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Optimum growth conditions of three isolated diatom species; *Skelatonema costatum, Chaetoceros calcitrans* and *Detonulla confervacea* and their utilization as feed for marine penaeid shrimp larvae

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Abstract

Microalgae are playing a very important role as live food for fish and shrimp larvae in marine hatcheries. In this study; three diatom species; *Skelatonema Costatum* (2-5 µm), *Chaetoceros calcitrans* (2-3 µm) and *Detonulla confervacea* (6-15 µm) were isolated from the Eastern Harbor of Alexandria, Egypt and evaluated as feed for shrimp larvae. Scanning electron microscope was used to support the identification process of the isolated diatom species. Effects of salinity, temperature, illumination and culture medium on growth of the three diatom species were conducted. The optimum salinity for highest growth rate of *S. costatum*, *C. calcitrans* and *D. confervacea* was achieved at 35 ppt, 30 ppt and 35 ppt, respectively; optimum temperature was achieved at 20ºC, 25ºC and 20ºC respectively; and optimum illumination (light duration and light intensity) was 24 h Light / 750 Lux for all species. *C. calcitrans* showed the highest growth rate with a modification of vitamin amount (Di- F/2 standard vitamin) and silicate amount (Tri- F/2 standard silicate). Mass culture in outdoor tanks for the three species was conducted at high temperature (27-34ºC) in summer season. *D. confervacea* showed the highest growth rate compared with other diatom species. The three diatoms were evaluated as feed for marine shrimp larvae *Penaeus japonicus* from the first protozoa stage to the first postlarvae stage. *D. confervacea* resulted in an equal survival, development and metamorphosis of penaeid shrimp larvae *Penaeus japonicus*, when compared with other diatoms. These results suggest that *D. confervacea* can be used as feed for marine shrimp larvae *Penaeus japonicus*, especially at high temperaturein summer season.

Keywords: Microalgae, marine shrimp larvae, *Skelatonema costatum, Chaetoceros calcitrans, Detonulla confervacea*

1. Introduction

Microalgae are playing a very important role as live food for fish and shrimp larvae in marine hatcheries. Not all algal species are equally useful in supporting the growth and survival of fish and shrimp larvae. Suitable algal species have been selected based on their mass-culture potential, cell size, digestibility, and overall food value for the feeding animal. However, out of the 80,000 species of microalgae, only 50-60 species are commercially important as live food and cultured as pure strains in intensive systems. There are several isolation methods techniques for isolation of microalgae and diatoms; serial dilution method (Allen &Nelson, 1952; Droop, 1954, Sournia1971), sicropipette washing technique (Phang and Chu 1999), centrifugation method (Phang and Chu 1999; M. Parvin 2007) and finally agar plating method (Phang and Chu 1999). Isolation of algal species is not simple because of the small cell size and the association with other epiphytic species. At present, most hatcheries produce their own microalgae on site and some of them have developed the process of selling algal concentrate to other hatcheries. Therefore, there are no doubts that isolated important microalgae play an important role in aquaculture development. Growth rate of microalgae is influenced by environmental conditions (S.M. Reneud *et al*., 2002). The main environmental conditions controlling microalgae growth rate are light, nutrients and temperature (Tzovenis *et al*., 1997; Zhu *et al*., 1997) but other conditions can be important to some species such as salinity (Chu *et al*., 1996). Silicate is specifically used for the growth of diatoms that utilize this compound for production of an external shell. For outdoor mass culture, it is important to choose a species of algae that tolerate the range of temperature likely to prevail at the site (Wendy and Kevan 1991). On the other hand, especially in hot season, these selected species must be give a high growth tare and a

high final yield. Payer *et al*. (1980) investigated the direct and indirect effect of temperature on a number of species and strains of algae in order to select those that would be suitable for hatcheries productions. In addition to find strain- specific results, they concluded that temperature however can be important in determining which species will predominate in open outdoor culture at local conditions (Goldman and Ryther 1977, Goldman 1979, Goldman and Mann 1980, De Pauw *et al*. 1980 and Witt *et al*. 1981)

Hudinaga (1942) cited that the diatoms are the best food that meets the requirements for penaeid shrimp larvae. The food used in most shrimp hatcheries for these larvae consists of one or more microalgae species supplied at different concentrations and with different feeding routines depending on the shrimp species, on the larval stage and also on the personal experience of the operators in charge of each hatchery (Aguirre-Hinojosa *et al*., 1999). The most common diatoms species used in larviculture of penaeid shrimp are *Chaetoceros calcitrans* and *Skeletonema costatum*. Phytoplankton density of larval rearing water is another interesting point to be discussed. Usually in the larviculture of penaeid shrimp larvae, the recommended plankton density varies with the larval stage, type of diatoms and culture system. Two questions of continuing importance's are; what is the best diatom source and what is the ideal cell concentration of diatom that should be introduced for each stage of penaeid shrimp larval.

The present study was conducted to investigate the effect of different important growth conditions (salinity, temperature, illumination "light duration and light intensity" and nutrients) on growth rate of the three isolated diatoms species. The present study investigated the survival, development and metamorphosis of penaeid shrimp larvae *Penaeus japonicus* that fed on six different cell concentrations of three isolated diatoms. The diatom *D. confervacea* was used for the first time as feed for marine shrimp larval *Penaeus japonicus*.

2. Material and Methods

2.1. Isolation and purification

The experiments of the present study were conducted in Invertebrate Culture Laboratory, National Institute of Oceanography and Fisheries (NIOF), Alexandria Branch. Isolation of diatoms was conducted in spring (March – April 2006) when the average of water temperature is 20± 2ºC and salinity 34 ppt. Scanning electron microscope (SEM- JEOL-JSM5300) was used to identify diatoms strains according to Ismael (1998). Three diatom strains; *Skelatonema costatum* (cell size 2-5 µm), *Chaetoceros calcitrans* (2-3 µm) and *Detonulla confervacea* (6-15µm) were isolated and purified by serial dilution method (Allen & Nelson, 1952; Droop, 1954 and Sournia, 1971) and Micropipette Washing method (Phang and Chu 1999) . Test tubes containing filtered seawater enriched with F/2 standard medium (Guillard and Ryther 1993) were inoculated with 1mL of diluted diatom culture. The colonies were kept to develop for 10 to 15 days under controlled conditions of temperature 20±1ºC and continuous illumination at 750 lux.

2.2. Optimum growth conditions (indoor)

2.2.1. General Growth Conditions

The growth experiments were conducted to investigate the effect of different salinity, temperature, illumination (light intensity and light duration), and the effect of modified F/2 nutrient medium (vitamins and silicate) on relative growth rate (according to Kratz and Myers, 1955) for the three isolated species. Initial cell concentrations of diatoms are shown in Table 1.

All experiments were conducted without aeration in conical flasks 250 ml (three replicates for each treatment) filled with 100 ml of culture medium. Cell count was examined daily by haemocytometer. All experiments ended when the growth rate had reached to the decline phase.

2.2.2. Effect of temperature

Temperature experiment was conducted at: 15°C, 20°C (control), 25°C, and 30°C. Each level was contacted under controlled conditions of salinity 34±1 ppt and continuous illumination at 750 Lux, using F/2 standard nutrients medium (Guillard and Ryther, 1993).

2.2.3. Effect of salinities

Salinity experiment was conducted at: 20 ppt, 25 ppt, 30 ppt and 35 ppt (control). Each level was tested under controlled conditions of temperature 20±1°C and continuous illumination at 750 Lux, using F/2 standard medium (Guillard and Ryther, 1993). Distilled water was used to dilute seawater to reach the three levels of salinities 20 ppt, 25 ppt and 30 ppt.

2.2.4. Effect of illuminations

Illumination was conducted at three levels of light intensities; 750 lux (control), 1500 lux and 3000 Lux at 24 h light duration. As well as, illumination was conducted at three levels of light durations; 6 h light: 18 h dark, 12 h light: 12 h dark and 24 h light: no dark (control), those were at light intensity of 750 lux. Light intensity was measured by Luxymeter. Illumination experiments were conducted by one to six fluorescent lamps 120 W and timer to control the light duration and light intensity. All illumination experiments were conducted under controlled conditions of temperature 20±1°C and salinity 34±1 ppt using F/2 standard medium (Guillard and Ryther 1993).

2.2.5. Effect of modified F/2 Nutrient Medium

The composition of F/2 medium was modified to examine the effect of increasing vitamins and silicate amounts on algal growth rate. Vitamins and silicate were added at 2 folds and 3 folds of the F/2 standard medium concentrations.

2.3. Mass culture (outdoor)

Outdoor mass culture experiment was conducted in nine plastic tanks (one ton capacity, three replicates for each species) filled with 500 L of seawater culture. The mass culture experiment was carried out during July 2007 at high temperature conditions ranged between 27-34°C, salinity 34±1 ppt and sunlight intensity of 25000-23000 Lux, using nutrient medium according to Ton Kay Heok (1982).

2.4. *Penaeus japonicus* methods

The three isolated diatoms species were used as feed for *Penaeus japonicus* larvae from the first zoea stage to the first post larvae stage.

2.4.1. Diatoms Cell Densities

Shrimp larvae were fed on diatoms with different cell densities as shown in Table 2

2.5. Larval rearing Experiment

Penaeus japonicus nauplii were obtained from a single matured female (hatched in Invertebrate Culture Lab., Alex. Branch, NIOF) at the stage of N5. When 90% of the larvae reached to zoea one stage, 450 vigorous zoea were collected and placed in separate containers (triplicates container for each treatment) with initial concentration of 150 larvae/L (Pina *et al.,* 2005) during experiment. Larvae were maintained under controlled conditions of (temperature 27 ± 1ºC, salinity 34 ppt, strong aeration and 1000 Lux / 24h.). Constant algae counts were maintained through all the experiment period. When the larvae move from stage to another (the first appearance of new development stage), samples of 10 – 15 larvae were collected to determine Development index (DI) and Total length (TL). Survival rate (SR) was calculated as a percent between live larvae and the total initial larvae in each treatment. Total length (TL, mm) was measured under a microscope with a calibrated eyepiece. Development index (DI) for larvae was calculated according to Villegas and Kanazawa, 1979 by the following equation:-

$$DI = n^{-1} \text{ SUM } ni$$

Were I = absolute value for each larvae stage (N5=0, Z1 =1 , Z2 =2 , Z3 =3 , M1 =4 , M2 =5 , M 3 =6 , PL1 =7), *ni* = number of larvae of each stage ; *n* = number of larvae in the sample.

3. Results

3.1. Isolations and identification of isolated diatoms

Three diatoms species; *Skelatonema costatum*, *Chaetoceros calcitrans* and *Detonulla converfacea* were isolated from the Eastern Harbor of Alexandria, Egypt (31º 13´ 48" N, 29º 53´ 12" E). Light and scanning electron microscope photos show the three isolated species in Figures (1, 2 and 3).

3.2. Optimum Growth Conditions (Indoor)

3.2.1. Effect of salinity

S. costatum

The highest growth rate was observed at treatment 35 ppt (control), (0.085±0.002 division/day) after three days and the lowest was observed at treatment 25 ppt (0.058±0.002 division/day) after five days, as shown in Figures (4, 5, 6 and 7)

D. confervacea

The highest growth rate was observed at treatment 35 ppt (control), (0.099±0.009 division/day) after twelve days and the lowest was observed at treatment 20 ppt (0.085±0.006 division/day) after four days, respectively, as shown in Figures (8, 9, 10, and 11).

C. calcitrans

The highest growth rate was observed at treatment 30 ppt (0.076±0.002 division/day) after four days and the lowest was observed at treatment 25 ppt (0.070±0.002 division/day) after six, as shown in figures (12, 16, 14 and 15).

3.2.2. Effect of temperature

S. costatum

The highest growth rate was observed at treatment 20ºC (control), (0.085±0.002 division/day) after three days and the lowest was observed at treatment 15ºC (0.058 ±0.002 division/day) after three days, as shown Figures (16, 17, 18 and 19).

D. Converfacea

The highest growth rate was observed at treatment 20ºC (control), (0.099±0.009 division/day) after twelve days and the lowest was observed at treatment 30ºC (0.070±0.006 division/day) after fife days, as shown Figures (20, 21, 22 and 23).

C. calcitrans

The highest growth rate was observed at treatment 25ºC (0.083±0.002 division/day) after three days and the lowest was observed at treatment 30ºC (0.073±0.002 division/day) after two days, as shown Figures (24, 25, 26 and 27).

Table 1: Initial cell concentrations of the isolated diatoms

Species	Cell Diameter (µm)	Cell Concentration (10^4cell/ml)	
		Indoor Experiment	*Outdoor Experiment*
S. costatum	2-5 µm	22 ± 1.4	9.5 ± 0.5
C. calcitrans	2-3 µm	25 ± 1.9	10 ± 0.8
De. confervacea	6~ 15 µm	6 ± 0.7	3.7 ± 0.5

Table 2: Cell concentration of the tested diatoms

Algal cell concentrations	1	2	3	4	5	6
D. confervacea	50000	100000	200000	300000	400000	500000
S. costatum	125000	25000	500000	750000	1000000	1250000
C. calcitrans	125000	25000	500000	750000	1000000	1250000

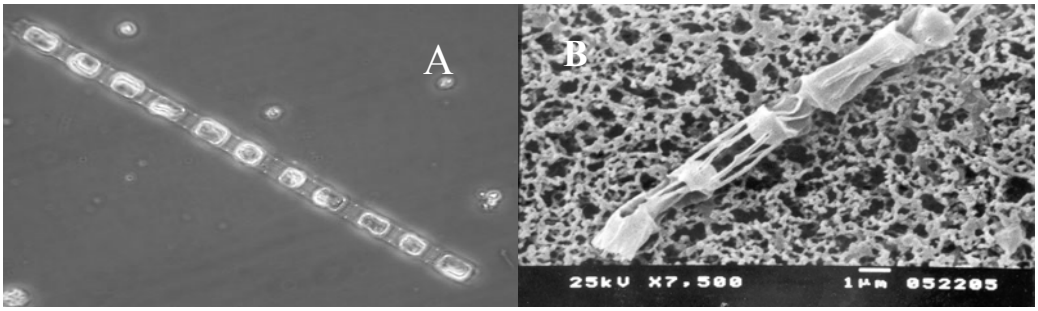

Figure 1: *Skeletonema Costatum* (6-15 µm), light microscope (A) and scanning electron microscope (B).

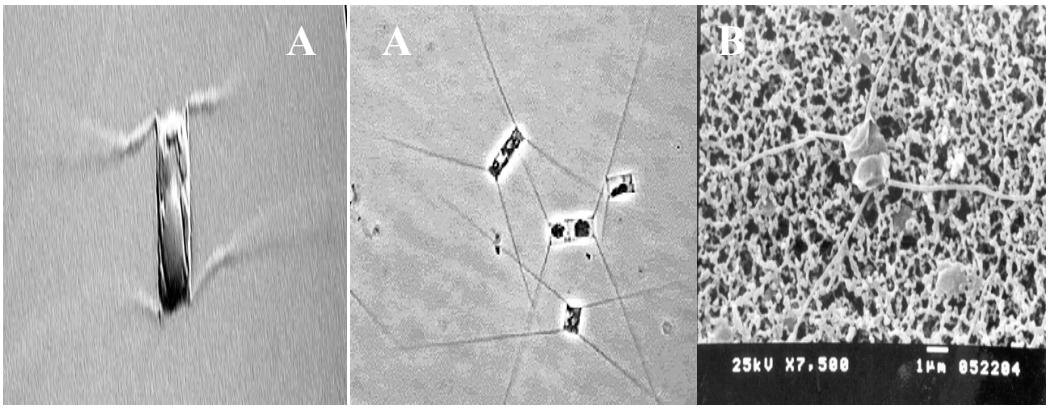

Figure 2: *Chaetoceros calcitrans* (2-5 µm), Light microscope (A) and scanning electron microscope (B).

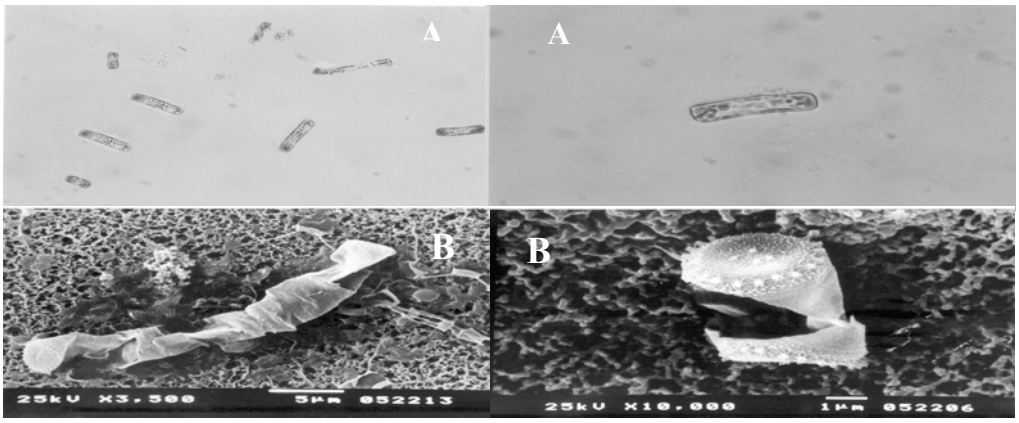

Figure 3: *Detonulla confervacea* (6-15 µm), Light microscope (A) and scanning electron microscope (B).

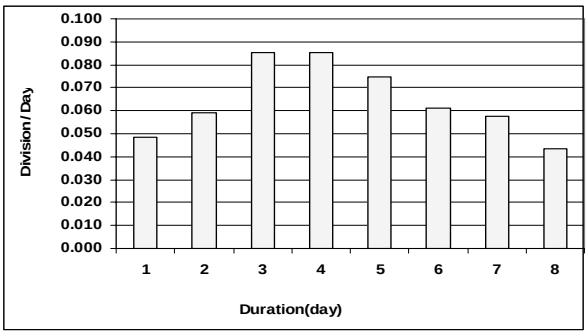

Figure 4: The effect of salinity 35ppt (control) on growth rate of *S. costatum*

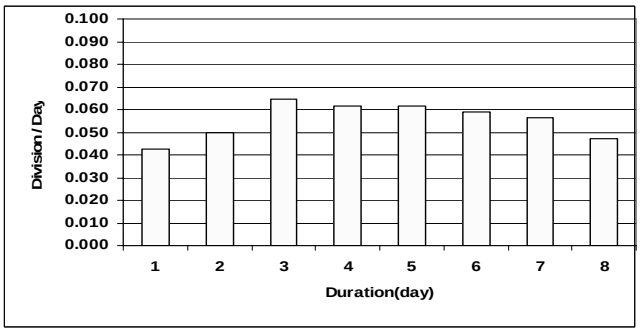

Figure 5: The effect of salinity 30ppt on growth rate of *S. costatum*

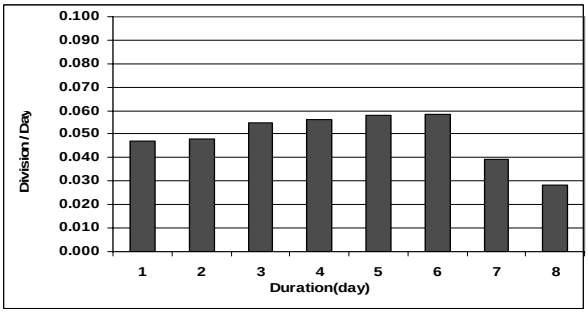

Figure 6: The effect of salinity 25 ppt on growth rate of *S. costatum*

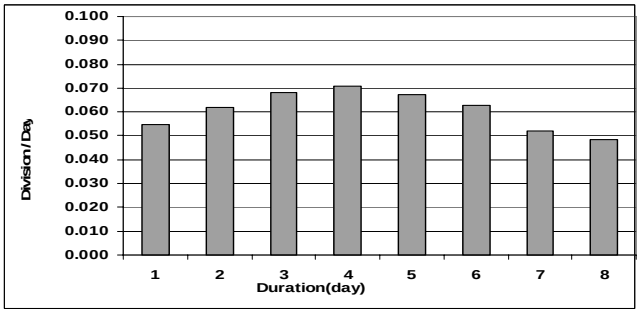

Figure 7: The effect of salinity 20 ppt on growth rate of *S. costatum*

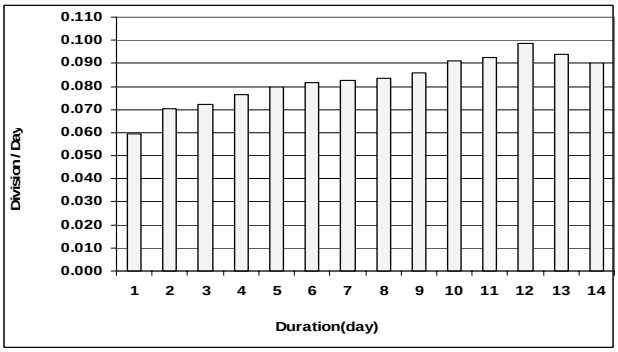

Figure 8: The effect of salinity 35ppt (control) on growth rate of *De. confervacea*

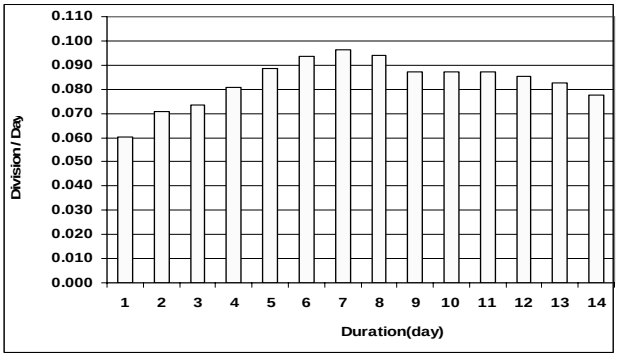

Figure 9: The effect of salinity 30 ppt on growth rate of *De. confervacea*

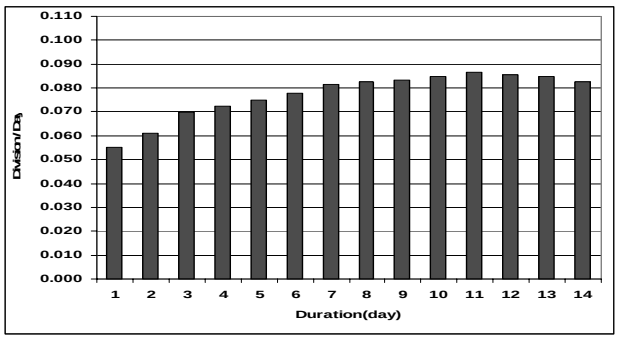

Figure 10: The effect of salinity 25 ppt on growth rate of *De. confervacea*

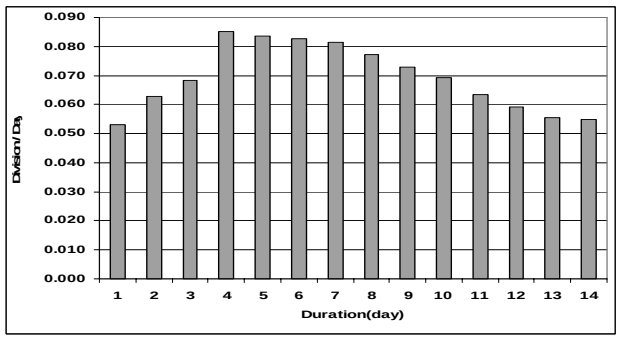

Figure 11: The effect of salinity 20 ppt on growth rate of *De. confervacea*

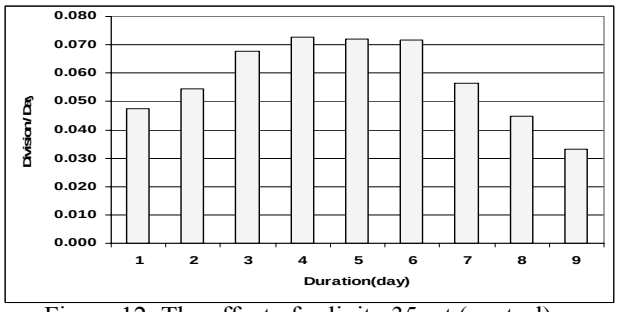

Figure 12: The effect of salinity 35ppt (control) on growth rate of *C. calcitrans*

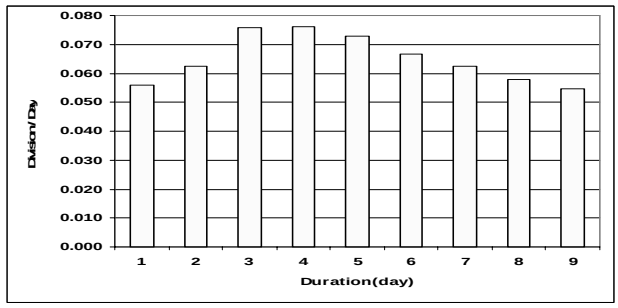

Figure 13: The effect of salinity 30ppt on growth rate of *C. calcitrans*

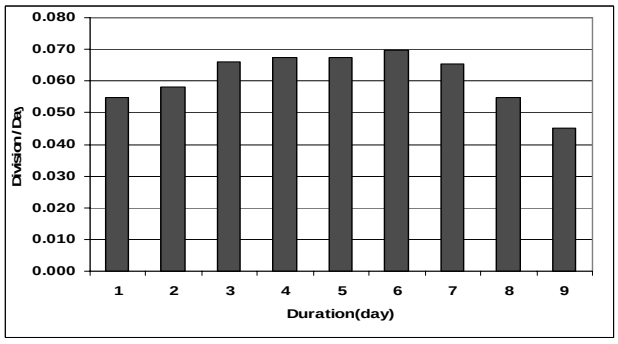

Figure 14: The effect of salinity 25 ppt on growth rate of *C. calcitrans*

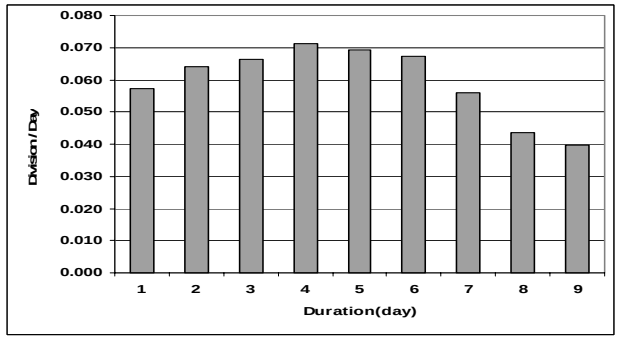

Figure 15: The effect of salinity 20 ppt on growth rate of *C. calcitrans*

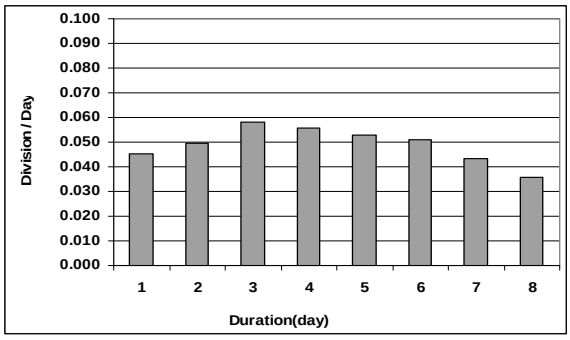

Figure 16: The effect of temperature 15ºC on growth rate of *S .costatum*

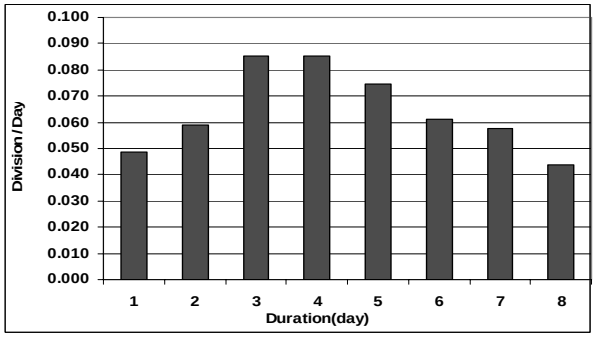

Figure 17: The effect of temperature 20ºC (control) on growth rate of *S .costatum*

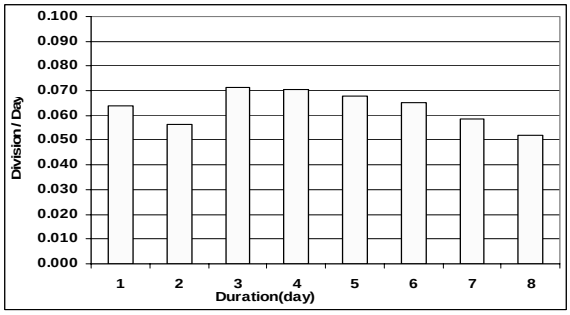

Figure 18: The effect of temperature 25ºC on growth rate of *S .costatum*

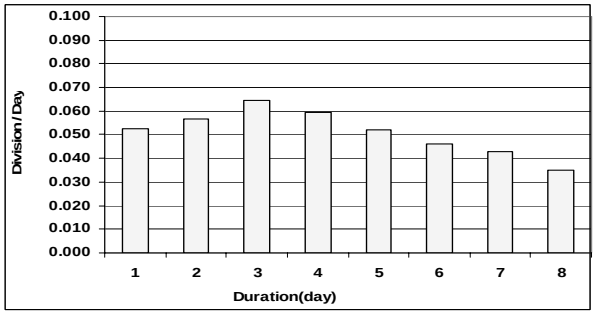

Figure 19: The effect of temperature 30ºC on growth rate of *S .costatum*

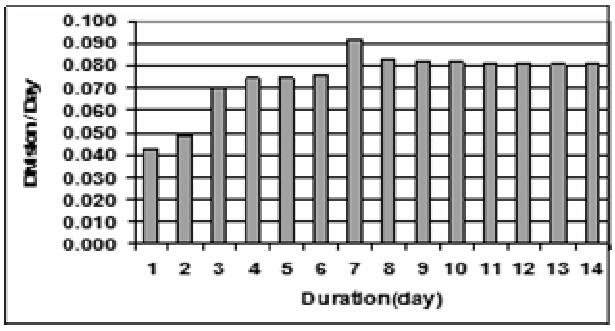

Figure (20): The effect of temperature 15ºC on growth rate of *De. confervacea*

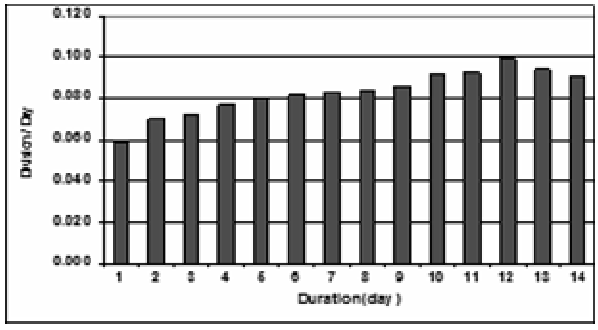

Figure (21): The effect of temperature 20ºC (control) on growth rate of *De. confervacea*

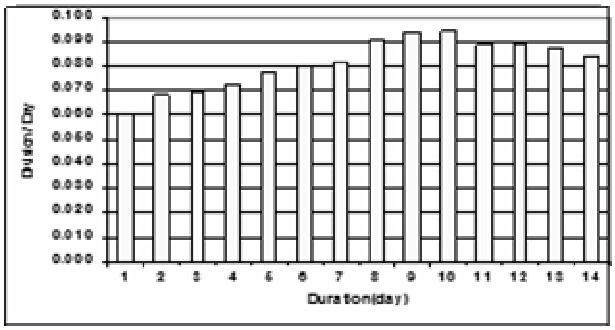

Figure (22): The effect of temperature 25±C on growth rate of *De. confervacea*

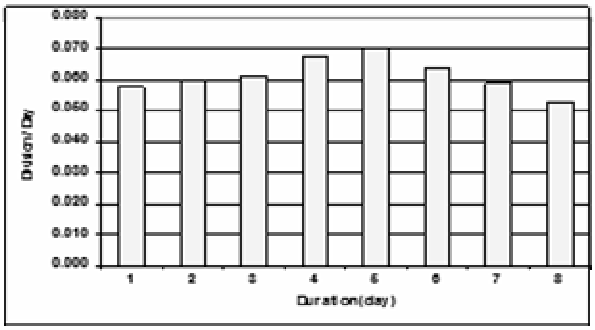

Figure (23): The effect of temperature 30±C on growth rate of *De. confervacea*

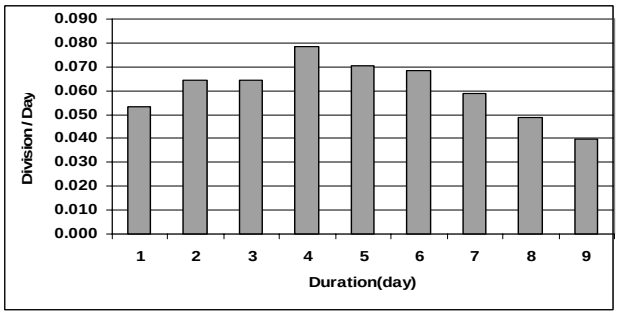

Figure 24: The effect of temperature 15ºC on growth rate of *C. calctirans*

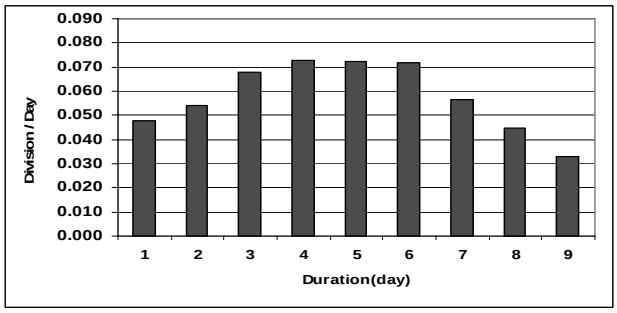

Figure 25: The effect of temperature 20ºC (control) on growth rate of *C. calcitrans*

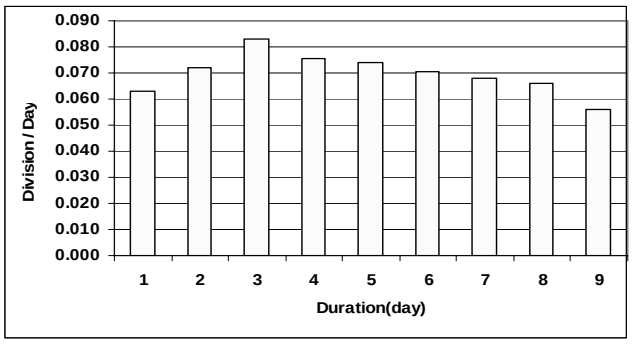

Figure 26: The effect of temperature 25ºC on growth rate of *C. calctirans*

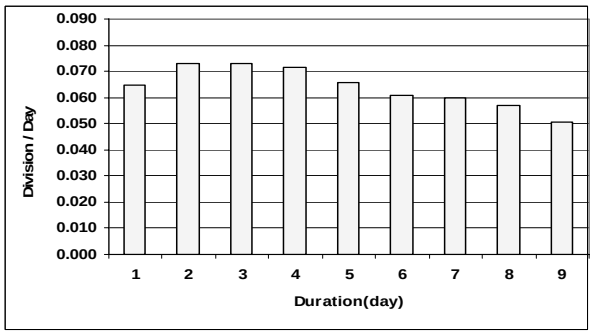

Figure 27: The effect of temperature 30ºC on growth rate of *C. calcitrans*

3.2.3. Effect of illumination

3.2.3.1. Effect of light duration

S. costatum

The highest growth rate was observed at treatment 24h light / 750Lux (control), (0.085±0.002 division/day) after three days and the lowest was observed at treatment 6h light: 18h dark / 750 Lux (0.047±0.001 division/day) after four days, as shown Figures (28, 29 and 30).

D. confervacea

The highest growth rate was observed at treatment 24h light / 750Lux (control), (0.099±0.009 division/day) after twelve days and the lowest was observed at treatment 6h light: 18h dark / 750 Lux (0.055±0.007 division/day) after three days, as shown in Figures (31, 32 and 33).

C. calcitrans

The highest growth rate was observed at treatment 24h light / 750Lux (control), (0.073±0.003 division/day) after four days and the lowest was observed at treatment 6h light:18h dark / 750 Lux (0.060±0.002 division/day) after nine days, as shown in Figures (34, 35 and 36).

3.2.3.2. Effect of light Intensity

S. costatum

The highest growth rate was observed at treatment 750Lux / 24h (control), (0.085±0.002 division/day) after three days and the lowest was observed at

treatment 3000 Lux / 24h (0.061±0.001 division/day) after four days, as shown in Figures (37, 38 and 39).

D. confervacea

The highest growth rate was observed at treatment 750 Lux/ 24h (control), (0.099±0.009 division/day) after twelve days and the lowest was observed at treatment 1500 Lux/ 24h (0.078±0.016 division/day) after four days, as shown in Figures (40, 41 and 42).

C. calcitrans

The highest growth rate was observed at treatment 24h light / 750Lux (control), (0.073±0.003 division/day) after four days and the lowest was observed at treatment 3000 Lux/ 24h (0.063±0.002 division/day) after nine days , as shown in Figures (43, 44 and 45).

3.2.4. Effect of modified F/2 Nutrient Medium

3.2.4.1. Effect of modified F/2 Vitamin Nutrient Medium

S. costatum

The highest growth rate was observed at treatment F/2 standard vitamin (control), (0.085±0.002 division/day) after three days and the lowest was observed at treatment Tri-vitamin (0.064±0.003 division/day) after four days, as shown in Figures (46, 47 and 48).

D. confervacea

The highest growth rate was observed at treatment F/2 standard vitamin (control), (0.099±0.009 division/day) after twelve days and the lowest was observed with addition of Tri-vitamin (0.090± 0.008 division/day) after thirteen days, as shown in Figures (49, 50 and51).

C. calcitrans

The highest growth rate was observed at treatment Di- vitamin (0.079±0.002 division/day) after three days and the lowest was observed at treatment Tri-vitamin (0.073±0.002 division/day) after three days, as shown Figures (52, 53 and 54).

3.2.4.2. Modified F/2 Silicate Nutrient Medium

S. costatum

The highest growth rate was observed at treatment F/2 standard silicate (control), (0.085±0.002 division/day) after three days and the lowest was observed with addition of Tri-silicate (0.075±0.002 division/day) after four days, as shown in Figures (55, 56 and 57).

D. confervacea

The highest growth rate was observed at treatment F/2 standard silicate (control), (0.099±0.009 division/day) after twelve days and the lowest was observed with addition of Tri-silicate (0.077±0.008 division/day) after eleven days, as shown in Figures (58, 59 and 60).

C. calcitrans

The highest growth rate was observed at treatment Tri-silicate (0.077±0.002 division/day) after three days and the lowest was observed with addition of F/2 standard silicate (control), (0.073±0.003 division/day) after four days, as shown in Figures (61, 62 and 63).

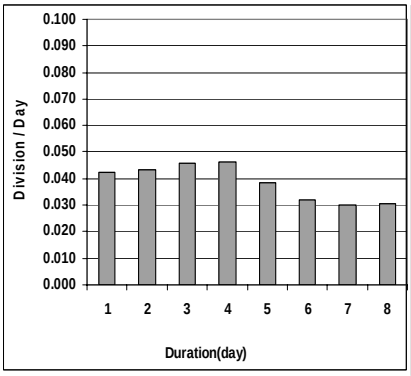

Figure 28: The effect of light duration 6h Light/18h Dark /750 Lux on growth rate of *S. costatum*

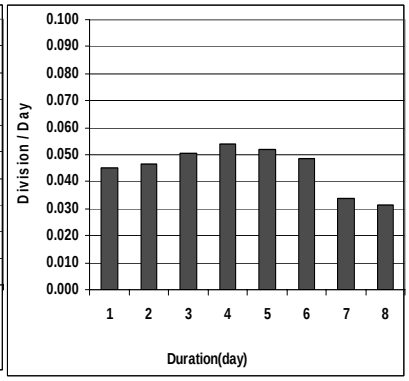

Figure 29: The effect of light duration 12h Light/12h Dark /750 Lux on growth rate of *S. costatum*

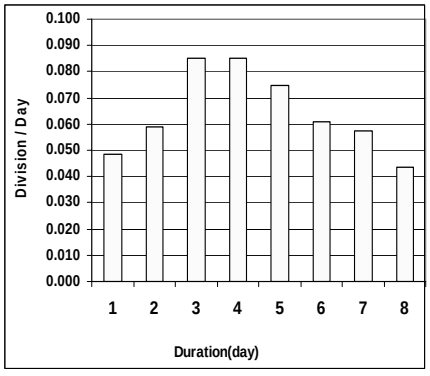

Figure 30: The effect of light duration 24 h Light/750 Lux (Control) on growth rate of *S. costatum*

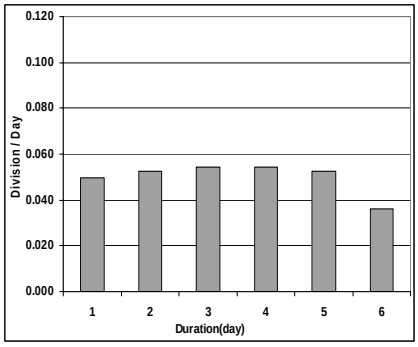

Figure 31: The effect of light duration 6h Light/18h Dark /750 Lux on growth rate of *De. confervacea*

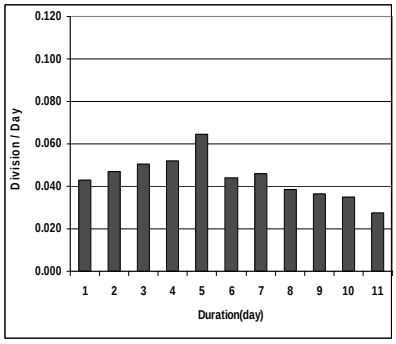

Figure 32: The effect of light duration 12h Light/12h Dark /750 Lux on growth rate of *De. confervacea*

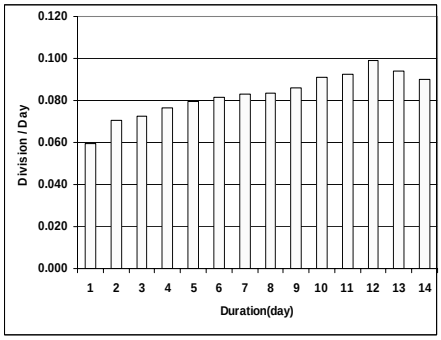

Figure 33: The effect of light duration 24 h Light/750 Lux (Control) on growth rate of *De. confervacea*

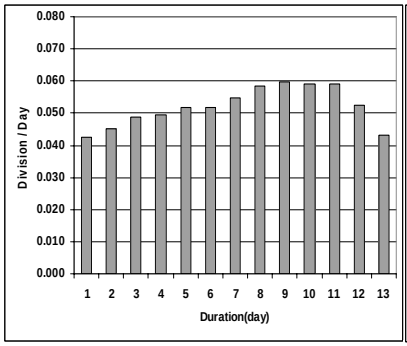

Figure 34: The effect of light duration 6h Light/18 h Dark /750Lux on growth rate of *C. calctirans*

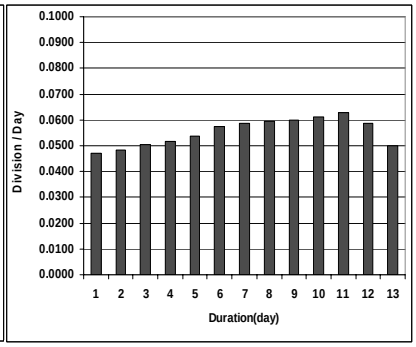

Figure 35: The effect of light duration 12h Light/12 h Dark /750 Lux on growth rate of *C. calctirans*

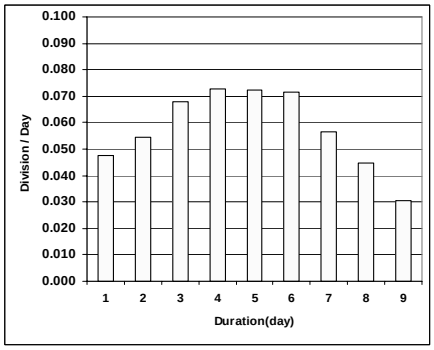

Figure 36: The effect of light duration 24 h Light/750 Lux Control) on growth rate of *C. calctirans*

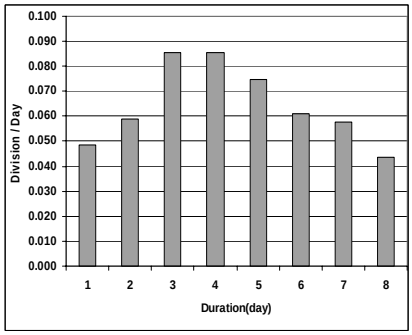

Figure 37: The effect of light intensity 750 Lux/24 h (control) on growth rate of *S. costatum*

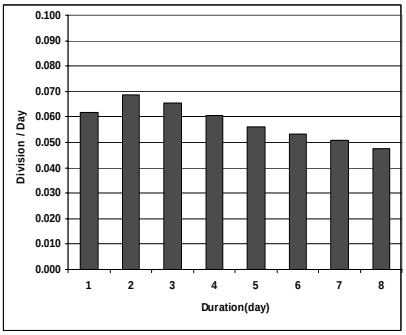

Figure 38: The effect of light intensity 1500 Lux/24 h on growth rate of *S. costatum*

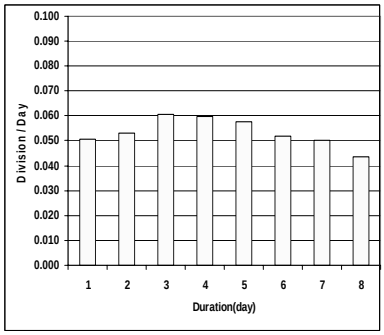

Figure 39: The effect of light intensity 3000 Lux/24 h on growth rate of *S. costatum*

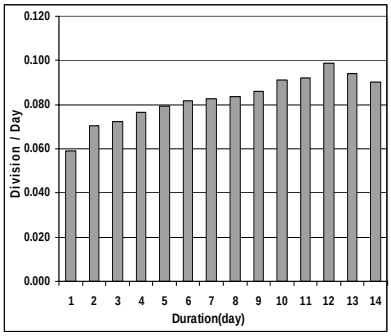

Figure 40: The effect of light intensity 750 Lux/24 h (control) on growth rate of *De. confervacea*

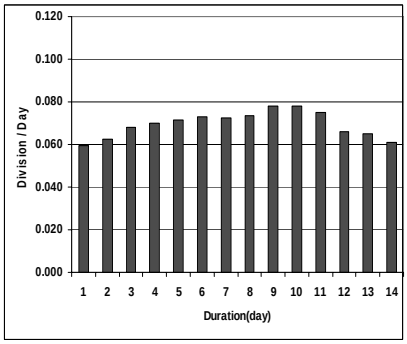

Figure 41: The effect of light intensity 1500 Lux / 24 h on growth rate of *De. confervacea*

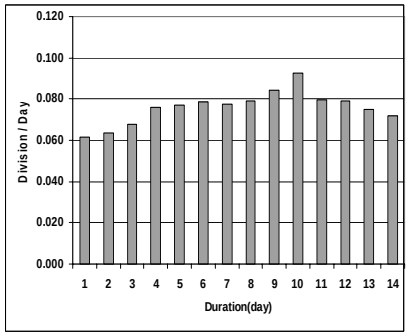

Figure 42: The effect of light intensity 3000 Lux/24 hon growth rate of *De. confervacea*

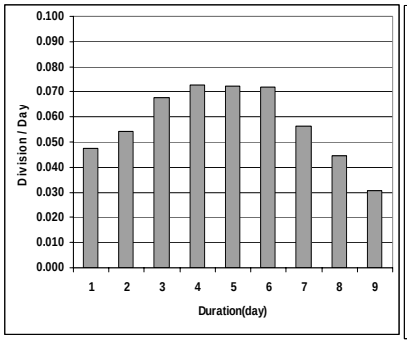

Figure 43: The effect of light intensity 750 Lux/24 h (control) on growth rate of *C. calctirans*

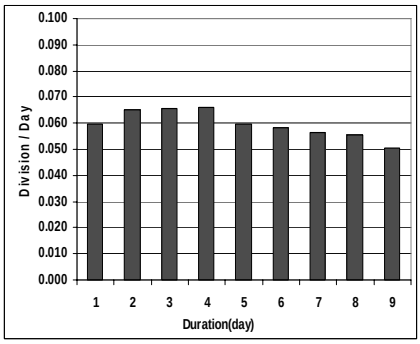

Figure 44: The effect of light intensity 1500 Lux /24 h on growth rate of *C. calctirans*

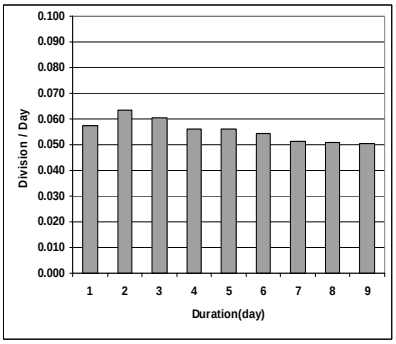

Figure 45: The effect of light intensity 3000 Lux /24 h on growth rate of *C. calctirans*

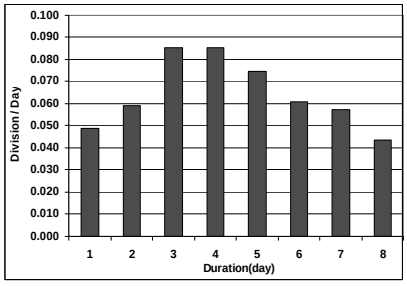

Figure 46: The effect of modified vitamin concentration (F/2 standard vitamin "control") on growth rate of *S. costatum*

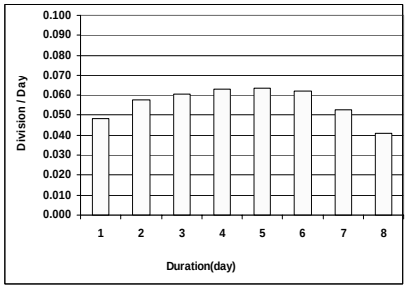

Figure 47: The effect of modified vitamin concentration (Tri-vitamin) on growth rate of *S. costatum*

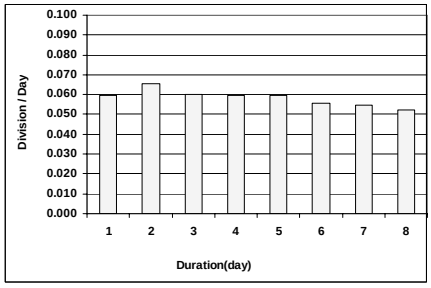

Figure 48: The effect of modified vitamin concentration (Di-vitamin) on growth rate of *S. costatum*

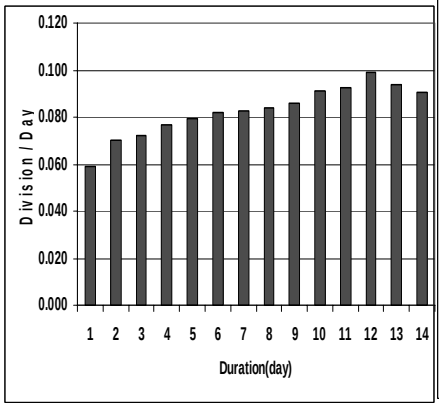

Figure 49: The effect of modified vitamin concentration (F/2 standard vitamin "control") on growth rate of *De. confervacea*

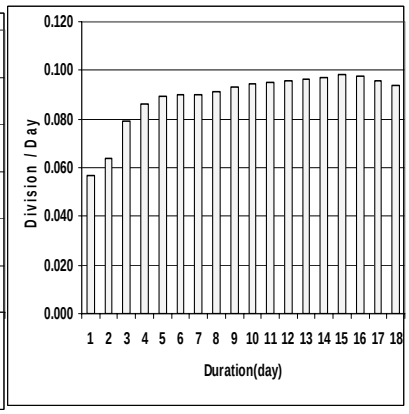

Figure 50: The effect of modified vitamin concentration (Di-vitamin) on growth rate of *De. confervacea*

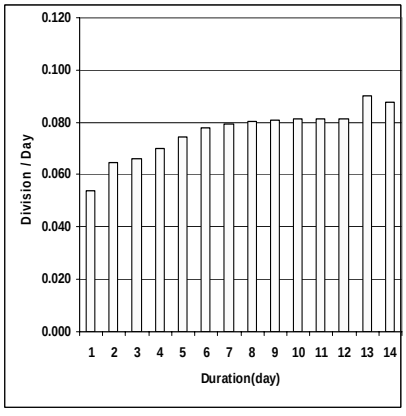

Figure 51: The effect of modified vitamin concentration (Tri-vitamin) on growth rate of *De. confervacea*

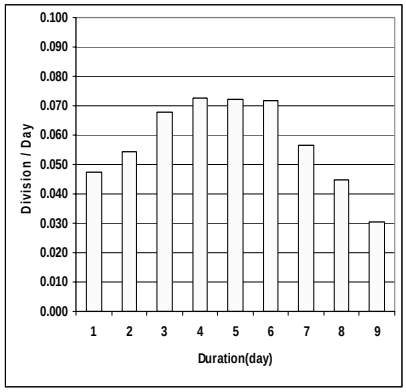

Figure 52: The effect of modified vitamin concentration (F/2 standard vitamin "control") on growth rate of *C. calcitrans*

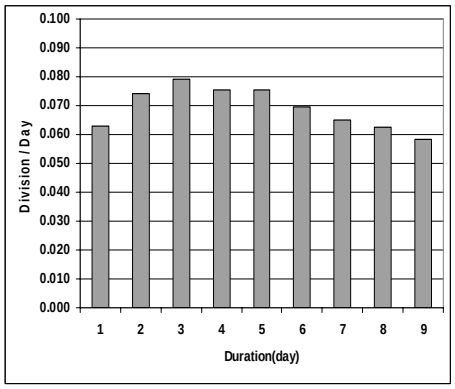

Figure 53: The effect of modified vitamin concentration (Di-vitamin) on growth rate of *C. calctirans*

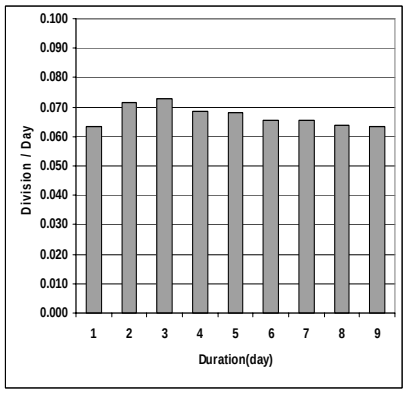

Figure 54: The effect of modified vitamin concentration (Tri-vitamin) on growth rate of *C. calctirans*

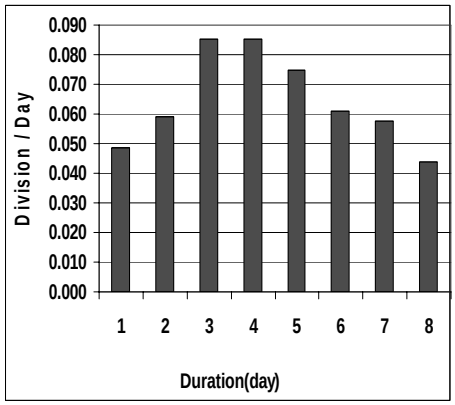

Figure 55: The effect of modified silicate concentration (F/2 standard silicate) on growth rate of *S. costatum*

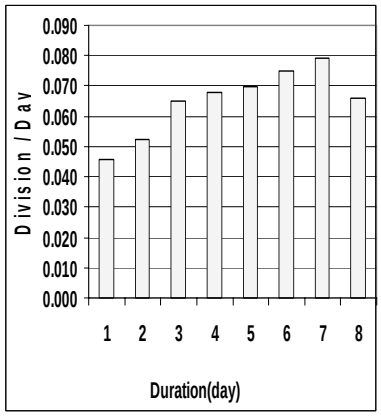

Figure 56: The effect of modified silicate concentration (D- silicate) on growth rate of *S. costatum*

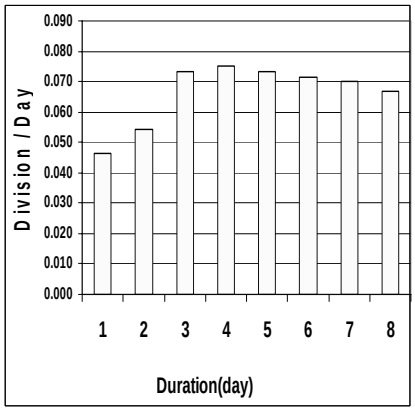

Figure 57: The effect of modified silicate concentration (Tri- silicate) on growth rate of *S. costatum*

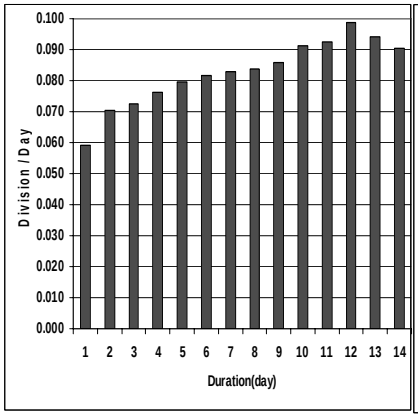

Figure 58: The effect of modified silicate concentration (F/2 standard silicate) on growth rate of *De. confervacea*

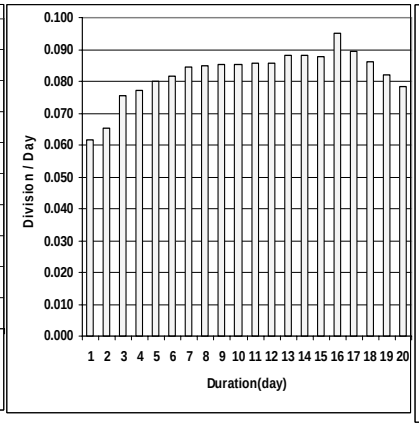

Figure 59: The effect of modified silicate concentration (Di- silicate) on growth rate of *De. confervacea*

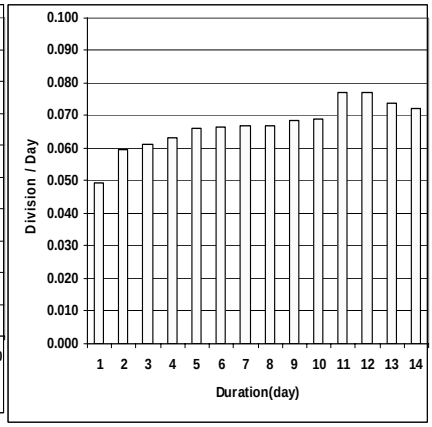

Figure 60: The effect of modified silicate concentration (Di- silicate) on growth rate of *De. confervacea*

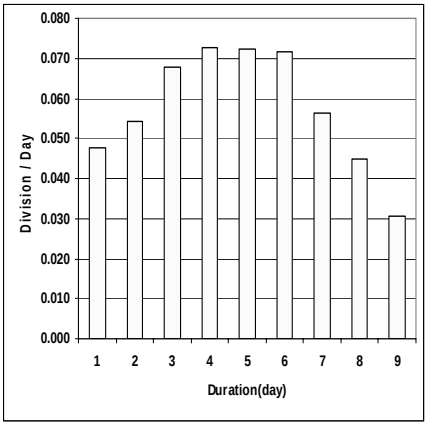

Figure 61: The effect of modified silicate concentration (F/2 standard vitamin) on growth rate of *C. calctirans*

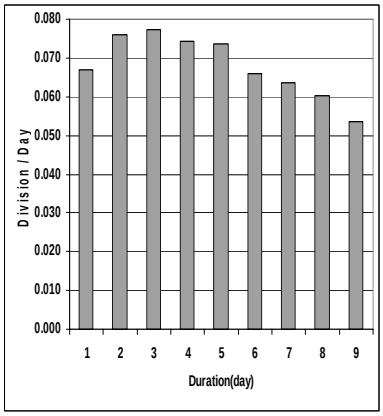

Figure 62: The effect of modified silicate concentration (Di-vitamin) on growth rate of *C. calctirans*

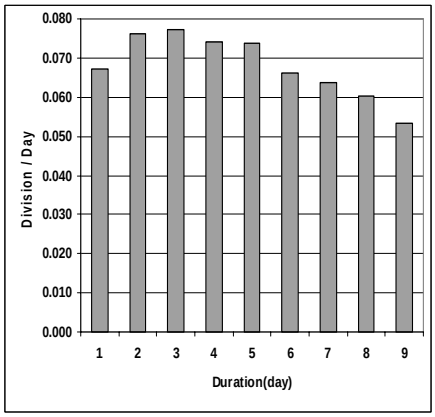

Figure 63: The effect of modified silicate concentration (Tri-vitamin) on growth rate of *C. calctirans*

3.3. Outdoor Mass Culture Results

The highest growth rate (0.140±0.011) was observed at *D. confervacea* after eleven days, followed by *S. costatum* (0.094±0.007) after three days and the lowest growth rate *C. calctirans* (0.092±0.008) was observed at *C. calctirans* after five days, as shown in Figure (64).

3.4. Feeding experiment of *Penaeus japonicus* larvae

STAGE Z1: Z2

Development index (DI)

The highest mean of DI noticed was in *D. confervacea* (1.76±0.08), followed by *C. calcitrans* (1.74±0.11) and the lowest was in *S. costatum* (1.64±0.25). The highest DI observed was in treatment number four (1.87±0.125) of *C. calcitrans* and four (1.87±0.001) of *S. costatum* too. The lowest DI observed was in treatment number six (1.34±0.054) of *S. costatum.* The duration time required for DI was equal (24±0.00 h) in all species and all treatments too, as shown in Figure (65).

Survival rate (SR)

The highest mean of SR noticed was in *D. confervacea* (83.35±7.77%), followed by *C. calcitrans* (66.58±22.55%) and the lowest was in *S. costatum* (50.66±32.06%). The highest SR observed was in treatment number three (96.00±4.00%) and four (92.66±6.00%) of *C. calcitrans* and the lowest was in treatment number one (11.75±1.54%), six (27.72±2.39%) and two (33.18±2.49%) of *S. costatum*, as shown in Figure (66).

Total length (TL)

The highest mean of TL observed was in *C. calcitrans* (1.77±0.13 mm), followed by *S. costatum* (1.62±0.26mm) and the lowest was in *D.* confervacea (1.52±0.07 mm). The highest TL observed was in treatment number four (1.91±0.001 mm) of *C. calcitrans* and the lowest was in treatment number six (1.25±0.034 mm) of *S. costatum*, as shown in Figure (67).

STAGE Z2: Z3

Development index (DI)

The highest mean observed of DI was in *D.* confervacea (2.81±0.12), followed by *S. costatum* (2.63±0.32) and the lowest was in *C. calcitrans* (2.43±0.17), as shown in figure (68). The highest DI observed was in treatment number five (2.95±0.010) of *S. costatum* and the lowest was in treatment number one (2.11±0.133) of *C. calcitrans* and one (2.11±0.130) of *S. costatum* too. The highest mean observed of required duration time for DI was in *S. costatum* (28 ± 9.79 h) and the low mean observed of duration time for DI was an equal in rest of species (24 h) and in rest treatments (24 h). The highest mean observed of required duration time was in treatment number one (48±0.00) of *S. costatum,* as shown in Figure (68).

Survival rate (SR)

The highest mean of SR noticed was in *S. costatum* (78.06±10.23%), followed by *C. calcitrans* (77.41±5.70%) and the lowest was in *D. confervacea* (75.52±5.00%). The highest SR observed was in treatment number two (96.66±0.66) of *S. costatum* and the lowest was in treatment number one (67.94±4.57%) of *D. confervacea* and one (67.90±1.46%) of *C. calcitrans* too, as shown in Figure (69).

Total length (TL)

The highest mean of TL observed was in *S. costatum* (3.01±0.09 mm) followed by *C. calcitrans* (2.66±0.20 mm) and the lowest mean of TL noticed was in *D. confervacea (*2.09±0.27 mm). The highest TL observed was in treatment number four (3.14±0.015 mm) of *S. costatum* and the lowest was in treatments number one (1.77±0.06 mm) of *D. confervacea* as shown in Figure (70).

STAGE Z3:M1

Development index (DI)

The highest mean observed of DI was in *D. confervacea* (3.45±0.33), followed by *S. costatum* (3.37±0.19) and the lowest was in *C. calcitrans* (3.26±0.12). The highest DI observed was in treatment number one (3.95±0.015) of *D. confervacea* and the lowest was in treatment number six (3.09±0.055) of *D. confervacea*. The highest mean observed of duration time of DI was in *C. calcitrans* (36±20.08 h), followed by *S. costatum* (32±12.39 h) and the lowest was in *D. confervacea* (24±0.00 h). The highest mean observed of duration time in all treatments was in treatment number one (72±0.00 h) of *C. calcitrans* followed by treatment number two (480.00 h) of *C. calcitrans* and treatment number one (480.00 h) and two (480.00 h) of *S. costatum*, as shown in Figure (71).

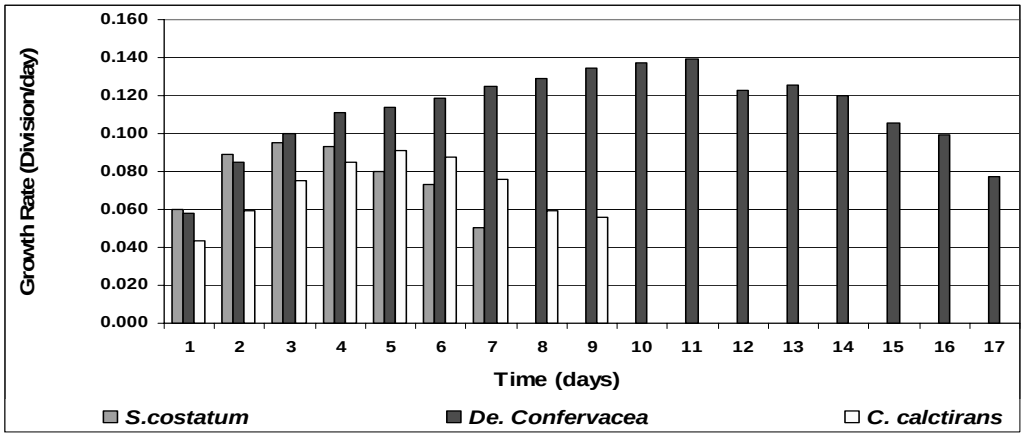

Figure 64: The effect of high temperature (27-34˚C) on growth rate of three isolated diatoms *S. costatum*, *D. confervacea* and *C. calcitrans* were cultured at outdoor mass

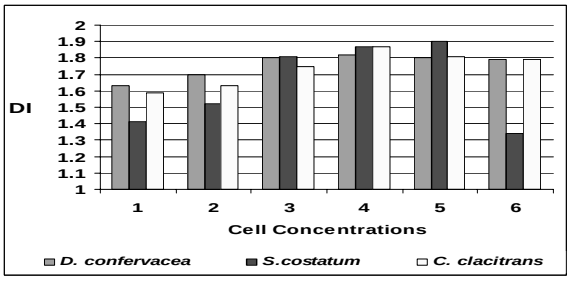

Figure 65: Development index (DI) of *Penaeus japonicus* larvae, stage of (Z1: Z2) fed with one to six concentrations

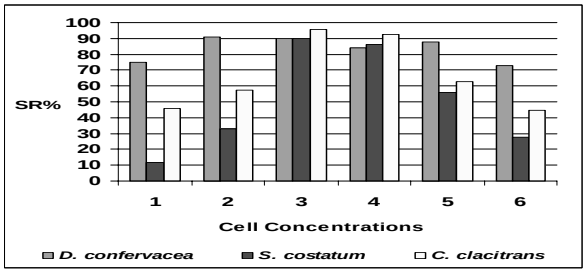

Figure 66: Survival rate (SR %) of *Penaeus japonicus* larvae, stage of (Z1: Z2) fed with one to six concentrations

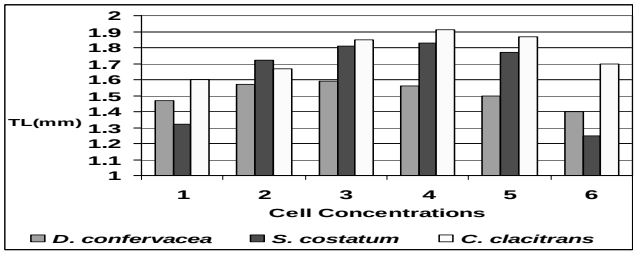

Figure 67: Total length (mm) of *Penaeus japonicus* larvae, stage of (Z1: Z2) fed with one to six concentrations

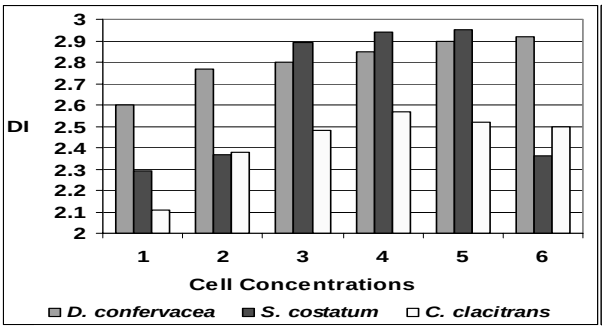

Figure 68: Development index (DI) of *Penaeus japonicus* larvae, stage of (Z2:Z3) fed with six concentrations

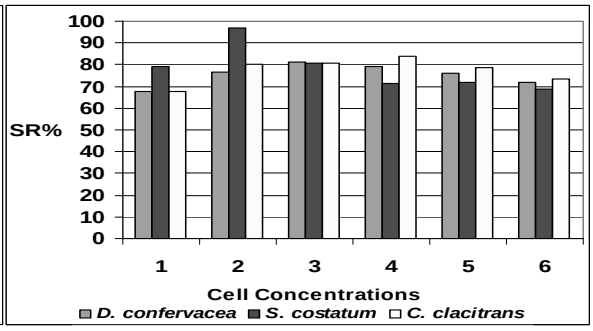

Figure 69: Survival rate (SR %) of *Penaeus japonicus* larvae, stage (Z2: Z3) fed with one to six concentrations

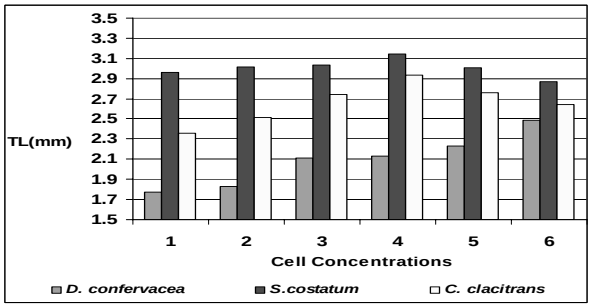

Figure 70: Total length (mm) of *Penaeus japonicus* larvae, stage of (Z2 Z3) fed with one to six concentrations

Survival rate (SR)

The highest mean of SR observed was in *D. confervacea* (77.60±6.44%) followed by *S. costatum* (70.97±7.26%) and the lowest was in *C. calcitrans* (65.58±14.00%). The highest SR observed was in treatment number five (83.95±1.05%) of *D. confervacea* and the lowest was in treatment number one (41.66±2.23%) of *C. calcitrans,* as shown in Figure (72).

Total length (TL)

The highest mean of TL observed was in *S. costatum* (3.77±0.23 mm) followed by *C. calcitrans* (3.48±0.17mm) and the lowest was in *D. confervacea (*3.44±0.27 mm). The highest TL observed was in treatments number four (3.97±0.030 mm) of *S. costatum* and the lowest was in treatment number one (3.11±0.140 mm) of *D. confervacea*, as shown in Figure (73).

STAGE M1:M2

Development index (DI)

The highest mean observed of DI was in *D. confervacea* (4.45±0.23), followed by *C. calcitrans* (4.37±0.12) and the lowest was in *S. costatum* (4.21±0.25). The highest DI observed was in treatments number two (4.73±0.240) of *D. confervacea* and the lowest DI observed was in treatment number six (4.05±0.230) of *S. costatum.* The highest mean observed of duration time for DI was in *C. calcitrans* (32±12.39 h). The highest mean observed of duration time for DI in all treatments was in treatment number one (48±0.00 h) and two (48±0.00 h) of *C. calcitrans*, as shown in Figure (74).

Survival rate (SR)

The highest mean of SR observed was in *D. confervacea* (67.65±8.94%) followed by *C. calcitrans* (60.02±23.77%) and the lowest mean of DI noticed was in *S. costatum* (54.14±15.17%). The highest SR observed was in treatment number three (78.90±4.20%) of *D. confervacea* and the lowest was in treatment number one (13.62±1.21%) of *C. calcitrans*, as shown in Figure (75).

Total length (TL)

The highest mean observed of TL was in *D. confervacea* (4.41±0.28 mm) followed by *S. costatum* (4.23±0.18 mm) and the lowest was in *C. calcitrans* (4.12±0.05 mm). The highest TL observed was in treatment number six (4.78±0.001 mm) of *D. confervacea* and the lowest was in treatments number six (4.02±0.040 mm) of *C. calcitrans*, as shown in Figure (76).

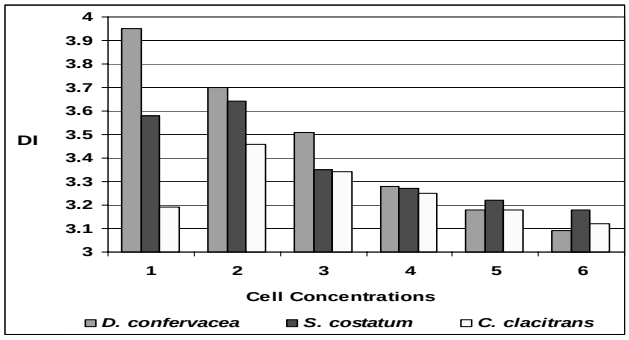

Figure 71: Development index (DI) of *Penaeus japonicus* larvae, stage of (Z3 : M1) fed with six concentrations

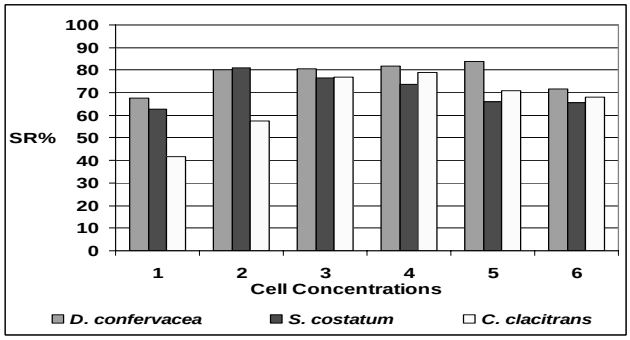

Figure 72: Survival rate (SR %) of *Penaeus japonicus* larvae, stage of (Z3:M1) fed with one to six concentrations

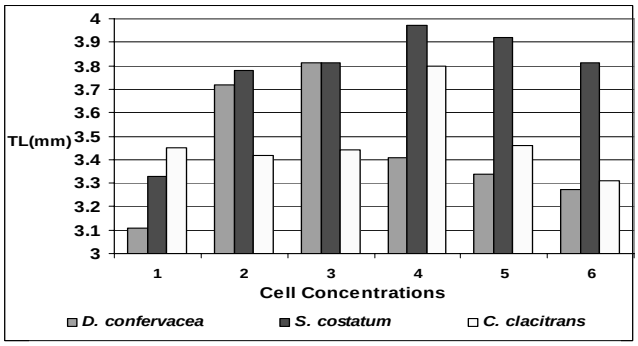

Figure 73: Total length (mm) of *Penaeus japonicus* larvae, stage of (Z3:M1) fed with one to six concentrations

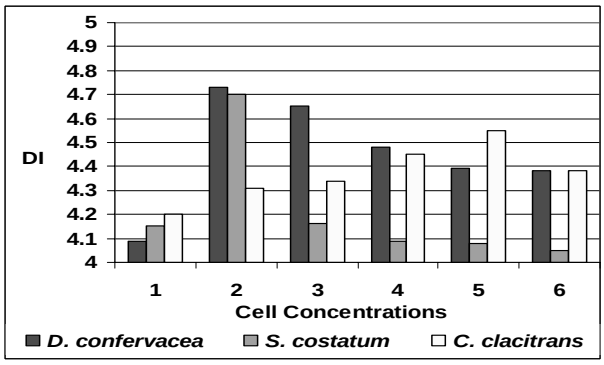

Figure 74: Development index (DI) of *Penaeus japonicus* larvae, stage of (M1:M2) fed with six concentrations

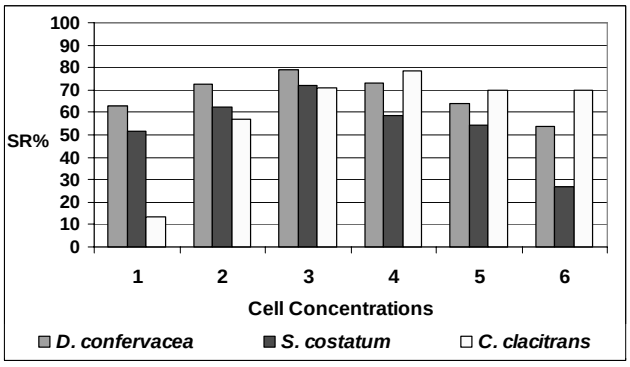

Figure75: Survival rate (SR %) of *Penaeus japonicus* larvae, stage of (M1:M2) fed with one to six concentrations

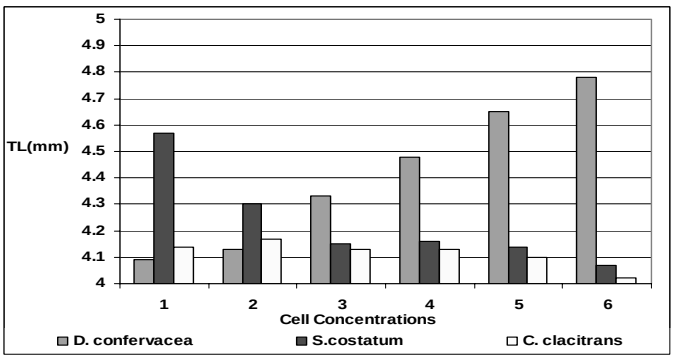

Figure 76: Total length (mm) of *Penaeus japonicus* larvae, stage of (M1:M2) fed with one to six concentrations

STAGE M2:M3

Development index (DI)

The highest mean observed of DI was in *S. costatum* (5.60±0.13) followed by *D. confervacea* (5.56±0.20) and the lowest was in *C. calcitrans* (5.35±0.10). The highest DI observed was in treatments number five (5.89±0.080) of *D. confervacea* and the lowest was in treatment number one (5.25±0.750) of *C. calcitrans* as shown in figure (80). The duration time for DI were equals (24±0.00 h) in all species and treatments, as shown in Figure (77).

Survival rate (SR)

The highest mean of SR observed was in *D. confervacea* (62.18±10.36%) followed by *C. calcitrans* (59.42±14.39%) and the lowest was in *S. costatum* (51.32±16.35%). The highest SR observed was in treatment number four (76.51±2.11%) of *C. calcitrans* and the lowest was in treatment number six (21.17±3.88%) of *S. costatum, as* shown in Figure (78).

Total length (TL)

The highest mean of TL observed was in *C. calcitrans* (4.69±0.43 mm), followed by *S. costatum* (4.65±0.39 mm) and the lowest was in *D. confervacea* (4.47±0.39 mm). The highest TL observed was in treatments number six (5.23±0.020 mm) of *C. calcitrans* and the lowest was in treatments number six (4.13±0.088 mm) of *D. confervacea*, as shown in Figure (79).

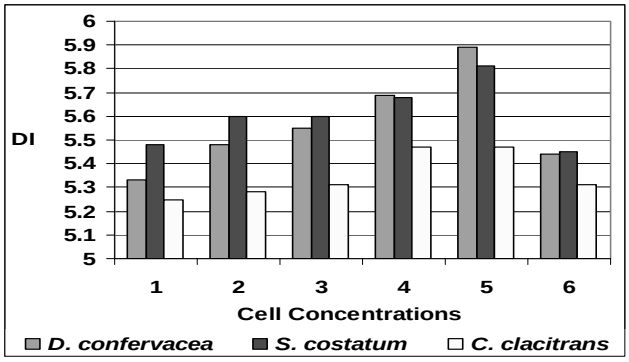

Figure 77: Development index (DI) of *Penaeus japonicus* larvae, stage of (M2:M3) fed with one to six concentrations

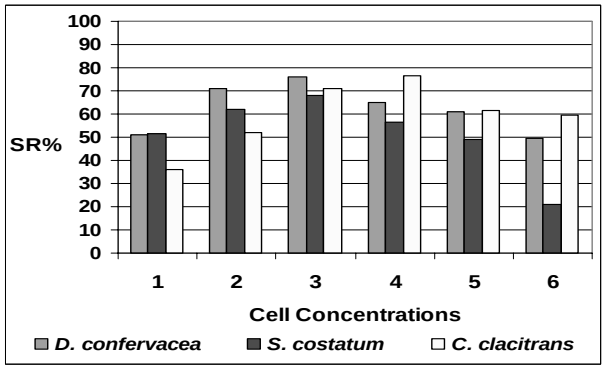

Figure 78: Survival rate (SR %) of *Penaeus japonicus* larvae, stage of (M2:M3) fed with one to six concentrations

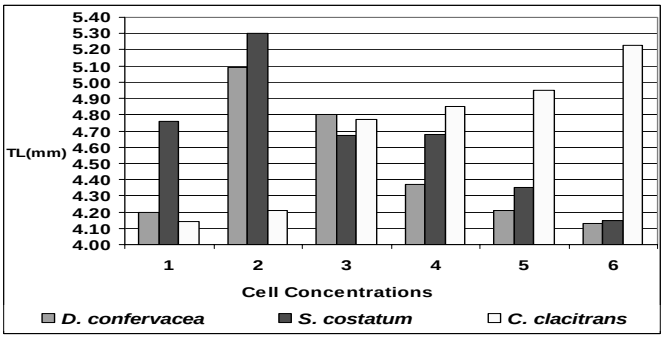

Figure 79: Total length (mm) of *Penaeus japonicus* larvae, stage of (M2:M3) fed with one to six concentrations

STAGE M3: PL1

Development index (DI):

The highest mean observed of DI was in *S. costatum* (6.30±0.17) followed by *D. confervacea* (6.18±0.15) and the lowest mean of DI noticed was in *C. calcitrans* (6.15±0.11). The highest DI observed was in treatment number five (6.53±0.40) of *S. costatum* and the lowest was in treatment number one (6.03±0.06) of *C. calcitrans*. The duration time for DI were equals (24±0.00 h) in all species and treatments, as shown in Figure (80).

Survival rate (SR):

The highest mean observed of SR was in *D. confervacea* (57.61±11.8%) followed by *C. calcitrans* (53.16±21.62%) and the lowest was in *S. costatum* (41.12±18.98%). The highest SR observed was in treatment number four (75.53±2.13%) of *C. calcitrans* and the lowest was in
treatment number one (14.58±1.40%) of *C. calcitrans*, as shown in Figure (81).

Total length (TL)

The highest mean of TL observed was in *S. costatum* (5.38±0.20 mm), followed by *D. confervacea* (5.09±0.17 mm) and the lowest was in *C. calcitrans* (5.02±0.28 mm). The highest TL observed was in treatment number three (5.47±0.010 mm) of *S. costatum* and the lowest was in treatment number one (4.71±0.020 mm) of *C. calcitrans*, as shown in Figure (82).

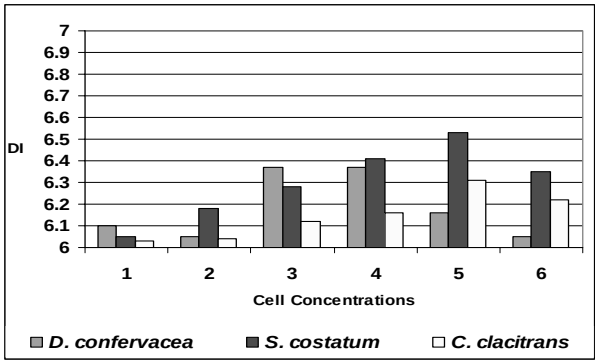

Figure 80: Development index (DI) of *Penaeus japonicus* larvae, stage of (M3:PL1) fed with one to six concentrations

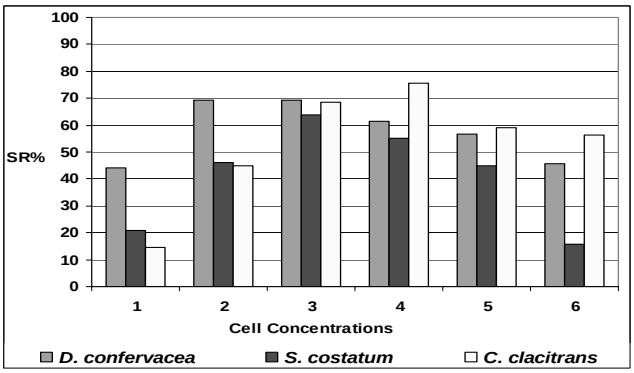

Figure 81: Survival rate (SR %) of *Penaeus japonicus* larvae, stage of (M3:PL1) fed with one to six concentrations

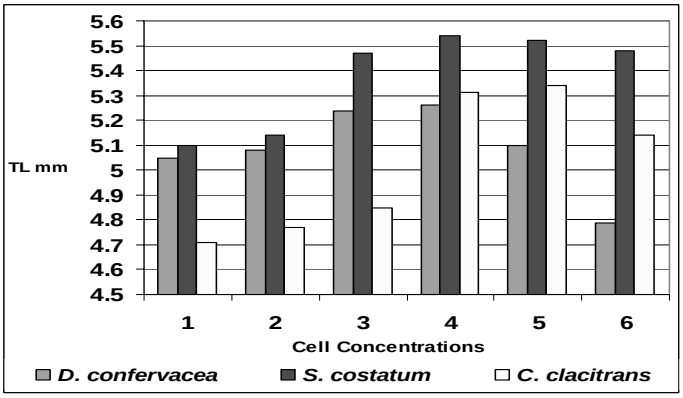

Figure 82: Total length (mm) of *Penaeus japonicus* larvae, stage of (M3:PL1) fed with one to six concentrations

4. Discussion

Three diatom species were isolated from the Eastern Harbor of Alexandria, Egypt. All isolated species were within size range suitable for ingestion by filter feeders (Webb & Chu 1983). There cell dimension (excluding spines and chains) ranged from 2-3µm (*C. calcitrans)*, 2-5 µm (*S. costatum)* or 6-15 µm (*D. converfacea*). *S. costatum* was predominantly a chain form had been comprised of about 5-20 cells, thin walls and usually straight. *C. calcitrans* was predominantly a single cell, thin walls, cells thin, cell extremely small and had straight seta.

D. confervacea sometimes had a single cell or a chain, chains comprised of about 2-5 cells, cells are long, thin chains, thin walls, forming bundles and inconspicuous intercalary bonds and had a large number of resting spores was observed by Durbin (2004)

The results of the present study showed that the optimum growth rate of *C. calcitrans* was at temperature 20ºC, salinity 30ppt and illumination of 750 Lux/ 24 h. From the modification of silicate and vitamin amount of F/2 standard medium, the results suggested that the highest growth rate was achieved with F/2 3-fold of silicate and 2-fold of vitamin content rather than other treatments. Kongkeo (1991) found that *C. calcitrans* grow well at temperature optimum temperature of 28ºC-30ºC, optimum salinity 22-28 ppt with an optimum light intensity >10.000 Lux/ 24 h. Hen (1991), Hirayama *et al*'(1991) and Okauchi (1991) cited that the optimal growth of *Chaetoceros sp.* was achieved at temperature 25- 35ºC, salinity 20–35 ppt and illumination of 8000- 10,000 Lux/24h

In this study; the optimum growth rate observed for *S. costatum* was at temperature 20ºC, salinity 35ppt and illumination of 750 Lux/ 24 h. From the modification of silicate and vitamin content of F/2 standard medium, the results suggested that the highest growth rate was achieved with F/2 standard silicate and vitamin content rather than other treatments. Uddin and Zafar (2006) found that *S. costatum* grow well at temperature ranging from 3ºC to 34ºC with an optimum temperature of 25ºC-27ºC, salinity 15-34 ppt with an optimum ranging from 25 to 29 ppt and with an optimum light intensity ranging from 500 to 10.000 Lux/ 24 h and declines at intensities exceeding 10.000 Lux (Liao& Huang, 1973). Kongkeo (1991) found that *S. costatum* grow well at temperature optimum temperature of 26ºC-28ºC, optimum salinity 27-30 ppt.

There are no information about indoor and outdoor growth conditions of *D. confervacea*. The results of this study showed that the optimum growth rate observed of *D. confervacea* was at temperature 20ºC, salinity 35ppt and illumination of 750 Lux/ 24 h. From the results obtained of the modification of silicate and vitamin content of F/2 standard medium, the results showed that the highest growth rate was achieved with standard F/2 . Mass culture experiments in outdoor tanks for the three isolated species were conducted in summer season at high temperature (27-34ºC).

D. converfacea showed the highest growth rate and the longest culture period compared with *S. costatum* and *C. calcitrans*.

The method used for outdoor mass culture of *S. costatum* is similar to that for *C. calcitrans,* except that *S. costatum* incubation period is short (Kongkeo 1991). Temperature can be important in determining which species will predominate in open outdoor culture at local conditions (Goldman and Ryther 1977, Goldman 1979, Goldman and Mann 1980, De Pauw *et al*. 1980 and Witt *et al*. 1981).

Chaetoceros calcitrans is a small species which reproduces at relatively low temperatures (10-20º C) (Okarche 1991). Baynes *et al*. (1979) cited that the *Chaetoceros* cannot be mass cultured easily in outdoor vessels for the following reasons: they require various vitamins and a stable temperature. According to Aekman *et al.* (1968), *Chaetoceros* is high in HUFAs and its overall nutritional value is also high. However, because of their population growth is not always constant (e. g. their lag phases are sometimes too long and their stationary phases are some times too short), their consistent mass culture in outdoor tanks is difficult.

According to Liao *et al*. (1991) in culture, the diameter of *S. costatum* decreases after cell division, thus, the *S. costatum* chains become thinner and thinner. As the diameter becomes smaller, the cell division rate increases, while the concentrated biomass and the duration of the exponential period decrease. A major problem in *S. costatum* culture is the short exponential growth phase and its tendency to perish after only short culture period.

The present study was conducted to evaluate the three isolated diatom species (with different cell densities) as feed for marine shrimp larvae *Penaeus japonicus* from the first protozoa stage to the first postlarvae stage.

The results of this study showed that shrimp larvae fed on *D. converfacea* showed in an equal survival, development and metamorphosis when compared with either *S. costatum* or *C. calcitrans* that are being used in most shrimp hatcheries (Samarasinghe *et al*., 1993)

These results suggest that *D. converfacea* (which could be mass cultured at high temperature) can be used as feed for marine shrimp larvae *Penaeus japonicus.*

5. Conclusion

The overall conclusions drawn from the present experimentation are summarized as follows:

1. All isolated diatom species have a cell size suitable for ingestion by zoea and mysis stages of penaeid shrimp larvae *Penaeus japonicus.*

2. Optimum growth rate observed of *S. costatum* was at temperature 20ºC,salinity 35ppt and illumination of 750 Lux/ 24 h, using F/2 standard medium.
3. Optimum growth rate observed for *C. calcitrans* was at temperature 20ºC, salinity 30ppt and illumination of 750 Lux/ 24 h, using F/2 nutrient medium with modification of F/2 silicate amount (3-fold) and F/2 vitamin amount (2-fold)
4. Optimum growth rate observed for *D. confervacea* was at temperature 20ºC, salinity 35ppt and illumination of 750 Lux/ 24 h, using F/2 standard medium.
5. In outdoor mass culture; *D. converfacea* showed the highest growth rate and the highest culture period "especially at high temperature (27-34ºC)" that when compared with *S.costatum* and *C. calcitrans*.
6. *D. converfacea* resulted in an equal survival, development and *metamorphosis* of penaeid shrimp larvae *Penaeus japonicus* when compared with either *S. costatum* or *C. calcitrans* that are being used in most shrimp hatcheries

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الظروف المثلى للنمو لثلاثة أنواع من الطحالب الدياتوميه المعزوله: *سكلاتونيما كوستاتم، كيتوسيرس كلاسترانس و ديتونيولا كونفيرفاسيا* ، واستخدامهم كغذاء ليرقات الجمبرى البحرى

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تقوم الطحالب الدقيقة بدور حيوى جدا فى تغذية يرقات الأسماك البحرية والجمبري في المفرخات. فى هذة الدراسة، تم عزل ثلاثة انواع من الدياتومات البحرية من الميناء الشرقى ،الاسكندرية، مصر ، وهذة الانواع هى: *سكلاتونيما كوستاتم* (2-5ميكرون)، *كيتوسيرس كلاسترانس* (2-3 ميكرون) و *ديتونيولا كونفيرفاسيا* (6-15 ميكرون) تم استخدام الميكروسكوب الالكترونى للمساعدة فى عملية التعريف بالانواع المعزوله.

تم اختبار الظروف المثلى للنمولكل منهم على النطاق المعملى والإنتاجي، حيث تم دراسة تأثير المستويات المختلفه من (درجة الحرارة، الملوحة، شدة الإضاءة، فترة الإضاءة، السليكات، الفيتامينات) على معدل النمو للثلاثة أنواع. درجة الملوحة المثلى والتى حققت أعلى معدل نمو كانت 35 ، 30 ، 35 جزء/ الف لكل من: *سكلاتونيما كوستاتم* ، *كيتوسيرس كلاسترانس*، *ديتونيولا كونفيرفاسيا* على التوالى. . درجة الحرارة المثلى والتى حققت أعلى معدل نمو كانت 20، 25، 20 درجة مؤية لكل من : *سكلاتونيما كوستاتم* ، *كيتوسيرس كلاسترانس*، *ديتونيولا كونفيرفاسيا* على التوالى. أفضل إضائة(شدة إضائة، فترة إضائة) كانت 750 لوكس/24 ساعة لكل الأنواع. *كيتوسيرس كلاسترانس* حقق أعلى معدل نمو عند تعديل المغذيات بمعدل ضعف كمية الفيتامينات وثلاثة أضعاف كمية السليكات. التجربة المزرعيد كانت فى فصل الصيف عند درجة حرارة مرتفعة(27-34 درجة مئويه)و وأظهرت هذة التجربة أن *الديتانيولا كونفيرفاسيا* أظهرت أعلى معدل نمو عند مقارنتها بالأنواع الباقيه.

أجريت تجارب لتقييم الأنواع الثلاثة المعزوله كغذاء ليرقات الجمبرى البحرى *بينيس جابونيكس* بداية من مرحلة البروتوزؤيا الى اليوم الاول لمرحلة ما بعد الطور اليرقى. أظهرت نتائج هذة التجربة أن *الديتانيولا كونفيرفاسيا* عند مقارنته بالأنواع الشائعه والمستخدمه فى تغذية الجمبرى البحرى (*سكلاتونيما كوستاتم* ، *كيتوسيرس كلاسترانس*) أعطى نتائج مرضية على كل من معدل الإعاشة ، النمو، التطور ليرقات. من هذة

النتائج نستنتج أن *ديتانيولا كونفيرفاسيا* يمكن إستخدامها كغذاء ليرقات الجمبرى البحرى *بينيس جابونيكس* وخصوصا فى فصل الصيف عند إرتفاع درجات الحرارة.

كلمات مفتاحية : الطحالب الدقيقة، يرقات الجمبرى البحرى، *بينيس جابونيكس*، *سكلاتونيما كوستاتم*، *كيتوسيرس كلاسترانس*، *ديتونيولا كونفيرفاسيا*