria at the level of the ambient natural environment. Each aquarium was supplied with aeration system. Twenty-eight liters of seawater that has been passed through a different filtration bags of 2, 3 and 5 µm, mesh size (to remove all algae) were put in each experimental aquarium. In order to determine the grazing rate of the different size class of *Tridacna maxima* at different feeding conditions, three different species of microalgae; *Nannochloropsis oculata*, *Isochrysis galbana* and *Tetraselmis suecica* were grown in the culture unit of the Marine Science Station. Two liters of each microalgae were added to each aquaria that contains the 28 liter of filtrated sea water. For short term grazing rate
experiments, sample of half liter were taken from each aquaria over a period of 24 hrs at the following time series: 7 am (initial), 11 am, 3 pm, 7 pm, 11 pm, 3 am and 7 am. For long-term experiments, samples of half liter were taken from each aquarium over a period of three days at the one day intervals. In both short and long-term experiments, half liter of sea water was taken from each aquarium immediately after the addition of the microalgae; for determination of the initial chlorophyll $a$ concentration. The collected samples were kept in a plastic container covered with a black plastic sheet to avoid exposure to sun light.

**Analysis**

Chlorophyll $a$ was measured spectrofluorometrically as described by (Arar and Collins 1992). Half liter of seawater was filtered through cellulose membrane filter (0.45 µm). Each filter was placed in a glass tube wrapped with aluminum foil. Ten ml of 90 % acetone was added into the test tube. The membrane filter was ground using a tissue grinder and then kept in a refrigerator at 4°C over night. The mixture was then centrifuged for 20 minutes at 5000 rpm, and Chlorophyll $a$ content was measured in a 13 mm glass test tube using the direct concentration calibration method. 90 % acetone was used as a blank.

**Culture of Microalgae**

Indoor and outdoor cultures of the microalgae *Isochrysis galbana, Nannochloropsis oculata, Tetraselmis suecica* were used in the present study for the continuous feeding of the giant clam juveniles and larvae. Indoor culture was used to produce small amounts of microalgae under controlled conditions of light, temperature and oxygen. Half liter of algal stock was added to every 6 liters of filtered seawater and mixed with one gram of nitrogen, phosphate, and potassium fertilizer (NPK). Ten liters from the indoor subculture were taken and added to the 500 l of filtered seawater in a large outdoor tank and mixed with 5 grams of NPK fertilizer (Le Borgne 1990)

**Veliger Feeding Experiment**

In order to determine the grazing rate of *Tridacna maxima* veliger under two feeding conditions on the microalgae *Nannochloropsis oculata*. A working solution of 300 cell. ml$^{-1}$ and 600 cell. ml$^{-1}$ density using cell count techniques was prepared from the original stock solution of *Nannochloropsis oculata*. A series of one liter capacity plastic bottles were filled with *Nannochloropsis oculata*. *Tridacna maxima* veligers were harvested from the mariculture unit of the Marine Science Station (MSS). The veliger density was checked before its use. Specific volumes from the harvested veliger batch were put in the plastic bottles to give veliger concentrations of 0, 3, 6 and 9 veliger. ml$^{-1}$. At the beginning of the experiment a sub-sample of 500 ml were taken for the determination of the initial chlorophyll $a$ concentration. The bottles were fixed on a rotating cylindrical wheel to maintain a continuous mixing of the algae inside the bottle. After 24 hr another sub-sample of 500 ml were taken from each bottle for chlorophyll $a$ measurements.

**Growth and Grazing Rate Measurements**

According to Landry and Hassett (1982), the rate of microalgae growth ($\mu$ d$^{-1}$) and grazing ($g$ d$^{-1}$) in the experimental bottles or aquaria are calculated from the observed change in the microalgae biomass, following a series of veliger density or a series of *Tridacna* Juveniles of different size, over a period of time ($t$). Microalgae biomass change with time can be represented appropriately by the exponential equation:

$$B_t = B_0 e^{(\mu-g) t}$$

where $\mu$ is the potential growth rate ($\mu$ d$^{-1}$) and $g$ is the potential grazing rate were calculated from initial and final chlorophyll $a$ in samples taken from the control enclosures where the grazing is equal to zero. Potential growth rate ($\mu$) was calculated according to the following equation:

$$\mu = \frac{\ln (B_f/B_0)}{t}$$

The grazing rate ($g$) was calculated according to the following equation:

$$g = \mu - \ln (B_f/B_0)/t.$$ In which:

- $\mu$: growth rate
- $B_0$: initial concentration of microalgae
- $B_f$: microalgae concentration after time ($t$).

3. RESULTS

**Statistical Analysis**

The one-way analysis of variance (ANOVA) was used to test the effect of feeding conditions, size of *T. maxima* and exposure time on the grazing rate of *T. maxima*. The results indicate that grazing rate of the clam on the different microalgae species is significantly different, grazing rate of the clam differs significantly among different size. In contrast, the result of the exposure time did not show significant effect on the
grazing rate of the clam. The same test showed that the effect of veliger number on the grazing rate was highly significant while, feeding conditions of the amount of available food (300 cell. ml\(^{-1}\) and 600 cell. ml\(^{-1}\)) did not show any significant effect on the veliger grazing rate.

**Tridacna Maxima Juvenile Feeding Experiments**

The mean grazing rates of *T. maxima* on the microalgae varied between different species. It ranged between 0.75 and 1.64 µg l\(^{-1}\) d\(^{-1}\) for natural phytoplankton and *Isochrysis galbana*. In between, the grazing rates on *Tetraselmis suecica* and *nanochloropsis oculata* were 1.22 and 0.96 µg l\(^{-1}\) d\(^{-1}\) respectively. Figure 2 shows the mean values of grazing rate (µg l\(^{-1}\) d\(^{-1}\)) ± standard deviation for different microalgae species.

![Figure 2](image1.png)

**Figure 2.** Mean grazing rate (µg l\(^{-1}\) d\(^{-1}\)) ± SD of *Tridacna maxima* under different feeding conditions of the microalgae species.

![Figure 3](image2.png)

**Figure 3.** Mean values of grazing rate (µg l\(^{-1}\) d\(^{-1}\)) ±SD of different size classes of *T. maxima*.

Grazing rate of the *Tridacna maxima* juveniles shell length size classes (3-5 cm), (5-7 cm) and (7-9 cm) was also variable. The 7-9 cm shell length (SL) size class, showed the higher grazing rate (2.197 µg l\(^{-1}\) d\(^{-1}\)) compared to the size classes of 5-7 and 3-5 cm which showed grazing rate of 1.805 and 1.570 µg l\(^{-1}\) d\(^{-1}\), respectively. Figure 3 shows the mean grazing rate (µg l\(^{-1}\) d\(^{-1}\)) of the different size classes of *T. maxima* (3-5cm), (5-7cm) and (7-9) cm, shell length.
Figure 4 shows the grazing rate of *Tridacna maxima* over 24 hr and of three days period experiment. The figure shows that there was an increase in the grazing rate after 12 and 16 hr from the beginning of the experiment, to the level of 2.02 and 1.96 $\mu$g l$^{-1}$ d$^{-1}$, respectively. A decrease occurred after 20 hrs (1.69 $\mu$g l$^{-1}$ d$^{-1}$). The mean value of the grazing rate during the three days period was higher at the second day of the experiment (1.21 $\mu$g l$^{-1}$ d$^{-1}$) compared to those of the first (1.06 $\mu$g l$^{-1}$ d$^{-1}$) and third day (1.14 $\mu$g l$^{-1}$ d$^{-1}$). However, the statistical analysis of the results showed that the grazing rates of *Tridacna maxima* at 4 hrs intervals over a period of 24 hrs did not differ significantly with time.

![Figure 4. Mean grazing rate ±SD of *Tridacna maxima* over a period of 24 hr and three days.](image)

**Veliger Larval Feeding Experiments**

Food concentration available to the bivalve larvae and feeding rates throughout the complete larval development of the bivalve's species are considered important keys in studying the nutrition of the bivalves but mainly giant clams. This part of the results shows the relationship between the number of the grazers (3, 6 and 9 veligers .ml$^{-1}$) and grazing rate under two different food concentrations (300 and 600 cell. ml$^{-1}$ of the microalgae *Nannochloropsis oculata*).

**Effect of Veliger Density and Feeding Condition**

A positive relationship has been observed between the number of veliger and the grazing rate in both feeding conditions. In case of 300 cell. ml$^{-1}$, the mean grazing rate was very high (0.127 $\mu$g l$^{-1}$ d$^{-1}$) for the bottles which contained 9 veliger .ml$^{-1}$. In contrast, the grazing rate was only 0.031 and 0.047 $\mu$g l$^{-1}$ d$^{-1}$ for the bottles of 3 and 6 veliger .ml$^{-1}$, respectively. The same trends were observed for the 600 cell. ml$^{-1}$ food concentration. The grazing rate was 0.038, 0.079 and 0.266 $\mu$g l$^{-1}$ d$^{-1}$ for the 3, 6 and 9 veliger. ml$^{-1}$ respectively. The mean grazing rate (0.13 $\mu$g l$^{-1}$ d$^{-1}$) was slightly higher under feeding condition of 600 cell. ml$^{-1}$ compared to the mean grazing rate of only 0.07 $\mu$g l$^{-1}$ d$^{-1}$ for the 300 cell .ml$^{-1}$ condition. Figure 5 (A & B) shows the grazing rate of different veliger numbers under 300 cell. ml$^{-1}$ and 600 cell. ml$^{-1}$ and the mean grazing rate under two feeding conditions.

![Figure 5. Mean grazing rate ±SD of *Tridacna maxima* over a period of 24 hr and three days.](image)
4. DISCUSSION

It has been reported that different microalgae vary in their suitability as food in mariculture (Landry and Hassett 1982). Filter-feeding and grazing rates of the giant clams (*Tridacna maxima*) juvenile on the microalgal species *Nannochloropsis oculata*, *Isochrysis galbana*, *Tetraselmis suecica* and on natural phytoplankton on both short-term (24 hrs) and long-term (three days) experiments. The results (Fig. 2) show that the grazing rate of *T. maxima* on the microalgal species *Isochrysis galbana* was higher than the grazing rate on *Tetraselmis suecica* and *Nannochloropsis oculata*. The results show also that feeding and grazing on the single cell species of microalgae differ significantly from feeding or grazing on the mixed natural phytoplankton of the Gulf of Aqaba. This can be attributed to the low phytoplankton concentrations in the oligotrophic waters of the Gulf of Aqaba (Lindell and Post 1995, Al-Najjar 2000). The comparison between the initial concentration (average) chlorophyll *a* of the single cell microalgae species and mixed natural phytoplankton used in the present experiment leads to the same conclusion. Similar results were reported by many other authors (Goreau et al., 1973; Fitt et al., 1986; Braley 1990; Dunstan et al., 1993 and Brown and Robert 2002). Braley 1990 and Nell and O'Connor 1991; McCausland et al., 1999). Heasman et al., (2000) reported that the *Isochrysis galbana* and *Tetraselmis suecica* as being suitable food for bivalve culture. Similarly, the *Nannochloropsis sp.* are commonly fed to *Artemia* or rotifers, which are fed later onto the larval stages of crustacean and fish larvae.

The main characteristics of the microalgae used in mariculture have been listed by many authors (Webb and Chu 1983; Jeffrey et al., 1992; Kawamura et al., 1998 and Brown and Robert 2002). They indicated that the shape and size (in addition to toxicity, digestibility and biochemical composition) of the microalgae can affect their value as food for *Tridacnids*. They suggested that microalgae must be of an appropriate size for ingestion, and noted that the suitable size for ingestion is 1 to 15 µm for filter feeders, and 10 to 100 µm for grazers.

The three *Tridacna* size classes used in the present study showed a considerable difference in their grazing rates. The grazing rate increased with increase of the body size between 3-5 cm shell lengths to the 7-9 cm size class. In the short-term experiment the grazing rates between the size classes were significantly different, it can be attributed to different factors that can affect and limit the process of grazing, among these factors is the available amount of microalgae, reduction in irradiance under natural conditions and sexual maturity differences between different species (Klumpp et al., 1994).

Klumpp and Griffiths (1994) studied the grazing rate of the four clam species and reported that grazing rate was strongly dependant on clam body size. At a weight of 1 g, *Tridacna gigas* showed the highest grazing rate of 3.68 l h⁻¹, compared to 0.58 for *Tridacna crocea* and 0.25 l h⁻¹ for *Hippopus hippopus* and only 0.32 l h⁻¹ for *T. squamosa*. A similar general pattern has been found in
the present study. The relationship between filter feeding and size of *Tridacna gigas* and *Hippopus hippopus* in simulated shallow inshore reef environment particulate organic matter (POM) can provide 113 % of the total carbon requirement of small *Tridacna gigas* (Shelley 1989). Klumpp and Lucas (1994) found that the *Tridacna crocea* and *T.squamosa* showed a different pattern, where POM contributed only about 10 % of the requirement of small clams, though this contribution increased with increasing body size. They found also that the resulting size- related changes in the rates of uptake of the absorbed particulate organic carbon (POC) by four species of tridacna, that *T.gigas* gained by far the most carbon from filter feeding except in the largest size class, which acquired the least.

Several comprehensive investigations have been made on the importance of various microalgae species to bivalve larvae (Davis and Guillard 1958; Walne 1963; Helm 1977; Fernández and Selma, 2004). Many of these investigations focused on the effect of size of different microalgae on the clearance rate. However, not much information is available on the effect of microalgae and veliger larval concentrations on the grazing rate of the veliger. In the present study, the experiments showed that the grazing rate of *Tridacna maxima* veliger is affected significantly by microalgae concentration. The grazing rate was higher when the microalgae concentrations was 600 cell .ml⁻¹ compared to that at concentration of 300 cell .ml⁻¹. This could be explained in the view of the fact, that ingestion rate increases with increasing food concentration up to an optimum concentration of microalgae (Fernandez and Selma, 2004). However, after the optimum concentration is reached, any increase in the concentration of microalgae will lead to inhibition of the grazing rate of the veliger. This inhibition appears to be due, at least in part, to a mechanical interference with feeding. Also, the response of the ingestion rate to particle concentration could be described by assuming increasing interference among particles that reduces the efficiency of capture by the veliger (Gallager 1988). Ukeles and Sweeney (1969) concluded that retention of microalgae *Isochrysis galbana* cells by *Crassostrea virginica* larvae was most efficient at 200 cell .ml⁻¹, while Malouf and Breese (1977) suggested that excessive formation of pseudofaeces caused inefficient feeding at high algal densities. The grazing experiments in the present study suggest that, the number of veliger larvae play an important role in the grazing rate on microalgae. Generally speaking, positive relationship between the number of *T.maxima* veliger and the grazing rate on the two microalgae (food) concentrations used in the experiment. This can be explained in the view that feeding of one larvae is independent on other larvae, despite of the change in the quantity of food available to Juvenile larvae. This means that the probability of a microalgae cell being grazed is a direct function of the rate of encounter of veliger with microalgae cell. This implies that the veliger is not food- satisfy at natural microalgae densities the number of microalgae ingested by a given veliger is linear to microalgae density; and the total grazing rate of the juvenile larvae in the experimental bottles is a direct function of a number of grazers.

5. CONCLUSIONS

The following conclusions are based on results obtained from the present experimental work on the heterotrophic nutrition of giant clam *T.maxima* under different feeding conditions of microalgae species *Isochrysis galbana*, *Nannochloropsis oculata*, *Tetraselmis suecica* and natural phytoplankton:

1. Highly significant difference in grazing rate was obtained between different microalgae species in both short-term (24hts) and long-term (three days) experiments.
2. The grazing rate of *T. maxima* on different microalgae species was in the following order: *Isochrysis galbana* > *Tetraselmis suecica* > *Nannochloropsis oculata* > natural phytoplankton.
3. The grazing rates between different shell-length classes was highly significant and in the following order: 3.5 > 5.7 > 7.9 cm.
4. Sampling time does not have any significant influence on the grazing rate of *T. maxima*.
5. A positive relationship between the grazing rate and veliger number were obtained.
6. The grazing rate of veliger larvae was significantly different under the two feeding conditions; it was 0.13 µg l⁻¹ d⁻¹ for the 600 cell. ml⁻¹ and 0.07µg l⁻¹ d⁻¹ for the 300 cell .ml⁻¹.

ACKNOWLEDGMENT

The authors would like to acknowledge the effort of Marine Science Station staff during this work, especially Omar Almomany and Ehab Eid. This study is part of the
pilot project on sustainable aquaculture of the giant clam (Tridacna spp.) in the Jordanian sector of the Gulf of Aqaba (Red Sea), in collaboration with the Centre of Tropical Marine Ecology (ZMT), Bremen, Germany.


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