Combined Effects of Temperature and Algal Concentration on Filtration and Ingestion Rates of *Crassostrea gigas*: Bivalvia

Mona,M. H.[1], Elkhodary, G.M.*[2] and Khalil, A.M

[1] Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt.
[2]Zoology Department, Faculty of Science, Damnhour University, Damnhour, Egypt.
[3]Zoology Department, Faculty of Science, Zagazig University, Zagazig, Egypt.
*gihankhodary@hotmail.com

Abstract: The effect of varying algal cell concentration, food items and temperature on the filtration and ingestion rates of the eyed larva of *Crassostrea gigas* were investigated under laboratory condition. The filtration and ingestion rates were measured according to the indirect method in which the flow of water into the passage cavity is inferred from the rate of removal of suspended particles. Cultures of the unicellular alga *Isochrysis galbana* and *Pavlovia lutheri* were used in the test solutions. Three different concentrations of the two algal species (100,50 and 25 cells/µl) and three temperature degrees (25 ºc, 20 ºc and 13 ºc) were used to study the effect of these factors at different time intervals from starting the experiments (1, 6 and 12 days) on the filtration and ingestion rates of the eyed – larvae. The filtration and ingestion rates of the larvae of *C.gigas* that recorded by using *I. galbana* were large than that of *P. lutheri.* These results might depend on the size of the filtered particles. Moreover, the larvae of *C. gigas* regulate filtration rates according to the particle concentration in the surrounding medium, filtration was more actively in lower concentrations (25 x 10^6 L^{-1}*I . galbana*) than at the higher ones (100 x 10^6 L^{-1}*I galbana*). The mean value of filtration and ingestion rates of *C.gigas* were significantly increased by the increase in temperature degree and 25ºc were considerable the perfect temperature to achieve high values of both filtration and ingestion rates if it is compared by 20 ºc and 13 ºc . These results help to explain the feeding behavior of *C.gigas*.
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1. Introduction

Filtration rate or clearance rate is defined as the volume of water filtered completely free of particles per unit of time and is also sometimes synonymously used as the pumping rate when all particles entering the mantle cavity are completely retained by gills (Winter, 1978). The ingestion rate or feeding rate is defined as the number of algal cells an organism consumes per unit time (Peters, 1984). Two principal methods are available to measure the rate of passage of water through the mantle cavity of a bivalve, the direct method on which the rate of pumping of water itself is measured , and the indirect method in which the flow is inferred from the rate of removal of suspended particles .The former method requires either a physical separation of the inhalant and the exhalant currents or a method of rendering visible the flow of water from the siphons by using dyes or other kinds of particles (Ali, 1970). Khalil, (1996) and Rajesh *et al*., (2001) measured the filtration and ingestion rates in *Tapes decussatus*, *Perna virids, Crassoostrea madrasensis and Paphia malabarica* using *Isochrysis galbana* and *Pavlovia lutheri* as unicellular algal food. Christina and Hans (2005) measured clearance rate of *Rhodomonas sp.* in *Mytilus edulis*. Luc *et al*., (2008) studies the filtration rate of *Mytilus edulis*and and *Crassostrea virginica* at low temperature using a mixed suspension of *Chaetoceros muelleri* and *Isochrysis galbana*. Filtration and ingestion rates of different larval stages of *Paphia malabarica* have been measured in relation to feeding on various species of unicellular algae (Raghavan, 2011).

Many countries in different regions of the world introduced the pacific oyster *Crassostrea gigas* into their water because of the over harvesting of their native oyster. *Crassostrea gigas* was introduced into Germany (Mexiner and Gerdener, 1976), Tawian (Li &Yuan, 1981), Chile (Winter *et al.*, 1984) and Mexico (Cardenas, 1984).

The present work was planned to construct the basis of an ideal hatchery upon which the pacific oyster can be introduced and planted in Egypt. The selection of this animal was based on the following.

1. It's highly valuable as a food where it is considered one of the most important inexpensive and rich sources of protein.
2. Its high tolerance to the different environment parameters (e.g temperature, salinity and food requirement).

3. The recent technical advances that facilitate its cultivation and maintenance.

This work aims to asses the effect of temperature and algal cell concentration of *Isochrysis galbana* and *Pavlovia lutheri* on filtration and ingestion rates of *Crassostrea gigas* eyed – larvae. The technique that used provided a means of carrying out acute measurements of grazing rate, and hence of detecting short – term responses to changes in the environment.

2. Material and Methods

The filtration rates, swept-clear-volumes, (ml/h/individual) and the ingestion rates, number of algal cells filtered out, (cells/h/individual) of eyed-larvae were measured as a function of different ecological factors. Three different concentrations of the two algal species and three temperature degrees were used to study the effect of these factors at different time intervals from starting the experiments on filtration and ingestion rates of the eyed–larvae.

Experimental animals:

The swimming larvae of *Crassostrea gigas* were collected from coast oyster Hatchery of Quilcen, Washington to the laboratory. The artificial sea water was removed from the culture vessels through a screen of sufficiently fine mesh to retain these small larvae. Larvae were fed on a mixture of algae *Isochrysis galbana and Pavlovia lutheri*. The algal culture methods followed the techniques adopted by Guillard and Ryther (1962). A mixture of the antibiotics Streptomycin Sulphate; Erythromycin Lactobionate and Cefotaxime (200,150,200mg, respectively \l sea water) was added to the petri dishes of larval culture at each water change. This process is very important to keep the larvae healthy and in an active swimming condition. All larvae used in experiments, were acclimated in aerated fresh seawater for 7 days in order to settle down after the disturbance caused by shipping them to the lab. Investigated samples in good condition were chosen from bunches of similar size and age and cleaned from any mud particles and any epizoic growth. Measurements of filtration rates were made at three different temperature degrees 13ºc, 20ºc and 25ºc, the required temperature was attained by holding the plastic dishes containing the algal cells and the larvae of *C. Gigas* in the analogous thermostat chambers. The larvae were brought to experimental temperature and left for 1 hour at this temperature before any readings of the filtration rates were begun.

Feeding:

Pure logarithmic phase from the cultures of the unicellular algae *Isochrysis galbana* and *Pavlovia lutheri* were used .These were grown in f/2 medium (Guillard and Ryther, 1962) and centrifuged from the culture medium and suspended again in filtered sea water. Cell density was determined by means of a blood –cell counting chamber (Thoma, 0.1 mm depth). Three levels of food concentration were used through these experiments: 100, 50, 25 cells /µl of the cultural media .To reach the actual concentration required, these steps were followed:

a. certain volume (v) of algal culture was poured in capped bottles.
b. The suspension was centrifuged to get ride of the supernatant that causes high mortality among the larval cultures.
c. Freshly prepared seawater was added to the algal cells that accumulated in the bottom of the bottles until reaching to the volume (v) again
d. The algal cells/ µl (c) were counted by using the blood cell counting chamber.
e. To deduce the required concentration (c) and volume (v) the following equation was applied c x v= c x v

Experimental Design:

Groups of 10 larvae were placed in 25 ml plastic dishes (to avoid the settlement and metamorphosis of these larvae which might result in other substrata other than plastic) containing 10 ml of algal culture. These groups of dishes were classified to fulfill the purpose of the following experiments:

(a) Experiments to measure the effect of temperature on the filtration rate and the ingestion rate.
(b) Experiments to measure the effect of food concentration on both filtration and ingestion rates.
(c) Experiments to measure the effects of time on filtration rate and ingestion rate.

All previous experiments were carried out on the eyed – larvae of *Crassostrea gigas* to compare the effect of the different parameters on their filtration rates. Three 1 litre Pyrex dishes of the larval cultures were prepared where the density of the larvae were 100 larvae / L and kept in 13 ºc, 20 ºc and 25 ºc thermostat chambers (one dish for each temperature) to be the stock of the different experiments.

To examine the effect of food concentrations (100, 50, 25 cells / µ1) on the filtration and ingestion rates, three groups, A, B and C were prepared where each food density was represented by one of these groups. Each group composed of 7 dishes, whereas 10 larvae were hold on 10 ml of algal suspension in 6 dishes while the seventh included only algae without animals as a control. This served to correct for any error which might result from sedimentation, flocculation or reproduction of the algae during the

experiments.

In clearance experiments, the larvae were continuously withdrawing particles and diluting the suspension with filtrate. To keep the initial concentration of the suspension nearly fixed, the suspension was exchanged with new one each 6 hours. To examine the effect of food items on the filtration rate, step 1 was repeated twice, one by using *pavlovia lutheri* and the other by using *Isochrysis galbana*. To examine the effect of temperature on the filtration rate step 2 was repeated three times for 13 ºc, 20 ºc, and 25 º c. To examine the effect of time on filtration rate, 10 larvae were pipetted by using the automatic micropipette from the stock dishes to 25 ml plastic dishes (where the experiments were conducted after 1,6 and 12 days for starting the experiments) then steps 1,2 and 3 were repeated.

Calculation:

All calculations were operated by methods of Coughlan (1969) and Peters (1984) where the following formulae were applied because of their easiness and reliance:

Ingestion rate= $\frac{(c_0 - c_t)\,V}{Nt}$ cells individual^{-1} hour^{-1}

Filtration rate= $\frac{(1nc_0 - 1nc_t)V}{Nt}$ ml. individual^{-1}hour^{-1}

Where:

C_o = Initial cell concentration at time zero.

C_t = Final cell concentration at time (t)

t = Time interval (hours)

N = Number of individuals in container.

V = Volume of the algal culture used.

Statistical analysis:

The results are presented as mean ± SD values. Two –way analysis of variance (ANOVA) was used to test the significance of filtration and ingestion rates in each food items. LSD test was used to analyze the mean comparisons among concentration of algal cell and temperature degree and paired T- test to compare between the different time intervals. All statistical analysis were preformed using the SPSS 17 soft ware (SPSS 2007)

3. Results

1.1. The effect of temperature, time and *I. galbana* concentration on filtration and ingestion rates of eyed larvae of *C. gigas*

By using 100 cells /µl of *I. galbana*, the filtration rate decreased significantly from 0.078 ml/h/larva at 25ºc to 0.005±0.001 ml/h/larva at 13 ºc and the ingestion rate from 3558.4±8.502 cells/h/larva at 25 ºc to only 1402.79±5.645 cells /h/larva at 13ºc (Tables,1&3). By comparing the filtration and the ingestion rates in relation to the time of experiments, a highly significant decrease (P≤ 0.001) in these rates was observed by the passage of the time.

At the concentration 50 cells/µl (figures 1, 2) the rates continued to decline significantly (P≤ 0.001) after 1, 6 and 12 days at all tested temperatures.

Table 1 elucidate that at the concentration 25 cells/µl, the filtration rates were greatly affected by temperature after one day from starting the experiments measured under the effect of different temperatures. They always increased significantly by increasing the tested temperatures (P≤ 0.001). The rates were larger at 25 ºc than that at 20 ºc and 13 ºc. At 25 ºc and 20 ºc, the filtration rate decreased significantly (P≤ 0.001) by increasing the duration of experiments and decreased not significantly at13 ºc.

On the other hand, the filtration rate of eyed –larva of *C. gigas* were significantly higher (P≤ 0.001) at concentration 25 cells/µl than concentration100 cells/µl meanwhile, the ingestion rates decreased significantly by decreasing these concentrations (Table, 2)

1.2 The effect of temperature, time and *P.lutheri* concentration on filtration and ingestion rates of eyed larvae of *C. gigas*.

Figures 3and 4 confine the predominance of 25ºc as a perfect temperature to achieve high significant values of both filtration and ingestion rates if it is compared by 20 ºc and 13ºc by using 100 cells /µl of *P.lutheri*. The highest values of these rates always recorded after one day from starting the experiments. At 20ºc, the passage of time of experiments also decreased these rates significantly (P≤0.001) but there was no significant effect (P≥0.05) for the time on these rates at 13ºc; the filtration (0.074±0.001 ml/h/larva) and the ingestion rates (1748.33±7.024 cells /h/larva) at the combination 25ºc, 50 cells/µl and one day significantly decrease than that at the combination 13ºc, 50cells/µl and 12 days, they were 0.003±0.001 ml/h/larva and 129.667± 2.517 cells /h/ larva respectively.

Tables 5 the filtration rate recorded when 25cells/µl was used as a food concentration were significantly high (P≤ 0.001) than that measured with 100 and 50 cells/ µl, yet these data were parallel to these of 100 and 50 cells/µl.

The previous data showed that the filtration rates and ingestion rates direct proportional with the temperature and inverse proportional with the time from starting the experiments. Meanwhile, the filtration rates inverse proportional with food concentration while ingestion rates direct proportional with the same parameter.

Table (1): The effect of different temperatures and different concentrations of *I. galbana* on the filtration rate (ml/h/larvae) of the eyed-larvae of *C.gigas*.

Time	Conc.		Temp.				LSD	
			25°C	20°C	13°C	Total	Comp.	P-value
T1	100 cells/µl	Mean	0.078	0.066	0.005	0.050	**100 cells/µl&50 cells/µl**	**0.000**
		SD	0.000	0.000	0.001	0.034	**100 cells/µl&25 cells/ µl**	0.000
	50 cells/µl	Mean	0.093	0.063	0.005	0.054	**50 cells/ µl &25 cells/ µl**	0.000
		SD	0.001	0.001	0.000	0.039	**25°C&20°C**	0.000
	25 cells/µl	Mean	0.112	0.085	0.008	0.068	**25°C&13°C**	0.000
		SD	0.000	0.001	0.001	0.047	**20°C&13°C**	0.000
	Total	Mean	0.094	0.071	0.006	0.057		
		SD	0.015	0.010	0.001	0.039		
T2	100 cells/µl	Mean	0.070	0.040	0.004	0.038	**100 cells/ µl &50 cells/ µl**	0.000
		SD	0.001	0.001	0.001	0.029	**100 cells/ µl &25 cells/ µl**	0.000
	50 cells/µl	Mean	0.077	0.052	0.003	0.044	**50 cells/ µl &25 cells/ µl**	0.000
		SD	0.001	0.001	0.001	0.033	**25°C&20°C**	0.000
	25 cells/µl	Mean	0.096	0.047	0.009	0.050	**25°C&13°C**	0.000
		SD	0.001	0.001	0.001	0.038	**20°C&13°C**	0.000
	Total	Mean	0.081	0.046	0.005	0.044		
		SD	0.012	0.005	0.003	0.032		
T3	100 cells/µl	Mean	0.017	0.010	0.003	0.010	**100 cells/ µl &50 cells/ µl**	0.000
		SD	0.001	0.001	0.001	0.006	**100 cells/ µl &25 cells/ µl**	0.000
	50 cells/µl	Mean	0.024	0.019	0.002	0.015	**50 cells/ µl &25 cells/ µl**	0.000
		SD	0.001	0.001	0.001	0.010	**25°C&20°C**	0.000
	25 cells/µl	Mean	0.042	0.020	0.007	0.023	**25°C&13°C**	0.000
		SD	0.001	0.002	0.000	0.015	**20°C&13°C**	0.000
	Total	Mean	0.028	0.017	0.004	0.016		
		SD	0.011	0.005	0.002	0.012		

Table (2): Two way analysis for variance on filtration rate of eyed- larvae of *C.gigas* fed on *Isochrysis gabana*

ANOVA 2-way	T1		T2		T3	
	F	P-value	F	P-value	F	P-value
Conc.	3019.179	0.000	1393.263	0.000	748.742	0.000
Temp.	69006.385	0.000	52247.933	0.000	2460.712	0.000
Conc. *Temp.	827.977	0.000	633.125	0.000	198.458	0.000

T1: time after one day. T1: time after 6days. T3: time after 12 days.

Table (3): The effect of different temperatures and different concentrations of *Isochrysis gabana* on the ingestion rate (cells/h/larvae) of the eyed-larvae of *C.gigas* at different time intervals.

Time	Conc.		Temp.				LSD	
			25°C	20°C	13°C	Total	Comp.	P-value
T1	100 cells/µl	Mean	3558.400	3340.800	497.333	2465.511	**100 cells/ µl &50 cells/ µl**	0.000
		SD	8.502	6.239	7.024	1479.151	**100 cells/ µl &25 cells/ µl**	0.000
	50 cells/µl	Mean	1845.067	1620.733	237.767	1234.522	**50 cells/ µl &25 cells/ µl**	0.000
		SD	10.621	9.012	4.676	753.887	**25°C&20°C**	0.000
	25 cells/µl	Mean	959.333	898.000	173.333	676.889	**25°C&13°C**	0.000
		SD	4.041	7.550	4.509	378.630	**20°C&13°C**	0.000
	Total	Mean	2120.933	1953.178	302.811	1458.974		
		SD	1144.311	1086.773	148.613	1213.278		
T2	100 cells/µl	Mean	3397.600	2600.200	375.933	2124.578	**100 cells/ µl &50 cells/ µl**	0.000
		SD	5.503	5.632	4.002	1356.182	**100 cells/ µl &25 cells/ µl**	0.000
	50 cells/µl	Mean	1760.367	1478.967	111.433	1116.922	**50 cells/ µl &25 cells/ µl**	0.000
		SD	5.040	7.919	10.304	763.929	**25°C&20°C**	0.000
	25 cells/µl	Mean	955.000	715.667	178.000	616.222	**25°C&13°C**	0.000
		SD	4.359	4.041	19.287	344.766	**20°C&13°C**	0.000
	Total	Mean	2037.656	1598.278	221.789	1285.907		
		SD	1077.937	820.935	119.665	1091.172		
T3	100 cells/µl	Mean	1402.767	925.933	240.667	856.456	**100 cells/ µl &50 cells/ µl**	0.000
		SD	5.645	6.001	4.041	505.916	**100 cells/ µl &25 cells/ µl**	0.000
	50 cells/ml	Mean	926.733	769.600	109.800	602.044	**50 cells/ µl &25 cells/ µl**	0.000
		SD	6.513	6.089	9.702	375.459	**25°C&20°C**	0.000
	25 cells/µl	Mean	645.667	388.000	152.667	395.444	**25°C&13°C**	0.000
		SD	5.132	4.583	2.517	213.580	**20°C&13°C**	0.000
	Total	Mean	991.722	694.511	167.711	617.981		
		SD	331.475	239.692	58.031	416.030		

Table (4): Two way analysis for variance on ingestion rate of eyed- larvae of *C.gigas* fed on *Isochrysis gabana*

ANOVA 2-way	T1		T2		T3	
	F	P-value	F	P-value	F	P-value
Conc.	144320.627	0.000	70343.022	0.000	13866.216	0.000
Temp.	173956.426	0.000	106971.294	0.000	45283.747	0.000
Conc. *Temp.	24044.330	0.000	13827.590	0.000	2926.363	0.000

T1: time after one day. T1: time after 6days. T3: time after 12 days

Table (5): The effect of different temperatures and different concentrations of *Pavlovia lutheri* on the filtration rate(ml/h/larvae) of the eyed-larvae of *C.gigas* at different time intervals.

Time	Conc.	Temp.					LSD	
			25°C	20°C	13°C	Total	Comp.	P-value
T1	100 cells/µl	Mean	0.061	0.037	0.003	0.034	100 cells/ µl &50 cells/ µl	0.000
		SD	0.000	0.001	0.001	0.026	100 cells/ µl &25 cells/ µl	0.000
	50 cells/µl	Mean	0.074	0.047	0.004	0.042	50 cells/ µl &25 cells/ µl	0.000
		SD	0.001	0.001	0.001	0.031	25°C&20°C	0.000
	25 cells/µl	Mean	0.085	0.066	0.002	0.051	25°C&13°C	0.000
		SD	0.001	0.001	0.000	0.037	20°C&13°C	0.000
	Total	Mean	0.073	0.050	0.003	0.042		
		SD	0.010	0.013	0.001	0.031		
T2	100 cells/µl	Mean	0.055	0.025	0.002	0.028	100 cells/ µl &50 cells/ µl	0.000
		SD	0.001	0.002	0.001	0.023	100 cells/ µl &25 cells/ µl	0.000
	50 cells/µl	Mean	0.056	0.036	0.003	0.032	50 cells/ µl &25 cells/ µl	0.002
		SD	0.001	0.001	0.001	0.023	25°C&20°C	0.000
	25 cells/µl	Mean	0.069	0.029	0.002	0.033	25°C&13°C	0.000
		SD	0.001	0.001	0.001	0.029	20°C&13°C	0.000
	Total	Mean	0.060	0.030	0.002	0.031		
		SD	0.006	0.005	0.001	0.024		
T3	100 cells/µl	Mean	0.013	0.008	0.003	0.008	100 cells/ µl &50 cells/ µl	0.000
		SD	0.001	0.001	0.001	0.004	100 cells/ µl &25 cells/ µl	0.000
	50 cells/µl	Mean	0.019	0.009	0.003	0.010	50 cells/ µl &25 cells/ µl	0.000
		SD	0.001	0.001	0.001	0.007	25°C&20°C	0.000
	25 cells/µl	Mean	0.030	0.009	0.002	0.014	25°C&13°C	0.000
		SD	0.001	0.001	0.002	0.012	20°C&13°C	0.000
	Total	Mean	0.020	0.009	0.003	0.011		
		SD	0.007	0.001	0.001	0.009		

Table (6): Two way analysis for variance on filtration rate of eyed- larvae of *C.gigas* fed on *Pavlovia lutheri*

ANOVA 2-way	T1		T2		T3	
	F	P-value	F	P-value	F	P-value
Conc.	1204.619	0.000	159.124	0.000	167.163	0.000
Temp.	20680.027	0.000	16166.796	0.000	1645.347	0.000
Conc. *Temp.	341.999	0.000	196.341	0.000	170.796	0.000

T1: time after one day. T1: time after 6days. T3: time after 12 days

4. Discussion

The comparison of the previous record filtration rates of many bivalve larvae with the present ones reveals that much of the existing data fall in the range of filtration rates determined in the present work. According to the present study it was found that, the colorimetric measurements for the initial and the final concentrations of the suspension tested by Ali (1970) were not exactly valid because the pseudofaeces rejected by the examined animals still have their own color and of course this will mislead to wrong reading. Although cell diameter of *P.lutheri* (6um) was larger

than that of *I.galbana* (3um) (Bayne 1965), the filtration and the ingestion rates that recorded by using *I.galbana* were larger than that of *P.lutheri*. These results were in agreement with that of Débora *et al*., (2009) who stated that, the differences in filtration rates of *Limnoperna fortunei* between the different types of food used (Algamac-2000® and *Scenedesmus sp*.) could be accounted for by the differences in algal cell size . Baker *et al*.,(1998) and Vanderploeg *et al*.,(2001) detected shifts in local phytoplanktonic communities following the introduction of the zebra mussel (*Dreissena. polymoroha*). The mechanism underlying those shifts seemed to be the selective removal of particles of specific sizes; with the rejected particles being returned to the water column in the form of unconsolidated pseudofaeces.

Moreover, This study also reported that the larvae of *C. gigas* regulate filtration rates according to the particle concentration in the surrounding medium, filtration was more actively in lower concentrations (25 x 10^6 L^{-1}*I . galbana*) than at the higher ones (100 x 10^6 L^{-1}*I galbana*). Similar data were recorded by Gerdes (1983) in relation to *C. gigas*. While ,Rajesh *et al*.(2001) reported that the filtration rate of *Perna viridis* increased with increasing algal cell concentration until 10^5 cells.ml^{-1}, after which there was a rapid decline.

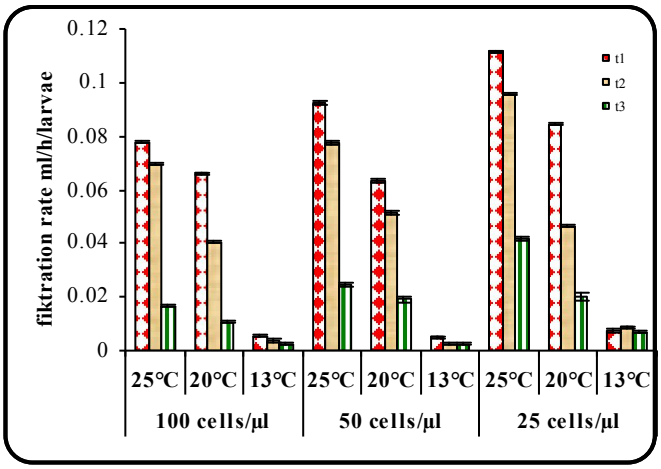
Table (7): The effect of different temperatures and different concentrations of *Pavlovia lutheri* on the ingestion rate(cells/h/larvae) of the eyed-larvae of *C.gigas*.

Time	Conc.		Temp.				LSD	
			25°C	20°C	13°C	Total	Comp.	P-value
T1	100 cells/ µl	Mean	3203.667	2427.333	267.333	1966.111	100 cells/ µl&50 cells/ µl	0.000
		SD	6.028	7.506	5.686	1317.697	100 cells/ µl &25 cells/ µl	0.000
	50 cells/ µl	Mean	1748.333	1434.333	280.000	1154.222	50 cells/ µl &25 cells/ µl	0.000
		SD	7.024	5.132	7.000	669.639	25°C&20°C	0.000
	25 cells/ µl	Mean	925.000	845.000	60.000	610.000	25°C&13°C	0.000
		SD	5.568	6.557	3.000	413.977	20°C&13°C	0.000
	Total	Mean	1959.000	1568.889	202.444	1243.444		
		SD	999.276	692.585	107.080	1023.420		
T2	100 cells/ µl	Mean	3074.000	1892.333	266.000	1744.111	100 cells/ µl &50 cells/ µl	0.000
		SD	8.718	7.024	5.292	1220.987	100 cells/ µl &25 cells/ µl	0.000
	50 cells/ µl	Mean	1564.667	1225.000	153.000	980.889	50 cells/ µl &25 cells/ µl	0.000
		SD	5.132	5.568	6.000	638.117	25°C&20°C	0.000
	25 cells/ µl	Mean	847.667	527.667	60.000	478.444	25°C&13°C	0.000
		SD	7.024	8.021	3.000	343.106	20°C&13°C	0.000
	Total	Mean	1828.778	1215.000	159.667	1067.815		
		SD	984.190	590.996	89.443	949.421		
T3	100 cells/ µl	Mean	1093.000	766.667	300.333	720.000	100 cells/ µl &50 cells/ µl	0.000
		SD	6.000	7.095	5.033	345.055	100 cells/ µl &25 cells/ µl	0.000
	50 cells/ µl	Mean	775.000	448.000	129.667	450.889	50 cells/ µl &25 cells/ µl	0.000
		SD	5.568	6.000	2.517	279.479	25°C&20°C	0.000
	25 cells/ µl	Mean	541.667	172.333	86.667	266.889	25°C&13°C	0.000
		SD	8.505	62.740	7.024	211.804	20°C&13°C	0.000
	Total	Mean	803.222	462.333	172.222	479.259		
		SD	239.743	259.523	97.974	332.301		

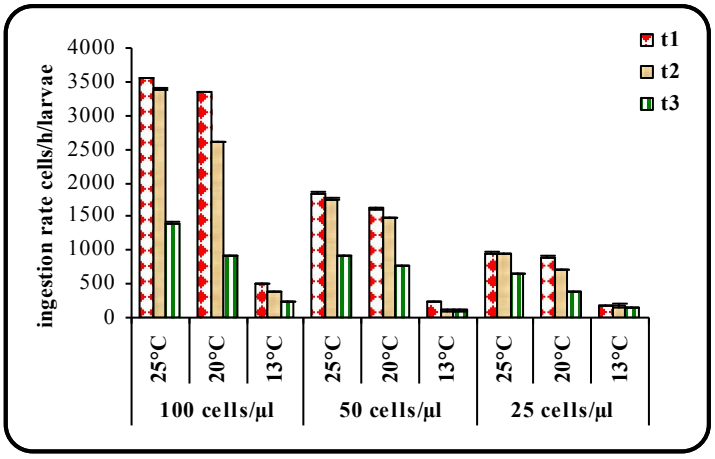
Table (8): Two way analysis for variance on ingestion rate of eyed- larvae of *C.gigas* fed on *Pavlovia lutheri*

ANOVA 2-way	T1		T2		T3	
	F	P-value	F	P-value	F	P-value
Conc.	113399.137	0.000	89073.342	0.000	991.378	0.000
Temp.	207160.524	0.000	156311.852	0.000	1904.352	0.000
Conc. *Temp.	23900.597	0.000	20078.818	0.000	70.093	0.000

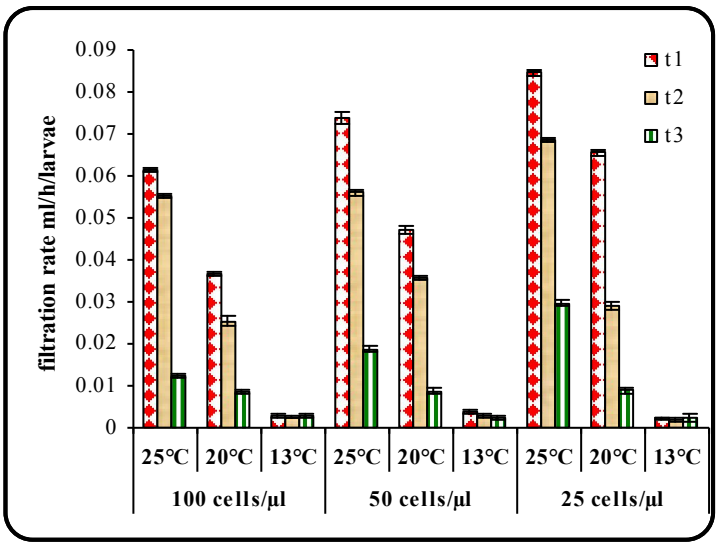
T1: time after one day. T1: time after 6days. T3: time after 12 days



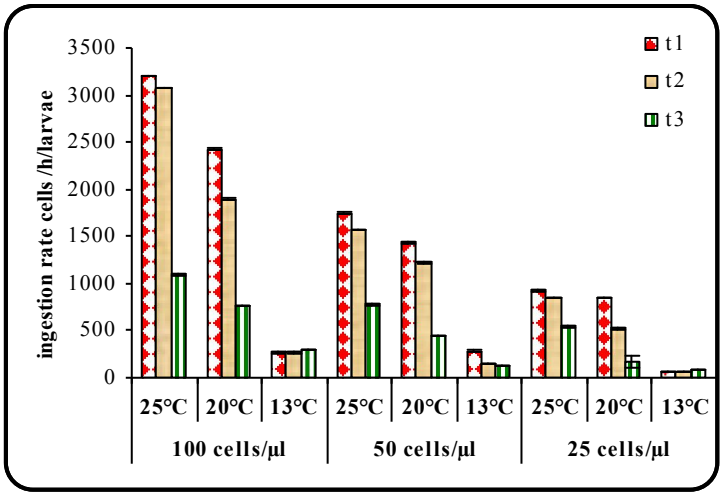
Fig(1): Mean ±SD of filtration rate of eyed larvae of *C.gigas* under the effect of temperature and food concentrations of *I .galbana* at one day (t1),six days (t2) and twelve days(t3).



Fig(2): Mean ±SD of ingestion rate of eyed larvae of *C.gigas* under the effect of temperature and food concentrations of *I .galbana* at one day (t1),six days (t2) and twelve days(t3).



Fig(3): Mean ±SD of filtration rate of eyed larvae of *C.gigas* under the effect of temperature and food concentrations of *P. lutheri* at one day (t1),six days (t2) and twelve days(t3).



Fig(4): Mean ±SD of ingestion rate of eyed larvae of *C.gigas* under the effect of temperature and food concentrations of *P. lutheri* at one day (t1),six days (t2) and twelve days(t3).

Many investigators elucidated the reduction rate on the basis of one of the following explanations: 1) the presence of the threshold concerning with the number of cells in suspension below which the oysters can not remove a maximum ration (Epifanio and Ewart, 1977), 2) the highly concentrated suspensions by which the gills of oysters became heavily covered with a mass of particles which would be rejected and removed as psuedofaeces (Ali, 1970), 3) the presence of inhibitory substances in the culture medium (Davids, 1964) and 4) the trying of the animals for adapting to food concentration to be typical of that of natural environments (Tenore and Dunstan, 1973). Horgan and Mills (1997) indicated that there was a positive correlation between the amount of the food available and filtration rates.

The present investigations showed that, for each *I. galbana* and *P. lutheri* the filtration rates were direct proportional with the ingestion rates. By increasing the algal cell concentration, the ingestion rates decreased. Strychar and Macdonald (1999) reported that in *Crassostrea virginica* ingestion was regulated as the concentration of particles increased both by producing pseudofaeces and reducing clearance rates even at low particle concentrations. Pseudofaeces production is an important mechanism

to regulate ingestion and has typically been shown to increase with elevated seston concentrations in most of the bivalves studied.

The result of herein experiment showed, the filtration and ingestion rates of eyed larvae of *C. gigas* increased by the increase in temperature degrees. The filtration rate was increased by increasing the temperature up to 15 ºc (Winter, 1969) and up to 15 ºc – 17 ºc (Theede, 1963, Ali, 1970). Working on *R. philippinarum*, Goulletquer *et al.* (1989) found that filtration of *R. philippinarum* was nearly constant between 12 and 20° C. Similarly, working on *R. philippinarum* and *T. decussatus*, Defossez and Daguzan (1995) showed no significant changes in pumping rates from 19 to 29 °C. The data of Gerdes (1983) and the herein data partially agreed with the mentioned published data concerning with algal concentrations but fully contradicted with the data concerning with the maximum temperature where the highest filtration rate and the cells that filtered out occurred at 25°c. Moreover, in the present study, the combination of high food concentration and temperature positively affected on the feeding rates and this of course played an important role in putting an available interpretation to the growth rates in the same conditions.

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Corresponding author

Mona, M.H.

Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt.

monamm3@yahoo.com

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