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EFFECT OF ENVIRONMENTAL FACTORS ON *CHRYSOCHROMULINA* GROWTH; THE MOST COMMON CAUSE OF FISH MORTALITY IN JEDDAH

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ABSTRACT

A phytoplankton bloom, dominated by the prymnesiophyte *Chrysochromulina sp.*, developed in two areas of the red sea coast, Al-Nawras and Al-Arbaeen lagoons (Jeddah, Saudi Arabia) in the fall of October and December 2016. *Chrysochromulina sp.* dominated at total cell densities of average 3×10^6 cells.ml^{-1}, and have caused variable degrees of mortality in fish. Fish gills were examined under a microscope and showed the presence of *Chrysochromulina sp* cells. This study was conducted to investigate environmental factors that affect the growth characters as a function of different salinity, pH, temperature, and light-regime (light duration and intensity). Light-regime showed the highest effect among all the factors tested, at 10:14h L:D for the light duration with a growth rate of (0.352 µ.d^{-1}), dry weight of (1552.601 ng), production per dry weight of (542.613 ng.d^{-1}), and chlorophyll-a content (0.221µg.ml^{-1}), as for light intensity, the best results were at around 60 to 70 µmol with a growth rate of (0.426 µ.d^{-1}), dry weight of (2213.086 ng), production per dry weight of (929.419 ng.d^{-1}), and chlorophyll-a content of (0.205 µg.ml^{-1}). Other factors were best at 15°C, 40 PSU, and 7 to 8 for the temperature, salinity, and pH, respectively. No acute toxicity was present. Therefore, the fish mortality was most likely related to the morphological aspect of the isolated species by clogging of fish gills accompanied with suitable environmental conditions, grazing and a very low dissolved oxygen level in both lagoons.

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Introduction

On Wednesday 12/ October, fall of 2016, a bloom of *Chrysochromulina sp.* occurred along the coast of Al-Nawras lagoon in Jeddah city, Saudi Arabia. The bloom killed thousands of sardine fish that entered the bay at the time and suffocated. Another bloom happened on Friday 2/ December 2016, along the coast of Al-Arbaeen lagoon in Jeddah city, Saudi Arabia. It also killed larger fish (milkfish and Tilapia) and some crabs. The Prymnesiophyta or Haptophyta are a group of uninucleate flagellates characterized by the presence of a haptonema between two smooth flagella. The prymnesiophyte genus *Chrysochromulina* presently embraces about 60 formally described species [1]. *Chrysochromulina* species have been found to make up 45 to 73.5 % of the identified species, and 2.5 to 50 % of the total number of nanoplankton cells [2]. Blooms threaten the environmental or public health and the fast development of economy, industry, and social life is one of the factors affecting negatively the environment. Sewage input into the coastal areas is the major problem along the coast of Saudi Arabia [3]. Jeddah is a major coastal city that has a network to collect municipal wastewater [4]. Some HABs are harmful by virtue of their sheer biomass, whereas some are capable of producing toxins. Other species are non-toxic to humans but harmful to fish and invertebrates (especially in intensive aquaculture systems) by damaging or clogging their gills [5, 6]. The aim of this study was to investigate several environmental factors in order to reveal possible effective factors in growth and bloom formation **(Figure 1)**.

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a)

b)

Figure 1. a) Fish kills at Al-Nawras lagoon on 12 October 2016. b) Fish kills at Al-Arbaeen lagoon on 2 December 2016.

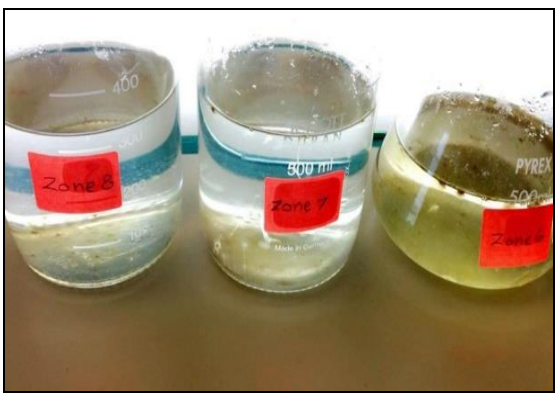
Materials and Methods

Samples Collection

Samples were obtained in the fall of 2016 (October and December) from surface water during blooms at the Red Sea coast in Al-Nawras lagoon on 12 October 2016 and in Al-Arbaeen lagoon on 2 December 2016. Using a water sampling bottle. The cells were examined using an Olympus inverted microscope equipped with a digital camera.

Stock Culture

After sample collection the isolation was achieved with the serial dilution culture method, using the standard pipette dilution. [7]. The batch culture was grown in a 1-L Erlenmeyer flask with IMR medium [8]. All cultures were grown under laboratory conditions at 22-23 ˚C and a continuous light using Extreme Cool Daylight tubes (PHILIPS TL-D 18W) **(Figure 2)**.



a)

b)

Figure 2. Culture flask.

Growth Measurement

Three replicates of all samples were used for measurement of dry weight by filtering 300 ml of microalgae, using glass microfiber filter 47 mm (Whatman). The growth rate was measured daily by using a Beckman Coulter Counter Multisizer™3, following the equation:

$$\text{Number of cells} = N \text{ X } \frac{1000}{N2} \quad (1)$$

Growth rate (GR) was calculated as:

$$GR(\mu, d^{-1}) = \text{Ln X D} \quad (2)$$

Cell volume was estimated at harvest day using the following equation:

$$CV\ (\mu m^3) = \frac{4}{3} \text{ X } \pi \left(\frac{W}{2}\right)^2 \text{X } \left(\frac{L}{2}\right) \quad (3)$$

Dry weight was estimated at harvest day by filtering 300 ml through Whatman (GF/F) glass fiber filter size 47 mm (pre-weighted). After filtration, samples were rinsed with cold sterilized distilled water. The filters were dried in an oven (JSR) at 80 ˚C for 4h and then weighed (3 times).

$$\text{Dry Weight (DW)} = W - W1 \quad (4)$$

Dry weight per cell (ng) was calculated by:

$$DW\ (ng) = \frac{Wmg}{N} \text{ X } 1000000 \quad (5)$$

For production per dry weight (ng.d^{-1}) was calculated by:

$$\text{Production per DW } (ngd^{-1}) = \mu \text{ X DWng} \quad (6)$$

For chlorophyll-a content determination, 100ml culture was filtered using 25mm Whatman GF/C filters. A solvent was added (90% acetone) and grounded with mortar and pestle until it appeared colorless. After preparation, the extract was centrifuged at 4000 rpm for 10 minutes on a Hettich centrifuge MIKRO 220R. After extraction, the absorbance of the solvent extract was measured at the given wavelengths, between the range of 400 and 700 nm against a solvent blank on a Genesys 10S UV/VIS spectrophotometer [9]. Chlorophyll content was estimated using the following SCOR-UNESCO (1966) equations:
Chlorophyll a:

µg chlorophyll/ml medium = (11.64A663 – 2.16 A645 + 0.10A630) v/ lV

where is:

Axxx = the absorbance at xxx nm, after removing the sample absorbance at 750 nm against a blank of the solvent used.

v = the volume of acetone used (ml).
l = the spectrophotometric cell length (cuvette) (cm).
V = the sample volume (ml).

Physical Analysis

The methodology of the physical factors experimentation and the toxicity test were according to [10-13]. Five factors were selected (temperature, pH, salinity, light duration, and light intensity) including five measurements degrees. These factors were measured directly after the sample collection using Apera PC60 multi-parameter, a Refractometer, and a digital lux meter.
In the salinity experiment, growth-medium was diluted with ion-free water (milli-Q) and raised with 0.1 M of NaCl to the following salinities: 8, 18, 28, 38, and 48 PSU. Average PFR was 230 µmol $m^{-1}s^{-1}$, the L:D cycle was 24:00 h, and the temperature was 23°C (± 1.5 °C).
In the temperature experiment, the flasks were placed in 5 different water baths using a tank water heater thermostat and air tubes for circulating the water around the flasks at the following temperatures: 10, 15, 20, 25, and 35°C. The average PFRs was 230 Ilmol $m^{-1}s^{-1}$, the L:D cycle 24:00 h, and the salinity 30 PSU.

In the light experiment, the flasks were placed on shelves in a culture room at the following PFRs: 24:00 h, 12: 12 h, 8:16 h, and 14:10 h LD cycles, the temperature was 23°C and the salinity 30 PSU.
In the intensity experiment, the flasks were placed on shelves at the following chosen levels: 25, 60, 70, 150, and 200 Ilmol $m^{-1}s^{-1}$ for light saturated growth.
In the pH experiment, the growth medium pH was lowered with the addition of 0.1 M HCl and raised with the addition of 0.1 M NaOH to the following pH levels: 6.5, 7, 7.5, 8, and 8.5. The temperature was 23°C and the salinity was 30 PSU.

Toxicity Test

Toxicity test was performed with nauplii of waterlife Artemia brine shrimp eggs. The test was carried out in a small Petri dish. About 10 ml of cysts were incubated for 24 h at room temperature in filtered and autoclaved seawater diluted to 25 PSU under gentle aeration and continuous illumination. Hatched nauplii were separated from the non-hatched cysts and incubated for another 24 h. After that, 10 nauplii were added with 10 ml of algal culture in a culture plate. The nauplii were defined as dead if they were immobile for 10 s or longer.

Statistical Analysis

Data were entered, coded, cleaned, and analyzed using statistical package for social science (IBM SPSS), version 25. The data were analyzed using descriptive statistics (mean, stander deviation, stander error). A normality test was used to determine sample data had been normally distributed, ANOVA test used for the data with normal distribution while Kruskal-Wallis was used for the data that were not normally distributed. Also, the LSD test was used to indicate where significant differences occurred. All graphs were performed using SigmaPlot software, version 14.0. Three-ways ANOVA for all growth characteristics were as follows: growth rate ($\mu.d^{-1}$), dry weight ($ng.cells^{-1}$, production per dry weight ($ng.d^{-1}$), and chlorophyll-a content ($\mu g.ml^{-1}$). In all analyses, the statistical significance was at $P \leq 0.05$.

Results and Discussion

Toxicity

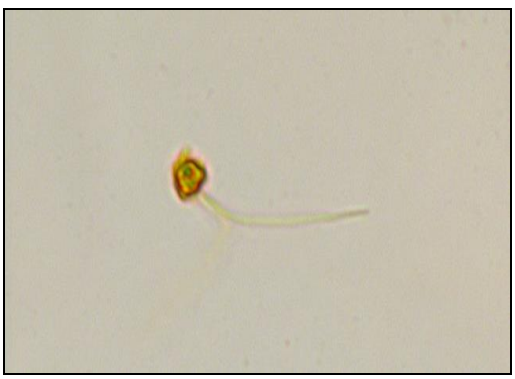
A total of 10 nauplii of Brine shrimp (Artemia) were exposed to 3×10^6 $cells.ml^{-1}$ of the isolated *Chrysochromulina sp*. No mortality was recorded, therefore, no acute toxicity was detected.

Bloom Measurements

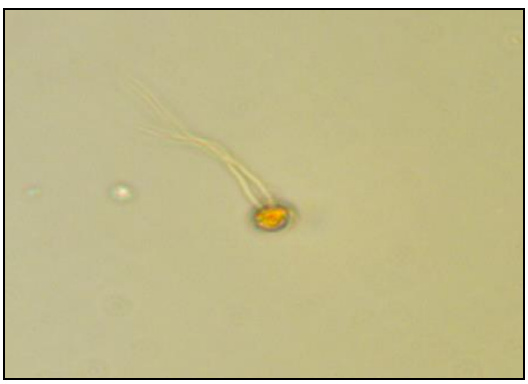
When both blooms formed, water and fish samples were analyzed from both lagoons. The results were confirmed by the Saudi Ministry of Environment, Water, and Agriculture according to the JFRC (Jeddah Fisheries Research Center) results. The water analysis showed that all samples had very low Dissolved Oxygen level, which was very much less than the allowable level. Salinity was about 50% less than the normal salinity of the open sea **(Table 1)**. The JFRC fish analysis showed that target viral, bacterial, and parasitic diseases were negative.

Chrysochromulina sp. Measurements

Microscopic images show cells appeared saddle shape with coiled haptonema between two long flagella and two parietal golden-brown chloroplasts **(Figure 3)** also, the Coulter Counter revealed some slight variation in the average cell size from 2.023 to 2.034 µm. Images also show evidence of *Chrysochromulina sp.* cells present inside the gills of dead fish samples **(Figure 4)**. **(Table 2)** provides an overview of characters that were used to distinguish some individual species of *Chrysochromulina* by light microscopy. The sample's characters mentioned in **(Table 1)** taken from Al-Nawras lagoon, are similar to Al-Arbaeen lagoon characters including the same saddle shape of the cell, length, width, flagella, haptonema, the position of haptonema, and the lack of toxicity.



a)



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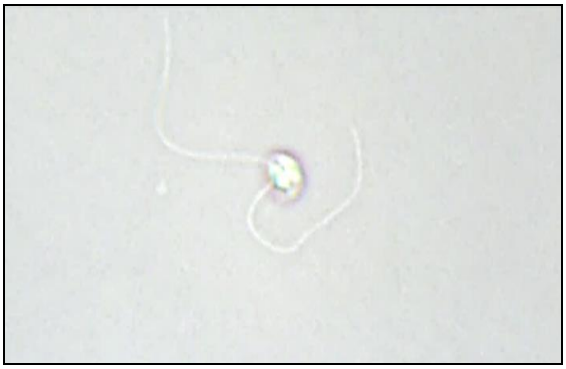
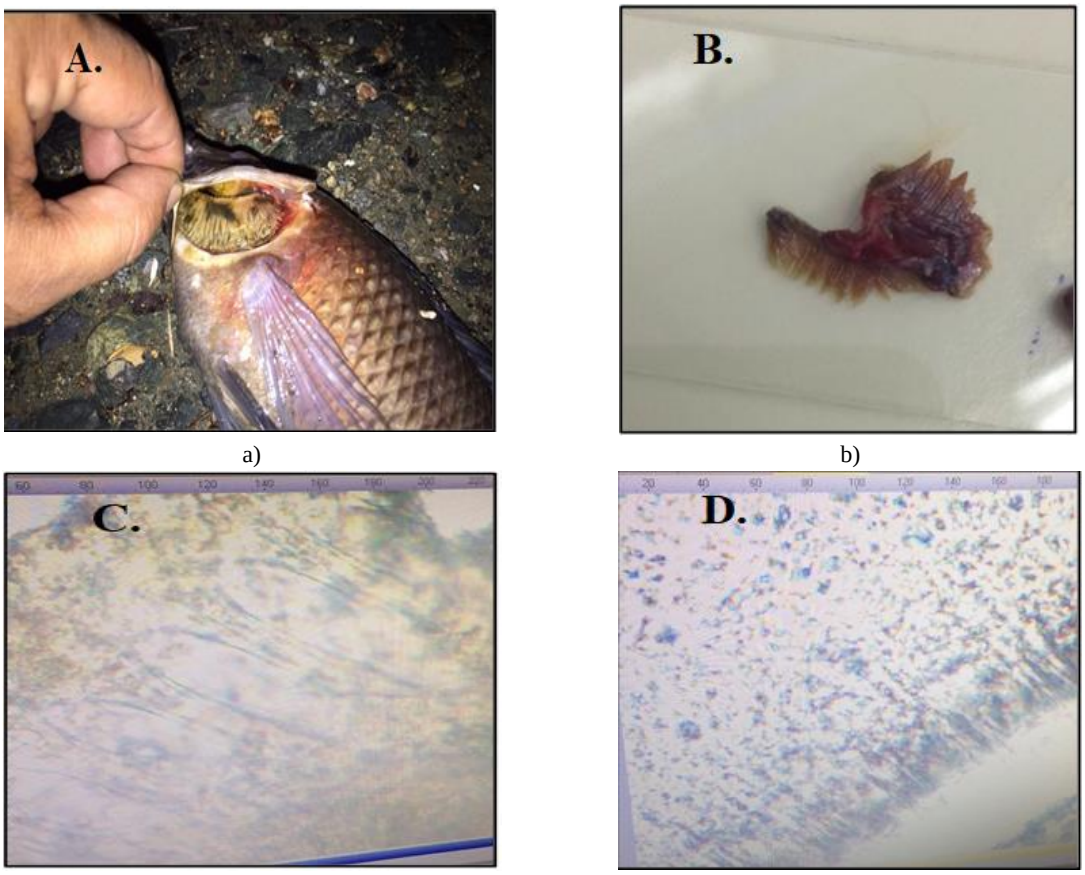


c)

Figure 3. Locally isolated *Chrysochromulina sp.* under microscope (×100). (photos were taken by Dr. Al-Sayegh using Leica microscope).



a) b)

c) d)

Figure 4. a) Growth on fish gills from the bloom. b) Sardine gills. and c) Smooth gills under a microscope (x60). d) Rough gills under a microscope shows algal cells within.

The growth characteristics tested were: growth rate ($\mu.d^{-1}$), dry weight (%), dry weight per cell (ng), production per dry weight ($ng.d^{-1}$), and chlorophyll-a content ($\mu g.ml^{-1}$) for isolated species *Crysochromulina sp.* as a standard in this work. Data showed that there is a significant difference between factors among growth rate, dry weight, production per dry weight, and chlorophyll-a content. And a significant difference between degrees among growth rate, dry weight, production per dry weight, and chlorophyll-a content. Also, there is a significant difference in the interaction between (factor and degree) among growth rate, dry weight, production per dry weight, and chlorophyll-a content. As for the two locations (Al-Nawras and Al-Arbaeen), data showed that there was no significant effect among them on all characteristics at differences of $P \leq 005$.

Temperature Experiment

The temperature was tested at five degrees: (10, 15, 20, 25, and 35 °C) for the isolated *Chrysochromulina sp.* at both zones of the bloom (Al-Nawras and Al-Arbaeen lagoons). Data showed that there are highly significant differences between the

five degrees at each growth character. Differences were between (10-15, 15-20 and 15-25 °C) among growth rate, (10-15 and 10-35°C) among dry weight, (10-15, 10-20, 10-25 and 10-35°C) among production per dry weight and (10-25 and 25-35°C) among chlorophyll-a content. The best temperature for growth rate was at 15°C, for both dry weight and production per dry weight the best temperature was at 10°C and the best for chlorophyll-a content was at 25°C **(Figure 5a)**.

Salinity Experiment

The salinity was tested at five degrees: (5, 10, 18, 25, and 40 PSU) for the isolated *Chrysochromulina sp.* at both zones of the bloom (Al-Nawras and Al-Arbaeen lagoons). Data showed that there are highly significant differences between the five degrees at each growth character. Differences are between (5-18, 5-25, 10-18, 10-25, 18-40 and 25-40 PSU) among growth rate, (5-25 and 5-40 PSU) among dry weight, (5-40 and 18-40 PSU), among production per dry weight, and (5-18 and 5-25 PSU) among chlorophyll-a content. The best salinity for growth rate, dry weight, and production per dry weight was at 40 PSU, and the best for chlorophyll-a content was at 25 PSU **(Figure 5b)**.

PH Experiment

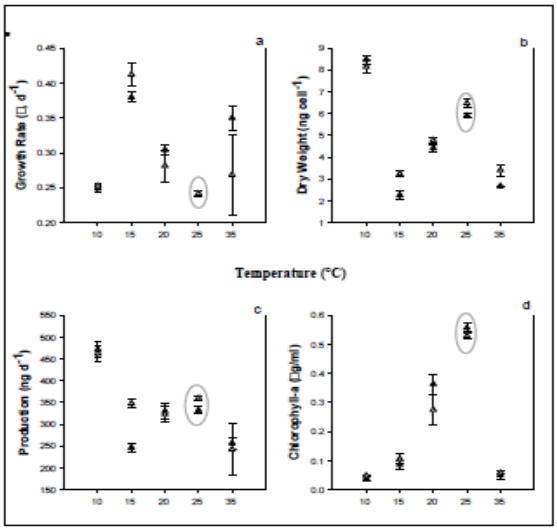
The pH was tested at five degrees: (6.5, 7, 7.5, 8, and 8.5 °C) for the isolated *Chrysochromulina sp.* at both zones of the bloom (Al-Nawras and Al-Arbaeen lagoons). Data showed that there are highly significant differences between the five degrees at each growth character except among production per dry weight because the overall test does not show significant differences across samples. Differences are between (6.5-8 and 7-8 pH) among growth rate, (6.5-8 pH) among dry weight, and (6.5-7.5 and 6.5-8 pH) among chlorophyll-a content. The best pH for growth rate was at 7 and the best for dry weight, production per dry weight and chlorophyll-a content was at pH 8 **(Figure 5c)**.

Light Duration

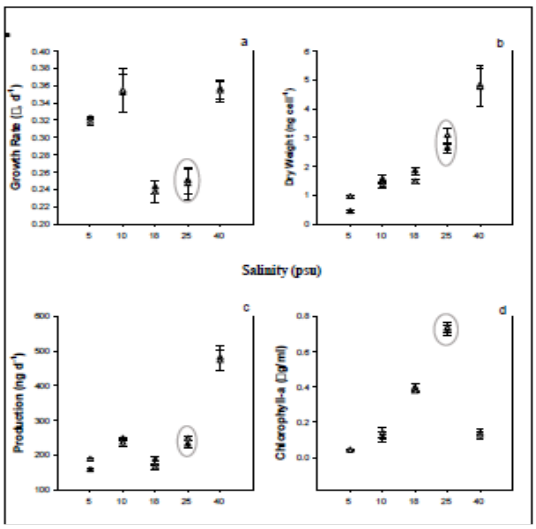
The light duration was tested at five degrees: (8:16, 12:12, 24:0, 10:14, and 14:10 hours) for the isolated *Chrysochromulina sp.* At both zones of the bloom (Al-Nawras and Al-Arbaeen lagoons). Data showed that there are highly significant differences between the five degrees at each growth character. Differences are between (8:16-10:14 and 12:12-10:14 h) among growth rate, (8:16-12:12 and 10:14-12:12 h) among dry weight, (8:16-14:10 h) among production per dry wight and (8:16-24:00 and 10:14-24:00 h) among chlorophyll-a content. The best light duration for both growth rate and chlorophyll-a content were at 10:14 h and both dry weight and production per dry weight were at 12:12 h **(Figure 5d)**.

Light Intensity

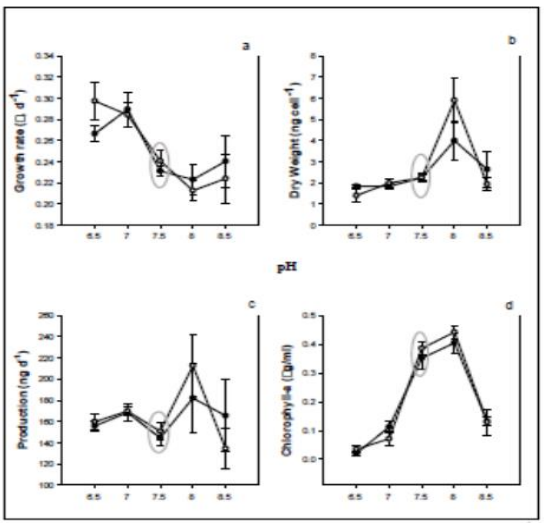
The light intensity was tested at five degrees: (25, 60, 70, 80, and 200 µmol $m^{-1}s^{-1}$) for the isolated *Chrysochromulina sp.* At both zones of the bloom (Al-Nawras and Al-Arbaeen lagoons). Data showed that there are highly significant differences between the five degrees at each growth character. Differences are between (60-70, 25-70, and 25-200 µmol) among growth rate, (25-60 and 25-70 µmol) among dry weight, (25-60, 60-70, and 60-200 µmol) among production per dry weight, and (25-70, 25-80, and 80-200 µmol) among chlorophyll-a content. The best light intensity for both growth rate and production per dry weight was at 60 µmol, for dry weight it was at 70 µmol, and for chlorophyll-a content, it was 80 µmol **(Figure 5e)**.



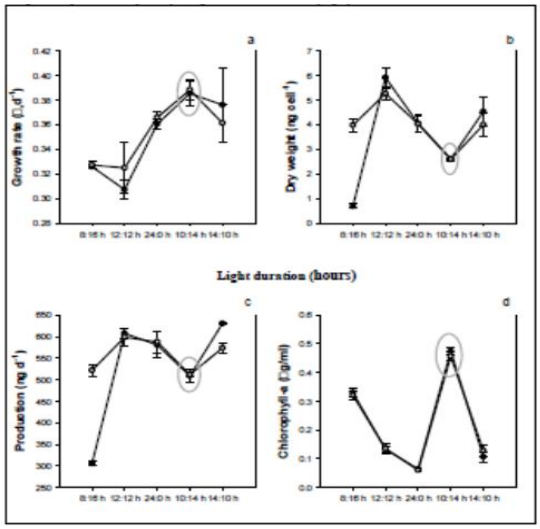
a) Function of temperature



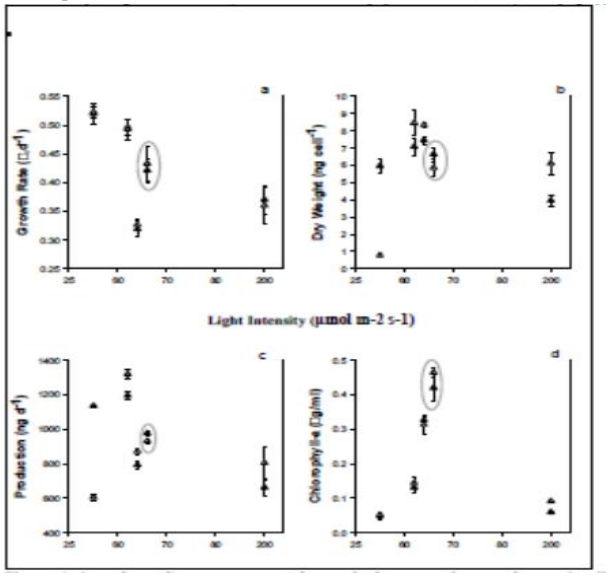
b) Function of salinity



c) Function of pH



d) Function of light duration



e) Function of light intensity

Figure 5. *Crysochromulina sp.* exponential growth characters (a. growth rate (µg.d^{-1}), b. dry weight (ng.cell^{-1}), c. production per dry weight (ng.d^{-1}), d. chlorophyll-a content (µg.ml^{-1}), in both zones of the bloom Al-Nawras (▲) and Al-Arbaeen (Δ), whereas the circles show the degree at the time of the bloom.

Table 1. A comparison of some recorded blooms related to *Chrysochromulina sp*. containing place of the bloom, time, cell count, temperature, salinity, chlorophyll-a (Chl-a), oxygen (O_2), and the type of bloom killings.

Place	Time	Cell count (cells.ml^{-1})	T (◦C)	Sali. (‰)	Chl-a (µg.ml^{-1})	O_2 (%)	Type of killing	Species	Source
Kattegat, Skagerrak area of the Norwegian Coast	May - June, 1988	5-10 × 10^4	10-15	13-28	0.4-2.8	-	Varies	*C. polylepis*	[14, 15]
Skagerrak area, Denmark	June, 1990 - 1991	-	-	-	-	-	-	*C. rotalis*	[16]
Lofoten-Vestfjorden area in northern Norway	May -June, 1991	1-3 ×10^9	9-17, Sunny calm weather	<33	0.6-1.1	86-110	Fish kills (salmon)	*C. leadbeateri*	[17, 18]
Southern coast of Norway	May-June, 1994-1995	-	-	-	-	-	-	*C. fragaria*	[12]

Borre Knob, Denmark	August, 1997	-	commonly <15	8-30	-	-	-	*C. ahrengotii*	[19]
Jiaozhou Bay, Yellow Sea, China	November, 2001	-	15.1	30	-	-	-	*C. planisquama*	[20]
Cantabrian Sea, northern Spain	March-September, 2003	-	18-24	32-34	-	590-790	-	*C. palbralis*	[21]
***Al-Nawras, Jeddah, Saudi Arabia**	October, 2016	2.5-4 × 10^4	22-26, sunny calm weather	11.5-20.6	0.34-0.46	14-35	Fish kills (81ardine)	*Chrysochromulina sp.*	Analysis results performed by the Ministry of Environment, Water Agriculture, Fisheries Research Center and Jeddah Fish Health & Safety Laboratory (2016)
***Al-Arbaeen, Jeddah, Saudi Arabia**	2December, 2016	2-3.5 × 10^6	23-30, partly sunny calm weather	22.2-23.7	0.34-0.44	30-55	Various	*Chrysochromulina sp.*	

This work showed a difference in the effect of physical factors (temperature, salinity, pH, light duration, and light intensity) on the locally isolated species *Chrysochromulina* used in this study. Our results indicate that there was a significant effect between all four growth characters and the physical factors, as demonstrated in the study by [22] during a 14-y time series analysis of the relationship between the annual abundance of *Chrysochromulina* sp. and environmental conditions.

As shown in **(Table 1)** measurements vary between blooms, cell count ranges from 5-10×10^4 cells.ml^{-1} [14, 15], to 1-3×10^9 cells.ml^{-1} [17, 18]. Our cell count in this study at the time of the bloom was 2-3.5×10^6 cells.ml^{-1} and within cultures, cell count was established at average 3×10^4 cells.ml^{-1}, which are both in the range of other similar blooms.

Cell size variation of *Crysochromulina sp.* **(Table 2)** appears to be a general pattern proved by [14], who showed that cells were larger in the evening than on the following morning and cell size decreased with depth. This pattern indicates that *Chrysochromulina sp*. divides for the most part during the dark period which confirms our results that light is the most influencing factor.

Table 2. A comparison of some *Chrysochromulina* species characters including habitat (Hab), dimensions and shape of the cell body, dimensions of flagella, haptonema (length, comments, and position), and toxicity of the species. (Redrawn from [7]).

Species	**Hab**	**Shape**	**Length**			**Width**			**Flagella ave.**	**Haptonema ave.**	**Haptonema comments**	**Position**	**Toxic**	**Source**
			Min.	**Ave.**	**Max.**	**Min.**	**Ave.**	**Max.**						
C. acantha	M	saddle	6	8	10	6	8	10	20	40	-	-	N	[23]
C. ahrengotii	M	saddle	4	5	7	4	5	6	16.5	64	Coiling, longer than flagella	-	N	[19]
C. apheles	M	saddle	3	4	4	3	5	6	11	30	-	-	N	[24]
C. brevifilum	M	spheroid	4	6	12	4	6	12	17	13	Rarely coiling	-	Y, N	[25]
C. camella	M	saddle	-	14	-	-	16	-	25	160	-	-	N	[26]
C. campanulifera	M	saddle	-	10	-	-	10	-	25	50	Longer than flagella	-	-	[27]
C. cymbium	M	saddle	-	7	-	-	7	-	20	60	-	-	-	[26]
C. discophora	M	spherical	-	10	-	-	10	-	25	30	>25µm	-	-	[28]
C. ericina	M	ovoid	5	8	12	4	7	10	18	37	coiling	All	N	[29, 30]
C. eyelash	M	saddle	10	11	12	10	11	12	22	7.5	Non-coiling	-	-	[31]

C. fragaria	M	spherical	4	6	4	4	6	8	13	6	Shorter than flagella, rarely coiling	-	N	[12]
C. hirta	M	-	-	6	12	-	6	12	20	30	Longer when extended	All	N	[32, 33]
C. kappa	M	spheroidal	4	6	10	4	6	10	11	14	A little longer	-	Y, N	[25]
C. latilepis	M	ovoid	-	9	-	-	6	-	15	25	Longer than flagella	-		[34]
C. leadbeateri	M	spherical	3	6	8	3	6	8	17	22	Coiling, longer than flagella	-	Y, N	[17]
C. minor	M	spheroid	3	4	8	3	4	8	11	9	Shorter than flagella	-	N	[25]
C. parkeae	M	elongate	10	20	30	5	8	10	20	5	Non-coiling	polar	-	[35]
C. polylepis	M	ovoid	6	9	12	5	7	9	23	11	-	-	Y, N	[36, 37]
C. pringsheimii	M	cylindrical	12	17	24	4	7	9	30	27	-	polar	N	[38]
C. rotalis	M	saddle	4	5	6	4	5	6	11	38.5	coiling	-	-	[16]
C. scutellum	M	saddle	4	7	9	4	5	8	16	46.5	-	-	N	[39]
C. simplex	M	-	-	6	-	-	5	-	14	78	-	-	N	[40]
C. spinifera	M	-	8	9	10	7	8	9	9	4.5	Non-coiling	all	-	[41]
C. tenuispina	M	globose	8	11	13	8	11	13	25	20	A little shorter than flagella	all	-	[32, 33]
C. throndsenii	M	saddle	5	6	6	5	6	6	12	41	-	-	N	[12]
C. vexillifera	M	-	-	8	-	-	6	-	20	25	>20µm	polar	N	[42]
C. breviturrita	F	spheroidal	6	10	16	6	10	16	22	12	-	-	-	[43]
C. parva	F	-	3	5	7	3	5	7	15	75	-	-	N	[44]
This study	M	saddle	2	3	4	1	1.5	2.5	17	22	Coiling, longer than flagella	polar	N	

Previous studies of light-regime (light duration and light intensity) growth generally worked with 12:12 h light and dark cycle and with saturation range from 100 to 250 µmol $m^{-2}s^{-1}$ [11, 20, 45, 46]. Our best light duration values for growth rate were found at 10:14 h L:D and for light intensity, result ranges from 60 to 70 µmol $m^{-2}s^{-1}$ similar to the range taken by [13], which both light results were the degrees at the time of the bloom.

Chrysochromulina sp. grew well over a wide temperature range. Blooms have been recorded from 10°C [14] to 24°C [21], and this species tolerated temperatures from 10°C to 35°C. The best growth rate value was found at 15°C, which is similar to many studies that used the same temperature for a close to optimum growth [11, 13, 18, 20, 45, 46].

According to [46] comparison growth is highest around moderate salinities 20-25 PSU, and this species survived all salinities from 5 to 40 PSU. The best growth rate, dry weight, and production per dry weight values were found at 40 PSU similar to the results found with high salinities by [11, 13, 18, 21, 46]. The high salinity is a characteristic of the Red Sea about 35 to 40.5 PSU [47], thus, explaining the growth at such high salinity.

PH as a factor in this study had the lowest effect on the growth values tested because pH can affect growth and survival of marine phytoplankton when it exceeds 9 to 9.5 according to [13].

Most likely the differences of our growth parameters are due to variation of strains, climate adaptation, and the grazing (feeding on bacteria) ability of this species since both areas (Al-Nawras and Al-Arbaeen) are contaminated by effluent discharge: sewage and groundwater sewage and not refreshed regularly according to the study by [48].

Conclusion

In conclusion, this study indicated that suitable light conditions have the most effect on increasing *Chrysochromulina sp.* growth rate and dry weight among other contributory physical factors. Moreover, results showed that both isolates from Al-Nawras and Al-Arbaeen lagoons had no significant differences and both are for the same species and strain. The results showed no acute toxicity present. Therefore, fish mortality is most likely related to the morphological aspect of the isolated species from the blooms. Whereas, the two long flagella and haptonema clogging of fish gills accompanied with suitable environmental conditions and a very low dissolved Oxygen level in both lagoons, all were the cause that leads to the fish mortality. Since the all growth count was lower than the count at the time of the bloom subsequently other auxiliary conditions contributed to bloom formation such as grazing combined with suitable climate conditions and low water oxygen. Thus, future studies should focus on suitable light conditions, grazing, nutrient limitation, and competition with other phytoplankton also DNA phylogenies, and morphology.

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