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EFFECTS OF TEMPERATURE, FOOD AND FOOD CONCENTRATIONS ON THE GROWTH OF THE LARVAE AND SPAT OF THE EDIBLE OYSTER *CRASSOSTREA GIGAS* (THUNBERG)

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ABSTRACT

The individual and the combined effects of temperature, food items and concentrations on the growth of the edible oyster larva and juveniles of *Crassostrea gigas* were studied. The effects of these factors are significantly related only as the limits of tolerance of either factor approached in relation to the veliger larvae. Sharply reduced and gradually increased in the growth rates were recorded in the eyed-larvae and juveniles respectively. In the veligers, the maximum growth rate (8.6 + 0.9 μm/day) was occurred at the combination 25°C and 100 cells/μl of a mixture of *I. galbana* and *P. lutheri* while the minimum one (1.1 + 0.2 μm/day) was observed at the combination of 13°C and 25 Cells/μl of *P. lutheri*. In the eyed-larvae, the maximum growth rate was 3.3 + 0.2 μm/day at the combination of 25°C and 100 cells/μl of the same mixture while the minimum was 0.3 + 0 μm/day at 13°C and 25 cells/μl of either of the two species. Finally the growth rate of the juveniles was maximum (2.4 + 0.1 μm/day) at 25°C and 100 cells/μl of the mixture and minimum (0.5 + 0.2 μm/day) at 25°C and 100 cells/μl of the mixture and minimum (0.5 + 0.2 μm/day) at 13°C and 25 cells of *P. lutheri*.

INTRODUCTION

IN RECENT years laboratory rearing of the shellfish larvae has assumed increasingly importance from both scientific and practical points of views and these potentialities were clearly presented by Loosanoff (1954, 1955). The biology of the larval molluscs have been studied since several years.

Many investigators studied the effects of different parameters independently on the growth rates of many bivalve larvae. The effects of food items and food concentrations on the growth of certain bivalve larvae have been investigated (Bruce *et al.*, 1940 ; Loosanoff, 1953 ; Walne, 1956, 1963, 1964, 1965). The effect of temperature on the growth rates was also assessed (Loosanoff *et al.*, 1951 ; Bayne, 1965 ; Malouf and Breese, 1977). While Nell and Holliday (1988) observed the effects of salinity. On the other hand certain investigations have been conducted on the combined effects of two or more environmental factors on adult marine animals, particularly molluscs. The combined effects of temperature and salinity was the important combination examined in detail (Medcof and Needler, 1941 ; Costlow *et al.*, 1960, 1962 ; Kinne and Kinne, 1962 ; Cain, 1973 ; Lough, 1974 ; 1975 ; Tettelbach and Rhodes, 1981). Brenko and Calabrese (1969) have studied the combined effects of temperature and salinity on the larvae of marine bivalves. MacInnes and Calabrese (1979) documented the effect of copper, as a

heavy metal, in combination with salinity on the growth of the larvae of the American oyster *Crassostrea virginica*. Robert *et al.* (1988) added the nutrition factor to the temperature and the salinity to study their effect, as a whole, on the growth of *Ostrea edulis*.

Although, similar work on Pacific oyster *Crassostrea gigas* has been undertaken in limited way by some investigators (Hori and Kusakabe, 1926; Imai and Hatanaka, 1949; Chan, 1950; Davis, 1950; Imai *et al.*, 1950 a, 1950 b; Loosanoff and Davis, 1963; Helm and Millican, 1977; Malouf and Breese, 1977; Gerdes, 1983), no detailed accounts have been given on its growth as a function of temperature and food requirements (food items and food concentrations) independently or in combination.

Therefore, the present study was planned to investigate the interaction of the individual and the combined effects of temperature, food item and food concentration on the growth rate of different stages of the larval period of *Crassostrea gigas* to clear up some of the existing gap in knowledge in this aspect and to stimulate further investigations on the culture of molluscs in Egypt in future firstly by giving a better understanding of the culture requirements of the larvae of this common edible oyster and secondly by finding positive growth relationship between the fastest growing and the earliest settling larvae, and the fastest growing juvenile oyster which will provide the oyster producers with several advantages.

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Material and Methods

Experimental animal

Experiments were conducted with the larvae of Pacific oyster *Crassostrea gigas* Thunberg. These larvae were classified according to their age into young larvae or veligers (150-250 μm), fully developed and competent larvae (285-215 μm) and post-metamorphosis larvae or juveniles (320-600 μm and more). The larvae were reared throughout metamorphosis according to the technique adopted by Loosanoff and Davis (1963) and Helm and Millican (1977).

Feeding

For all experiments, pure unicellular algae *Isochrysis galbana* Park and *Pavlova lutheri* Droop were used. These algae were grown in f/2 medium (Guillard and Ryther, 1962) and centrifuged from the culture medium and then suspended again in freshly prepared sea water, density was determined by means of a blood-cell counting chamber (thoma, 1 mm depth). Four levels of food concentrations were used throughout the growth rate experiments : 100, 50 and 25 cells/μl of the culture media besides zero cell/μl level which was used as a control.

Temperature and salinity

From available literature concerned with such subject (Stickney, 1964; Calabrese, 1969; Davis and Calabrese, 1969) three temperature degrees (25°C; 20°C and 13°C) were chosen to conduct all the experiments. Vast and large chambers with temperature control devices were selected to give the required temperature, one chamber for each degree. Only one salinity degree (28‰) was chosen to represent the optimum salinity for rearing the larvae of *Crassostrea gigas* (Gerdes, 1983).

Examination

A dissecting microscope supported by calibrated ocular micrometer was used through

all stages of experiments to measure the growth by measuring the maximum length (μm). All measurements were determined at high magnification (50 × objective × 10 × ocular).

Calculation

Growth rate (μm/day) was calculated by subtracting the initial length (L1) in μm from the final length (L2) μm after 12 days according to the following simple equation :

$$\text{Growth rate} = \frac{L2\text{-}L1}{t}\ \mu\text{m/day}$$

The significance of the differences between growth rates was used by an analysis of the variance (ANOVA) within and between the samples at different time intervals, different food concentrations and different temperatures for each larval period. Apple Mac II computer was used to compute the ANOVA by using ' Stat View 512 Statistical Program '.

Scanning Electron Microscopy Fixation : Samples for SEM were fixed in the parducz, s. instant fixative (Parducz, 1967) which composed of 6 parts OsO_4 (2%) and 1 part of salt $HgCl_2$.

Dehydration : The samples were dehydrated through an ethyl alcohol series.

Critical point drying (CPD) : Samples were critical point dried using Samdri Critical Point Dryer in which the CO_2 was used as a transitional fluid.

Coating : The samples were coated with palladium-gold in evaporative coater with a rotating planetary stage.

Results

Veliger larvae

Table 1 shows that the combination of 25°C with 100 cells/ μ l of any used algae has a noticeable effect on the growth rates of the veligers, but the type of the algae was distinguishable where the mixture (1 : 1) of *Isochrysis galbana* and *Pavlova lutheri* achieved the best growth rate (8.6 + 9.9 μm/day.)

At all temperature the growth rates resultee in using *I. galbana* and *P. lutheri* alone werd similar at all concentrations.

Generally, the food concentration was remarkably significant on the growth rates at all temperatures and food items, the lowest growth rate (1.1 + 0.2 μ m/day) resulted in the combined effect of 13°C, 25 cells / μ l of *P. luthri*. At each food concentration, the temperature interferred to change the value of the growth rate according to the value of the used temperature. The growth rates were maximum at 25°C then decreased by reducing the temperature to 20°C and stabilized (4.5 μ m/day) when the temperature reached 13°C whatever the kind of the food item.

At 13°C, the growth rates greatly relie on the food concentration. They were 4.2+0.4, 2.9 + 0.3 and 1.1 + 0.2 μ m/day at the concentrations 100, 50 and 52 cells/μl respectively. It must be noted that the growth rate at zero cell/μ l (control) and all temperature degree was 0.3 + 0.1 μ m/day *i.e.*, the veligers still grew even at 13°C if a reasonable value of food was occurred (in this study it was 25 cells/ μ l).

Eyed-larvae

A trend is seen in Table 1 indicating that the effect of different treatments on the rate of growth of the late veligers (eyed-larvae) was greatly different from that recorded with the early veligers.

From the first sight, it is clear that the growth rates with all combinations moved with slower velocity than that of veligers. The highest growth rate (3.3 + 0.2 μ m/day) was observed at the combination of 25°C, 100 cells/ μ l of the mixture.

At 13°C, the growth rate did not change whatever the food concentrations and the food items (*i.e.* the eyed-larvae differed from veligers in their failure in distinguishing between the kind of food). The growth rate here fluctuated near the control zone (0.3 + 0.1 μ m/day).

centration, while it was changed only when the food concentration raised into 100 cells/ μ l in case of using 20°C and 25°C. Table 1 indicates that the highest growth rate (2.4 + 0.1 μ m/day) resulted as a combination of 25°C and 100 cells/ μ l of a mixture of *I. galbana* and *P. lutheri*.

TABLE 1. *The effect of different concentrations (cells/μl) of three food items on the growth rates (μm/day) of the larvae and spat of* C. gigas *at different temperatures*

Food conc. (cells/μl)	Average Growth Rates (μm/day)								
	I. galbana & *P. lutheri*			*I. galbana*			*P. lutheri*		
	25°C	20°C	13°C	25°C	20°C	13°C	25°C	20°C	13°C
100	8.6+0.9	6.8+0.3	4.6+0.3	6.5+0.3	5.3+0.2	4.5+0.4	6.1+0.3	5.0+0.1	4.2+0.4
50	5.3+0.7	4.3+0.5	3.2+0.2	4.2+0.1	4.5+0.5	3.1+0.2	4.3+0.5	4.2+0.2	2.9+0.3
25	3.4+0.3	3.4+0.4	1.9+0.2	2.9+0.4	2.0+0.2	1.1+0.2	1.7+0.3	2.0+0.2	1.1+0.2
100	3.3+0.2	2.0+0.2	0.6+0.1	1.8+0.1	1.2+0.1	0.4+0.1	1.4+0.1	0.9+0.1	0.4+0.1
50	1.6+0.2	1.3+0.1	0.4+0.1	1.2+0.1	0.8+0.1	0.3+0.1	1.1+0.1	0.8+0.1	0.3+0.0
25	0.9+0.1	0.8+0.1	0.5+0.1	0.7+0.1	0.6+0.1	0.3+0.1	0.5+0.1	0.5+0.1	0.3+0.0
100	2.4+0.1	2.5+0.1	0.9+0.1	3.1+0.1	2.5+0.2	0.4+0.1	2.1+0.2	2.6+0.2	0.7+0.1
50	2.1+0.2	1.5+0.1	0.7+0.1	1.4+0.2	1.9+0.2	0.5+0.1	1.3+0.1	1.3+0.1	0.5+0.1
25	1.7+0.2	1.2+0.1	0.5+0.1	1.0+0.2	1.3+0.2	0.4+0.1	0.9+0.1	1.0+0.2	0.5+0.2

At 25°C and 20°C, it is different a little bit where there was a noticeable differentiation in the growth rates, resulted in the different food concentrations of the mixture only.

Juveniles

The combined effect of temperature and 100 cells/μl was distinguishable, because the resulted growth rate increased progressively as the temperature increased from 13°C to 20°C and 25°C while in 50 and 25 cells/ μl, it was not affected by increasing the temperature from 20°C to 25°C. Meanwhile, the growth rate increased twice by increasing the temperature from 13°C to 20°C. From the other side, the growth rate at 13°C was not affected under the induction of food con-

The effect of the previous ecological factors was very highly significant ($P>0.0001$) on the growth of the veligers and the juveniles (Table 2, 4) while their effects on the eyed-larvae (Table 3) changed according to the type of these factors, the effect of either *I. galbana* or *P. lutheri* alone was not significant on the growth rate ($P>0.05$), likewise, the effect of the combination of temperature and time was highly significant ($P=0.0001$) at the mixture, significant ($P<0.005$) at *P. lutheri* and not significant at *I. galbana* ($P>1$). The combination of temperature, food concentration and time was highly significant ($P=0.0001$) at the mixture, significant ($P<.05$) at *I. galbana* and not significant at *P. lutheri* ($P>0.4$).

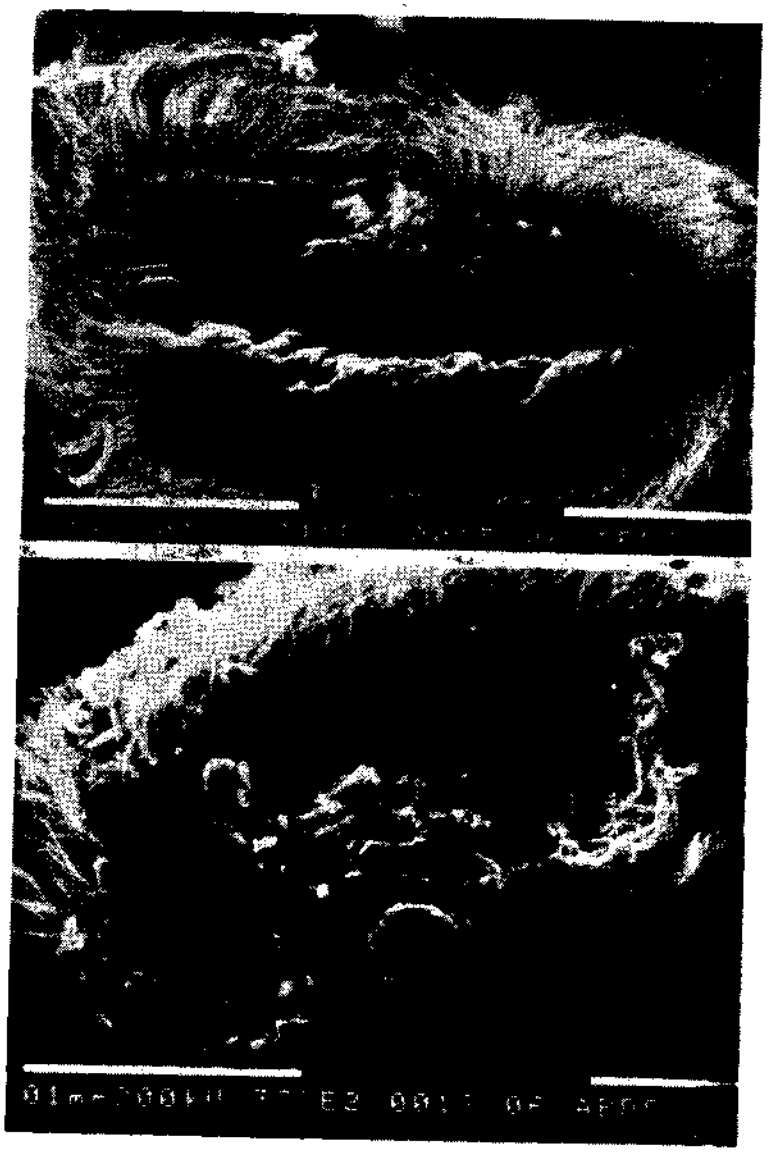

PLATE I. Scanning electron micrographs showing the degradation of the inner ciliary ring responsible for feeding in *C. gigas*.

Discussion

First of all it is worthy to mention that the experiments carried during this work indicated that the larvae of *C. gigas* are capable of tolerating wider range of environmental conditions. Their responses to factors such as temperature, food density and food item closely parallel to those of larvae of Coot clam *Mulinia lateralis* reviewed in Calabrese (1969) and *Mytilus edulis* in Bayne (1965). This study also demonstrates the importance of controlling environmental conditions during different larvae periods. The selection of temperatures did not come randomly, but they were picked from the literature according to their biological importance. Walne (1965) suggested 'a biological zero temperature of 13°C, at which no growth should occur in the larvae of *Ostrea edulis* from his determination on 24-hour growth rates and he concluded 'In the pelagic phase the time taken to grow from 175-250 μm

Table 2. *Three-way ANOVA Table for growth rate between the veligers of* C. gigas *fed on three food items, three concentration levels for each, under the effect of three temperature degrees and at three time intervals*

Source		df	Sum of squares	Mean square	F-test	P value
Concentration (A)	..	2	308.36	154.18	487.766	.0001
Temperature (B)	..	2	96.474	48.237	152.603	.0001
AB	..	4	5.226	655.605	4.133	.0035
Time (C)	..	2	414.466	207.233	655.605	.0001
AC	..	4	114.103	28.526	90.245	.0001
BC	..	4	83.752	20.938	66.24	.0001
ABC	..	8	44.961	5.62	17.78	.0001
Error	..	132	41.724	.316		

Table 3. *Three-way ANOVA Table for growth rate between the eyed-larvae of* C. gigas *fed on three food items, three concentration levels for each under the effect of three temperature degrees and at the three time intervals*

Source		df	Sum of squares	Mean square	F-test	P value
Concentration (A)	..	2	9.431	4.716	110.71	.0001
Temperature (B)	..	2	20.778	10.389	243.915	.0001
AB	..	4	3.507	.877	20.582	.0001
Time (C)	..	2	.046	.023	.54	.5840
AC	..	4	.208	.052	1.222	.3044
BC	..	4	.304	.076	1.782	.1361
ABC	..	8	.871	.109	2.555	.0126
Error	..	135	5.75	.043		

decreases from 14 days at 17°C to 5 days at 25°C. Similar experiments have been conducted on different larval period of *C. gigas* to see if similar data will be repeated or not. The present data have been shown that the growth of larvae was achieved even at 13°C and ofcourse there is no agreement between them and those obtained by Walne (1965). Davis and Calabrese (1969) reported that the growth of the larvae of *Ostrea edulis* was satisfactory (70% or more of maximum) only within the range from 17.5°C to 30°C; at temperature above and below this range growth rates were to slow to be practical for swimming for more than 30 days, but they did not feed and there were no growth after the first three days. Davis (1953) and Davis and Guillard (1958) concluded that the presence and thickness of cell walls and degree of toxicity of metabolites are important factors in determining usability of photosynthetic microorganisms as the larvae of American oyster food. They also showed that the naked flagellates *I. galbana* and *P. lutheri* were approximately equal value as food and induce more rapid growth than any other tested species. Davis also believed that *I. galbana* and *P. lutheri* produced little, if any,

TABLE 4. *Three-way ANOVA Table for growth rate between the juveniles of* C. gigas *fed on three food items three concentration levels for each under the effect of three temperature degrees and three time intervals*

Source		df	Sum of squares	Mean square	F-test	P-value
Concentration (A)	..	2	15.696	7.848	29.355	.0001
Temperature (B)	..	2	53.06	26.53	99.236	.0001
AB	..	4	22.574	5.643	21.109	.0001
Time (C)	..	2	8.646	4.323	16.17	.0001
AC	..	4	13.047	3.262	12.201	.0001
BC	..	4	38.976	9.744	36.447	.0001
ABC	..	8	17.59	2.199	8.224	.0001
Error	..	135	36.091	.267		

shellfish. Ukeles (1961) demonstrated that temperatures of 27°C or higher destroyed the cells of *Isochrysis galbana* and *Monochrysis lutheri* (which was corrected by Droop to be *Pavlova luthert*). Upon this theory, the 25°C was selected here as a maximum temperature to avoid the expected destroying of the algae resulted from high temperature. Davis and Calabrese (1964) suggested that failure of bivalue larvae to grow at low temperature appeared to be caused by their in ability to digest available food. Bayne (1965) carried out experiments at temperature from 5°C to 22°C. He found that the larvae remained toxic external metabolities which unfavourably affected the larvae. Loosanoff and Davis (1963) found that the food value of microorganisms also depended, in part, upon how completely they meet the food requirements of larvae. The result of this study agreed with that of Loosanoff and Davis (1963), where the mixture of *I. galbana* and *P. lutheri* indiced more rapid growth of both clam and oyster larvae than did equal quantities of any of these alone. The same finding was also observed by Bayne (1965) who documented that when an equal mixture of *I. galbana* and *Monochrysis lutheri* was introduced to the

larvae of *Mytilus edulis*, their growth will be larger than using either of them alone. High magnification scanning electron micrograph of the velum of the late veligers (eyed-larvae) taken during this study confirmed the results that obtained by Bayne (1965) where the growth rate of these larvae reduced to the minimum value (sometimes nearly to zero). As a matter of fact, the scanning electron micrographs showed the degeneration of the inner ciliary ring responsible for feeding (Pl. I).

In this study, the gap resulted in the lack of the literature that discuss the effect of the combination of the ecological conditions with the food on the growth rate of the oyster larvae has been filled. Only one work (Robert *et al.*, 1988) has been already published, dealt with the growth rate of *Ostrea edulis*. Nevertheless, certain literature discussed the combined effect of combinations were referred in other place in this paper.

Unfortunately, there is little information on the growth rate in relation to the body size beyond the size 500 μm. Thus it is strongly recommended to conduct many experiments in the future to fill this gap.

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