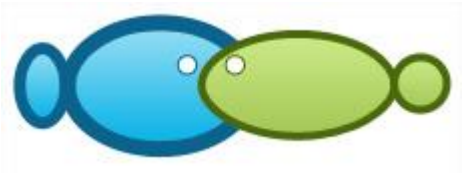

Effect of temperature, irradiance and nutrients on the growth of *Amphidinium* cf. *carterae* from Atlantic Morocco and bioassay on *Artemia salina*

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Abstract. Some environmental parameters such as temperature, irradiation, salinity, pH, nutrient concentration can be classified as important factors to promote the growth and proliferation of toxic or harmful microalgae. Studies on the effect and variation of these environmental parameters on the growth of benthic dinoflagellates such as *Amphidinium carterae* in Moroccan Atlantic waters are rare. Therefore, in this study, which is the first in this region of Morocco, the growth of *Amphidinium* cf. *carterae* and its lethal effect after 24 h on the brine shrimp *Artemia salina* was evaluated. *A.* cf. *carterae* cultures were exposed to three temperatures (15, 20 and 25°C), three irradiation levels (20, 80 and 130 µmol m^{-2} s^{-1}) and five concentration (µM L^{-1}) ratios of nitrate/phosphate in the medium (882.4/36.2; 1,323.6/54.3; 1,764.8/72.4; 2,206/90.5 and 2,647.2/108.6). At the end of our experiment, the specimens were able to survive all temperatures, irradiances and nutrient concentrations tested. The highest growth rate was 0.86 day^{-1} recorded at a temperature of 20°C, an irradiation level of 130 µmol m^{-2} s^{-1} and a nitrate/phosphate concentration of 882.4/36.2. The cell density, in all cultures, was approximately 10^5 cell mL^{-1}. The lethal effect of *Amphidinium* on *A. salina* started at a concentration of 1.15×10^6 cell mL^{-1} with 10% mortality, up to 100% for 9.2×10^6 cell mL^{-1}. The LC_{50} obtained was 2.4×10^6 cell mL^{-1}. This study established a first approach in the understanding of the bloom of the harmful benthic dinoflagellate *A.* cf. *carterae*. The aim was to strengthen the monitoring of toxic/harmful species and to anticipate potential blooms along the Moroccan coast by delimiting the periods and areas at risk.
Key Words: benthic dinoflagellate, growth rate, batch culture, toxicity.

Introduction. *Amphidinium* is a genus of dinoflagellates that is widely distributed throughout the world in a wide variety of habitats and temperatures (Murray & Patterson 2002). They grow easily in culture and can be scaled up to larger mass volumes. The genus is known to form non-pelagic blooms in tropical and temperate coastal waters (Gómez et al 2015). But, their mechanism of toxicity in the environment remains to be proven. However*, Amphidinium* produces toxins and bioactive compounds that may have harmful effects, haemolysins 1 and 2. These compounds have been shown to be haemolytic. Cytotoxic and ichthyotoxic effects have also been attributed to them. Rhodes et al (2010) administered culture extracts intraperitoneally or orally to mice, which caused respiratory paralysis. On the other hand, culture supernatants caused the death in the fish species *Fundulus heteroclitus* (Lassus et al 2016). Also in Australia, in a coastal lagoon, a bloom of *Amphidinium carterae* co-occurred with the death of more than 300 individuals of different fish species (Murray et al 2015). According to Baig et al (2006), the toxins produced by *Amphidinium* could increase the effect of ciguatera poisoning, as *Amphidinium* is often found in association with the ichthyotoxic species *Gambierdiscus*. *A. carterae* is also known to produce compounds with antifungal and antimicrobial properties, called "the amphidinols" (Nuzzo et al 2014). Until 2016, amphidinol-3 was considered the most active secondary metabolite produced by *Amphidinium* cf. *carterae* (Aquino-Cruz & Okolodkov 2016) and shows potent haemolytic

activity against human erythrocytes and antifungal activity against the ascomycete fungus *Aspergillus niger* van Tieghem (Vicente et al 2006).

According to Lassus et al (2016), the production of endotoxin or exotoxin appears to be a response to different kinds of stress, caused either by particular environmental conditions or by predatory behavior, and moreover it cannot be excluded that these species are involved in the transfer of toxins to other marine organisms. Among the environmental conditions in benthic ecosystems, changes in water temperature determine the seasonal trends and densities of microalgae communities (Edwards et al 2006). Seawater temperature plays an important role in the physiological activities of various algae. It has been suggested that ocean warming and eutrophication may cause the distribution of toxic microalgae species to expand from tropical areas to higher latitude areas (Wells et al 2015). Irradiance is another important environmental factor that influences the physiology of microalgae. Furthermore, it would seem that the light intensity required by a clone reflects its habitat more than the phylogenetic class to which it belongs (Brand & Guillard 1981). Although the harmful effects of this benthic genus in Moroccan waters have not yet been reported, its study remains no less important. According to the National Agency for the Development of Aquaculture (ANDA) (https://www.anda.gov.ma/), the aquaculture sector in Morocco has a significant potential estimated at 380,000 tons year^{-1} of confirmed capacity along the coastline with several hectares available to date for the realization of aquaculture farm projects.

Given the limited data available on harmful benthic dinoflagellates along the Moroccan Atlantic coasts, many uncertainties remain as to how environmental parameters might accelerate the growth of benthic dinoflagellates and their toxicity. This research aimed to determine the effect of increasing temperature, irradiance and nutrient concentration (nitrates and phosphates) on the growth of *A.* cf. *carterae* and to determine its toxic effect on *A. salina,* in order to strengthen the monitoring of toxic/harmful species and to prevent potential cases of bloom on the Moroccan Atlantic coast.

Material and Method

Sampling and establishment of cultures. The dinoflagellate *A.* cf. *carterae* was kept apart from seawater samples from the Tamri Cape Ghir shellfish growing area in the South Atlantic of Morocco (30°42'41N/09°51'44W-30°37'17N/09°52'47W). Isolation was made by capillary pipette (Tsuchikane et al 2018) under the microscope. Microalgae monocultures were started in Elisa microplates: 96-well plates were filled with L1 medium (Guillard & Hargraves 1993) without silica. The medium was prepared with 0.2 µm filtered seawater and then autoclaved at 121°C for fifteen minutes (Guillard & Morton 2003). Elisa microplates were placed in a culture chamber (Binder model KBW 240) with programmable day and night cycles, at a temperature of 20±1°C, 12:12h light-dark cycle. Illumination was provided by white fluorescent lamps, under a photon flux density of 80 µmol m^{-2} s^{-1}. The cells were examined every single day under an inverted microscope (Leica microsystems, DMi8) to ensure the purity of the strains. Subsequently, the growing cells in the wells were transferred to 100 mL Erlenmeyer flasks filled with L1 medium without silica, placed under the same conditions. All monocultures were axenic.

Growth experiments: determination of optimal growth conditions. The growth experiments were done in batch culture with single strains of *Amphidinium*. All growth experiments were performed in sterilized Erlenmeyer flasks containing 50 mL of culture medium. They were conducted in the KBW 240 culture chamber, in L1 medium without silica, with a salinity of 36 psu and a 12:12 h light-dark cycle. Each growth experiment was done in triplicate (n=3). Cultures were exposed to three temperatures 15, 20 and 25°C. The irradiance in all three temperatures was 20 µmol m^{-2} s^{-1}. The temperature that gave a statistically significant growth will be maintained for the rest of the experiments. In the opposite case (non-significant difference) the temperature that gave the highest growth rate will be used. It will be the same for the irradiance and the concentration of nitrates and phosphates of the medium L1. They are three irradiances that were tested

20, 80 and 130 µmol m^{-2} s^{-1} (the value of 20 µmol m^{-2} s^{-1} was used in the temperature experiment). The different irradiances were measured using a Quantum Apogee Underwater Full Spectrum Meter MQ-510. After determining the optimal temperature and irradiance, four other nitrate and phosphate concentrations will be tested simultaneously (knowing that the L1 medium without silica has already been tested for the previous experiments). A N:P ratio equal to 24.4 was maintained in the preparation of the media. The concentrations of the L1 medium tested were 1.5×(L1), 2×(L1), 2.5×(L1) and 3×(L1), corresponding to $NaNO_3$/NaH_2PO_4 ratios of: 1323.6/54.3 µM, 1764.8/72.4 µM, 2206/90.5 µM and 2647.2/108.6 µM, respectively.

Estimation of cell abundance. Cultures were sampled every 2 days throughout the growth phase, until the beginning of the decline phase. Erlenmeyer flasks of culture were gently stirred near an electric burner. Culture subsamples (200 µL) were collected in Eppendorf tubes and fixed with lugol solution. Cell density was determined by microscopic counting, using a Malassez cell (Blau Brand, Germany) under a light microscope (Leica DM 1000). Instantaneous growth rates (µ; expressed in day^{-1}) were calculated over the exponential growth phase using the following equation (Guillard 1973):

$$\mu = \frac{(lnN1 - lnN0)}{t1 - t0}$$

Where:
N1 and N0 - the cell abundances at the time t1 and t0.

Cell abundances in each subsample were quantified in triplicate. Growth curves were generated from the average cell density data.

Identification of the species. Cell identification was based on visual observation of general characters and of remarkable morphological attributes of the cells, i.e., according to shape, size, presence of a theca or not and by comparison to reference materials (Hoppenrath et al 2014; Taylor et al 2003; Murray & Patterson 2002), in order to determine the cells size. Cells were fixed with acid lugol (final concentration 1%) and examined under an inverted microscope (Leica microsystems, DMi8) at ×40 magnification. Measurements were taken using the progressive microscope cameras software analysis tool, JENOPTIK. A total of 30 cells were measured in length and width among the pre-cultures.

Cell lysis. The method for cell lysis was adapted from Pagliara & Caroppo (2012) and Moreira-González et al (2019). *A.* cf. *carterae* cells from an exponential phase culture were collected by centrifugation (30 min at 3,500×g). To the pellet, 1 mL of distilled water was added and the suspension was subsequently sonicated with an ultrasound probe (Branson Ultrasonics Coperation) 3 times for 10 min on ice. The sonicated sample was checked under a microscope to ensure cell lysis and a cell count was performed to estimate the number of remaining intact cells, which were then subtracted from the initial cell density to calculate the cell equivalence of the extract. Then, the sample was left on ice for 1 h in the dark before centrifugation at 20,000×g for 30 min. The lysate supernatant was recovered and stored at -20°C until use.

Bioassay on Artemia salina. Three hundred milligrams of *A. salina* cysts were hatched in 300 mL of seawater at 25°C with constant light and aeration. After 24 h, the *Artemia nauplii* (1 day old) were collected. Ten *A. nauplii* were contacted with 100 µL of the cell lysate at different concentrations of the lysate; a dilution factor of 2 was applied. The concentrations tested were 1.84×10^7 cell mL^{-1}, 9.2×10^6 cell mL^{-1}, 4.6×10^6 cell mL^{-1}, 2.3×10^6 cell mL^{-1}, 1.15×10^6 cell mL^{-1}, 5.75×10^5 cell mL^{-1} and 1.15×10^5 cell mL^{-1}. Filtered and sterilized seawater was used as negative control. The test was performed in 96-well Elisa plates, incubated at 25°C in the dark. For each concentration, three replicates were

performed. After 24 hours, *A. nauplii* showing no appendage movement during 10 seconds of observation were considered dead and results are expressed as percentage of dead nauplii after 24 hours (Laza-Martinez et al 2011). For the *A. salina* bioassay, the mortality rate ± SEM for each concentration was calculated based on the 24 h control. The LC_{50} value was determined using probit analysis as described by Finney (1971) with Microsoft Excel version 2013 software.

Statistical analysis. The calculated growth rates were tested for statistical differences by the Anova one way test ($p<0.05$). The homogeneity of group variances was checked by Levene's test and normality of residuals for each group by Shapiro-wilk test. The Kruskal-Wallis test, the nonparametric equivalent of the one-way ANOVA test, was used when homogeneity and normality were not confirmed. The SPSS v. 22.0 statistic software for windows (IBM) was used for all statistical tests.

Results

Condition and identification of the cultures. *Amphidinium* cultures induced blooms of stained water of a brown-orange color (Figure 1). The cells obtained in the culture are naked species of small size, oval at the hypocone and flattened dorso-ventrally with an average length of 13.66±0.95 µm, average width of 9.77±0.76 µm and average length/width ratio of 1.4±0.1 (Table 1). They possess a small crescent-shaped epicone asymmetrically oriented to the left in dorsal view (Figure 2). According to these various characteristics, the species obtained would be *Amphidinium* cf. *carterae.*

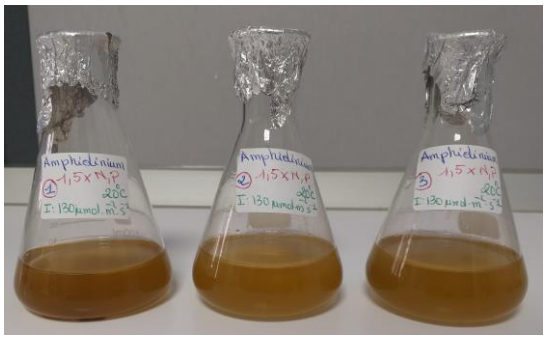

Figure 1. Colored water bloom induced by *Amphidinium* cf. *carterae* culture.

Table 1

Size of *Amphidinium* cf. *carterae* isolated in the Cape Ghir area (Moroccan Atlantic) n=30

Number	*Length*	*Width*	*Length/width*	*Number*	*Length*	*Width*	*Length/width*
1	15.01	10.16	1.5	17	14.44	10.83	1.3
2	14.99	10.57	1.4	18	13.99	9.29	1.5
3	15.32	11.87	1.3	19	13.39	9	1.5
4	12.88	9.83	1.3	20	11.6	8.98	1.3
5	13.25	9.01	1.5	21	13.48	8.21	1.6
6	15.18	10.38	1.5	22	14.95	11.37	1.3
7	16.61	9.07	1.4	23	12.54	8.96	1.4
8	13.37	9.44	1.4	24	12.05	8.5	1.4
9	15.59	11.29	1.4	25	12.42	9.05	1.4
10	12.97	9.09	1.4	26	14.19	10.49	1.4
11	13.49	10.26	1.3	27	13.17	9.81	1.3
12	15.18	10.85	1.4	28	15.28	9.39	1.6
13	13.4	9.55	1.4	29	13.46	9.3	1.4
14	13.03	9.52	1.4	30	11.6	9.81	1.2
15	14.05	10.6	1.3	mean	13.66	9.77	1.4
16	12.9	8.6	1.5	SD	0.95	0.76	0.1

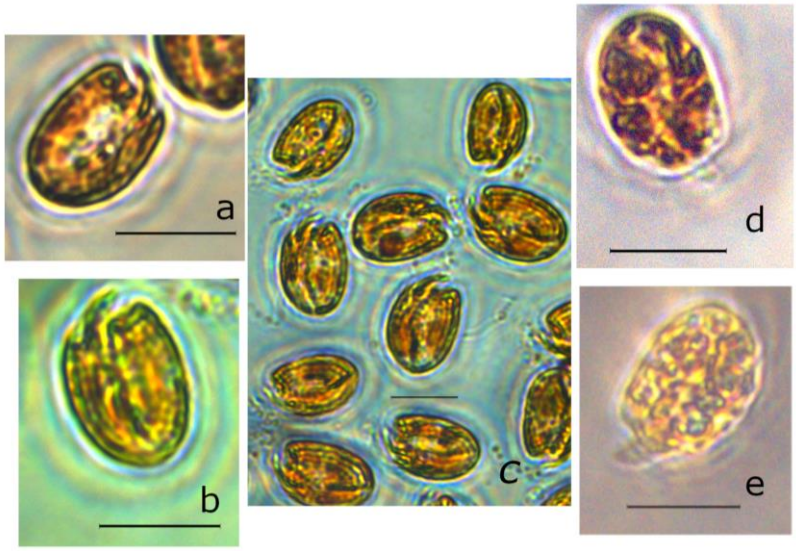

Figure 2. Culture of *Amphidinium* cf. *carterae* magnification ×40, dorsal view with epicone deflected to the left (a), ventral view (b), ventral view with distinct flagella (c), presence of chloroplast, probably single with multiple lobes in the cell (d, e). Scale bar: 10 µm.

Determination of the optimal temperature. The growth curves of average cell densities of *A.* cf. *carterae* (Figure 3) at 20 and 25°C are higher than that at 15°C throughout the growth period until the day of their decline. However, at 15°C the culture has a higher maximum cell density (1.026×10^6 cell mL^{-1}) followed by that at 20°C (9.14×10^5 cell mL^{-1}) and 25°C (7.76×10^5 cell mL^{-1}). The culture at 15°C was the slowest to reach its highest cell density on day 23 followed by those at 20°C on day 16 and 25°C on day 14. During the exponential growth phase, which lasted from day 2 to 11 for 15 and 20°C and from day 2 to 9 for 25°C, the maximum growth rate over the exponential growth phase of *A. carterae* varied from 0.53 to 0.69 day^{-1} (Figure 4). The maximum growth rate was recorded at 20°C (0.69 day^{-1}) followed by 15°C (0.59 day^{-1}) and 25°C (0.53 day^{-1}). However, despite different growth rates, there was no statistically significant difference ($p>0.05$) in the growth of *A.* cf. *carterae* at 15, 20 and 25°C. Therefore, our *A.* cf. *carterae* withstood temperatures ranging from 15°C to 25°C with a decrease in growth at 25°C.

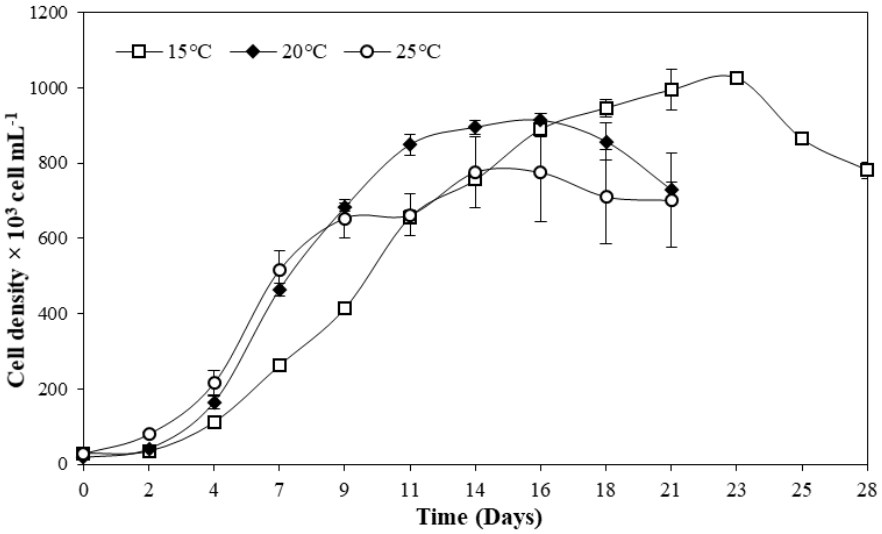

Figure 3. Growth curves of average cell densities of *Amphidinium* cf. *carterae* cultures under temperatures of 15, 20 and 25°C in L1 medium at 20 µmol m^{-2} s^{-1}. The error bars indicate the standard error.

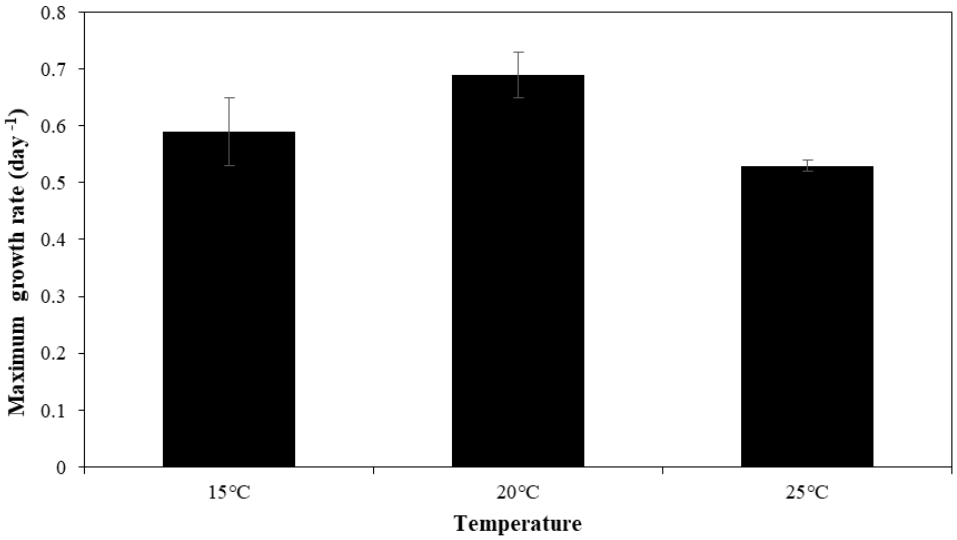

Figure 4. Maximum growth rate of *Amphidinium* cf. *carterae* at temperatures of 15, 20 and 25°C in L1 medium at 20 µmol m^{-2} s^{-1}. The values represent the mean and the error bars indicate the standard error.

Effect of different irradiances at the optimal temperature on the growth of A.* cf. *carterae. The growth curve of *A.* cf. *carterae* at an irradiance level of 80 µmol m^{-2} s^{-1} is higher over the entire experiment, than at 20 and 130 µmol m^{-2} s^{-1} (Figure 5). The level of 80 µmol m^{-2} s^{-1} also has a slightly higher cell density (1.020×10^6 cell mL^{-1}), followed by the level of 130 µmol m^{-2} s^{-1} (9.61×10^5 cell mL^{-1}) and of 20 µmol m^{-2} s^{-1} (9.14×10^5 cell mL^{-1}) curve. The culture under an irradiance level of 80 µmol m^{-2} s^{-1} was the first to reach its maximum cell density on day 11 followed, by the level of 20 µmol m^{-2} s^{-1} on day 16 and the level of 130 µmol m^{-2} s^{-1} on day 21. The exponential growth phase goes from day 2 to 11, day 2 to 9 and day 2 to 7 for the irradiance levels of 20, 80 and 130 µmol m^{-2} s^{-1}, respectively. Under the three irradiances, the growth rates varied from 0.69 to 0.88 day^{-1} (Figure 6). The maximum growth rate was recorded for an irradiance of 130 µmol m^{-2} s^{-1} (0.88 day^{-1}) followed by 80 µmol m^{-2} s^{-1} (0.77 day^{-1}) and 20 µmol m^{-2} s^{-1} (0.69 day^{-1}). Despite the positive correlation between irradiance and the maximum growth rate, the effect on the growth of the species remains almost the same, for the three tested levels (ANOVA; $p>0.05$).

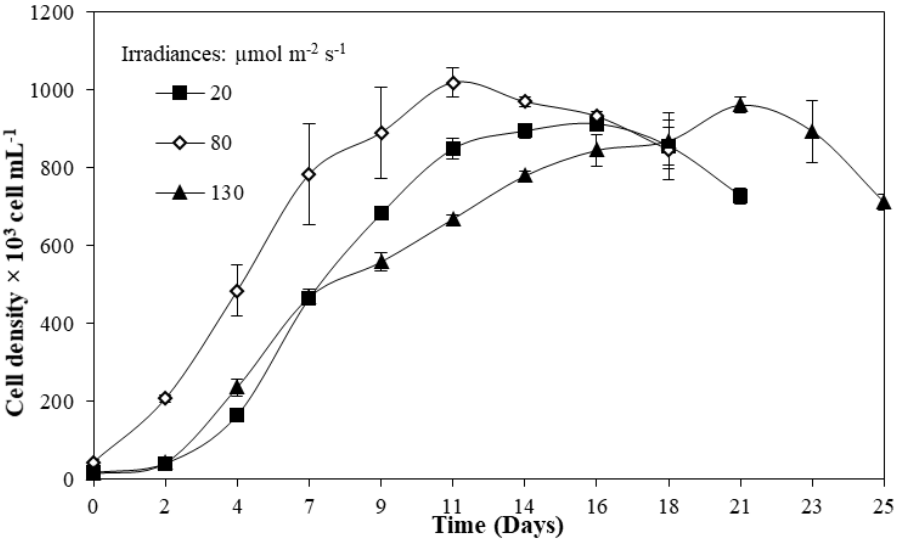

Figure 5. Growth curves of average cell densities of *Amphidinium* cf. *carterae* cultures under irradiances of 20, 80 and 130 µmol m^{-2} s^{-1} in the medium L1 at 20°C. The error bars indicate the standard error.

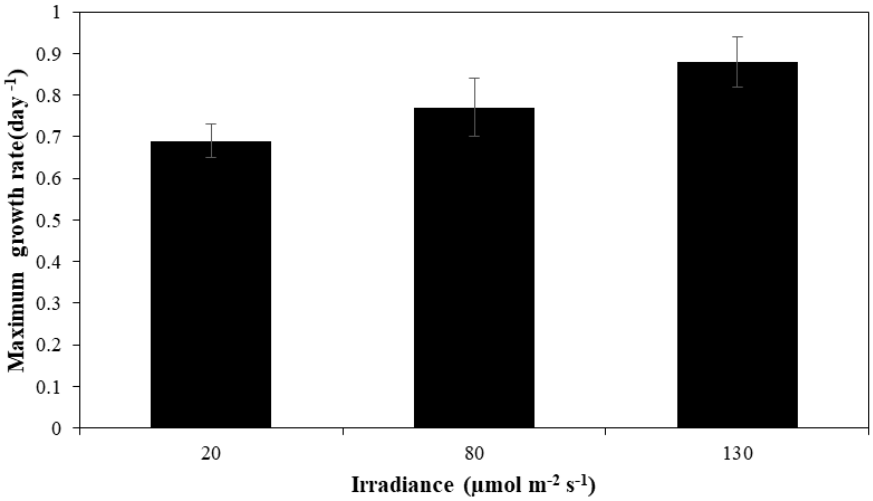

Figure 6. Maximum growth rate of *Amphidinium* cf. *carterae* under irradiances of 20, 80 and 130 µmol m^{-2} s^{-1} in the medium L1 at 20°C. The values represent the mean and the error bars indicate the standard error.

Effect of different nutrient concentrations. At a temperature of 20°C and an irradiance of 130 µmol m^{-2} s^{-1}, the growth curves of *A.* cf. *carterae* at different concentrations of the medium show that until day 11 of growth the curves almost blend, except for 3×L1 which merges until day 7 (Figure 7). L1 has the highest maximum cell density (9.61×10^5 cell mL^{-1}), followed by 2.5×L1 (8.49×10^5 cell mL^{-1}), 2×L1 (8.37×10^5 cell mL^{-1}), 3×L1 (8.00×10^5 cell mL^{-1}) and finally 1.5×L1 (7.45×10^5 cell mL^{-1}). On the other hand, *A.* cf. *carterae* from 1.5×L1; 2.5×L1 and 3×L1 were the first ones to reach their maximum cell density on day 14, followed by those from 2×L1 on day 16 and finally those from L1 on day 21. Of the five medium concentrations tested, the maximum growth rates ranged from 0.57 day^{-1} to 0.88 day^{-1} (Figure 8). L1 medium gave the highest maximum growth rate with 0.88 day^{-1}, followed by 1.5×L1 medium (0.77 day^{-1}); 2×L1 (0.69 day^{-1}); 2.5×L1 (0.58 day^{-1}), and finally 3×L1 (0.57 day^{-1}). All maximum growth rates occurred on day 4. We also notice that the higher the nutrient concentration, the lower the maximum growth. That means that, there is a negative correlation between the concentration of nitrates and phosphates and the growth rate of *A.* cf. *carterae* species. However, there is no significant difference on the growth of *A.* cf. *carterae* for the five nutrient concentrations tested (Kruskal-Wallis test, $p>0.05$).

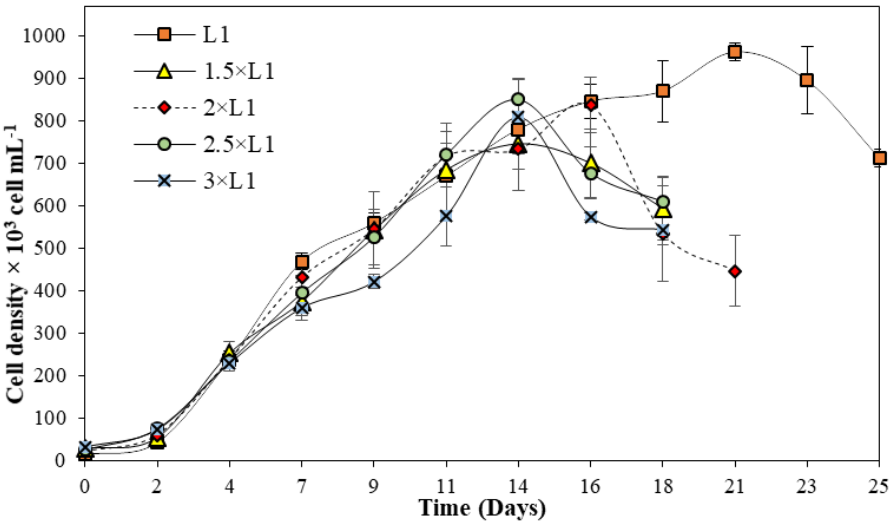

Figure 7. Growth curves of average cell densities of *Amphidinium* cf. *carterae* cultures under different nutrient concentrations in 1; 1.5; 2; 2.5 and 3×L1 at 20°C and 130 µmol m^{-2} s^{-1}. The error bars indicate the standard error.

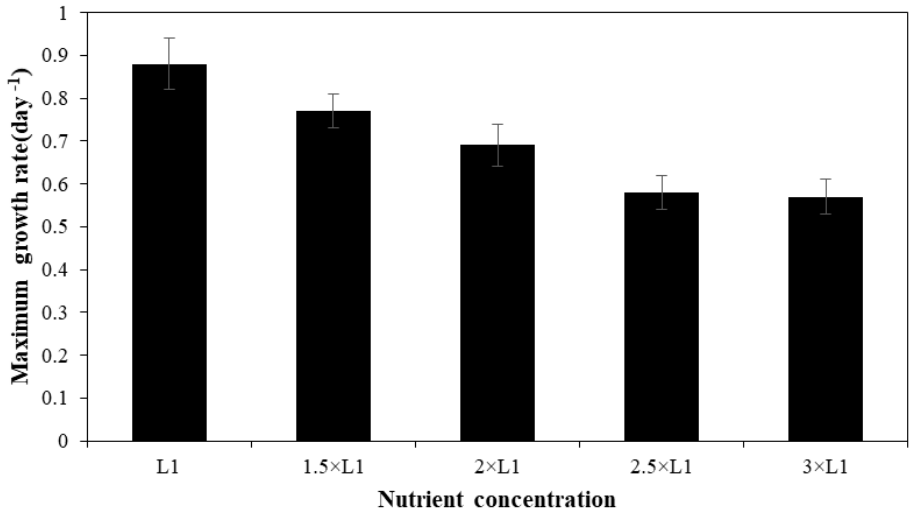

Figure 8. Maximum growth rates of *Amphidinium* cf. *carterae* at different nutrient concentrations in 1; 1.5; 2; 2.5 and 3×L1 at 20 °C under 130 µmol m^{-2} s^{-1}.

Bioassay on Artemia salina. In the *A. salina* lethality bioassay, *A.* cf. *carterae* cell lysate was lethal from 1.15×10^6 cell mL^{-1} with 10% mortality (Figure 8). Cell lysates of the order of 10^6 achieved very high to total mortality rates of 97% and 100% for the concentrations of 4.6×10^6 cell mL^{-1} and 9.2×10^6 cell mL^{-1}, respectively. The lysate of 1.15×10^5 cell mL^{-1} showed no lethal effect, as did that of 5.75×10^5 cell mL^{-1}, and was therefore ignored. The LC_{50} value obtained after 24h was 2.4×10^6 cell mL^{-1}. Also, it is found that there is a strong positive correlation of 0.89 between the concentration of *A* cf. *carterae* cell lysate and the lethal effect on *A. salina*.

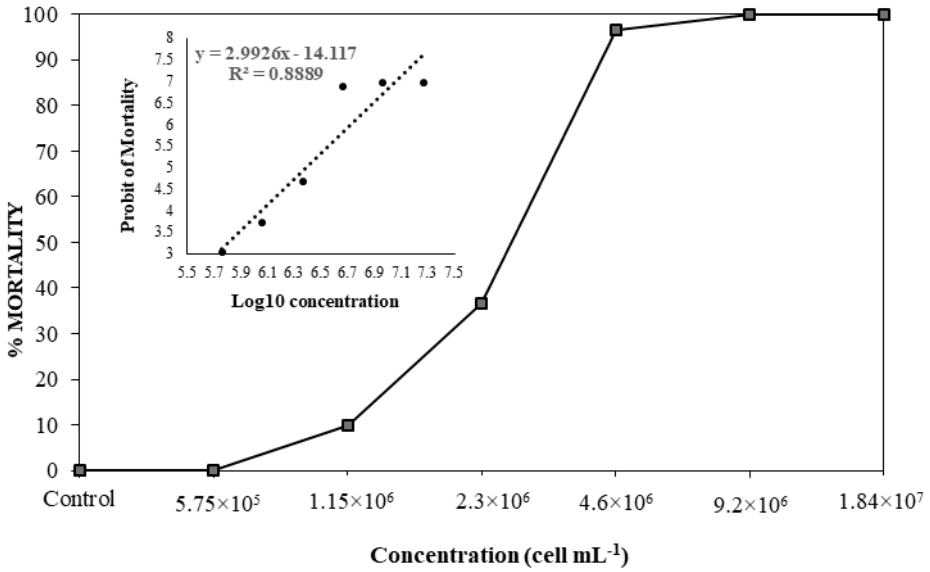

Figure 9. Percentage mortality curve of *Artemia salina* exposed to *Amphidinium* cf. *carterae* cell lysate at concentrations of 5.75×10^5-1.84×10^7 cell mL^{-1} after 24 h. Results expressed as mean±SEM for the six experiments (full curve and grey squares) and linear regression based on probit analysis of percentage mortality and log10 of *Amphidinium* cf. *carterae* cell lysate concentrations (inner graph).

Discussion. *A. carterae* belongs to the genus *Amphidinium* which is a group of very diverse atecal dinoflagellates. They are naked dinoflagellates with cells generally flattened dorso-ventrally, oval in ventral view, with a reduced epicone, crescent-shaped or triangular, and often deviated to the left. *A. carterae* according to Hoppenrath et al

(2014) have a length of 10-17 µm, a width of 7-13 µm and a length to width ratio of 1.2-1.6 µm. According to Murray & Patterson (2002), they have a length of 11-17 µm (most cells having only 13-15 µm), a width of 9-13 µm, a length to width ratio of 1.2-1.6. This description matches perfectly to the species kept apart in the waters of Cape Ghir, in view of the size and particular characteristics related to the species (Table 2).

The results of this study showed that the species *A.* cf. *carterae* tolerates temperatures ranging from 15 to 25°C with optimal growth at 20°C. This is in agreement with the work of Han et al (2004), who found that maximum growth of *A.* cf. *carterae* occurred at 18°C in the China Sea. Similarly, in the southern Gulf of California, Gárate-Lizárraga (2012) reported pelagic proliferation of *A.* cf. *carterae* at a water temperature of 20°C. Ismael et al (1999) found that the species withstood temperatures ranging from 20 to 30°C, with an optimal growth at 25°C in eutrophic waters of Alexandria (Egypt). Aquino-Cruz & Okolodkov (2016) found that temperatures ranging from 25 to 30°C were related to the optimal growth of *A.* cf. *carterae*, which corresponded to their summer surface water temperature of 25°C. Studies led by Somoue et al (2013) at the Moroccan south Atlantic showed that in summer, the maximum temperature was 21.4°C. This temperature is very near the optimal temperature for growth of *A.* cf. *carterae* found in this study (20°C). The species is able to proliferate at water temperatures of 32°C in some areas, such as the Gulf of Mexico (Okolodkov et al 2007). However, Lee et al (2003) also reported survival of *Amphidinium* at temperatures of 34°C, but temperatures above this resulted in death (cell lysis) of *A.* cf. *carterae*. The species could not only proliferate at high temperatures, but also maintain continuous cell growth at 5°C (Aquino-Cruz & Okolodkov 2016). This reflects a remarkable physiological adaptability of the species for colonization and survival in various habitats ranging from warmer tropical waters to temperate or even colder coastal waters. Thus, *Amphidinium* can survive in the different Moroccan marine waters as well as in those of the Mediterranean or the Atlantic. In particular, it has been detected in the Mediterranean waters of M'diq Bay, where summer temperatures are quite warm (Rijalleblad 2012), and as well in those of the South Atlantic, particularly those of Dakhla (Elghrib et al 2012). Indeed, the optimal growth temperature of marine phytoplankton is species or strain specific and is also influenced by different provenances as well as different culture conditions (Boyd et al 2013).

A. *carterae* is a benthic genus and although statistically there was no difference in growth between irradiances, nevertheless it was at the highest irradiance that it gave an optimal growth rate. Indeed 130 µmol m^{-2} s^{-1} gave the best growth rate (0.88 day^{-1}) than the irradiances 80 µmol m^{-2} s^{-1} (0.77 day^{-1}) and 20 µmol m^{-2} s^{-1} (0.69 day^{-1}). This is in agreement with the work of Valenzuela-Espinoza et al (2011), who showed that the species *A.* cf. *carterae* gave higher growth rates at high illumination than at low illumination, thus passing growth rates from 0.40 to 0.66 day^{-1} for irradiances of 50 to 750 µmol m^{-2} s^{-1}. The same is true for Han et al (2004), who tested light intensities of 2,400 and 3,800 lux and found a maximum growth rate at the highest intensity. Ismael et al (1999) also found that the optimal growth intensity of *A*. *carterae* occurred in the highest light intensities of their study (2,900 and 3,420 lux). On the other hand, for the benthic species *Gambierdiscus australes*, their optimal light intensity was 208 µmol m^{-2} s^{-1} and an irradiance of 10 µmol m^{-2} s^{-1} was required to maintain its growth (Yoshimatsu et al 2016). In this study, an irradiance of 20 µmol m^{-2} s^{-1} at the three tested temperatures 15, 20 and 25°C, varied the growth rate by 0.59, 0.69 and 0.53 day^{-1}, respectively, showing that *A.* cf. *carterae* from the Moroccan Atlantic grows also at low irradiances but with optimal growth occurs at higher irradiances.

Growth experiments at different nutrient concentrations ($NaNO_3$ and NaH_2PO_4. $9H_2O$) showed that as the nutrient concentration increased, the growth rate decreased, although there were no significant differences in growth ($P>0.05$) between the different media. In this same context, Valenzuela-Espinoza et al (2011) showed that the growth rate was mainly influenced by the increasing irradiances, rather than by the different concentrations of nitrates and phosphates, at the same cells density.

Table 2

Grouping of sizes and morphological characteristics of *Amphidinium carterae* from the literature and the result of the present study

Species	*Length (µm)*	*Width (µm)*	*Ratio: L/W*	*Shape*	*Species location*	*References*
Amphidinium carterae	12-20	8-10	nd	Oval in shape and flattened dorsoventrally. Only one chloroplast is present which is peripheral and branched with a single prominent centrally placed pyrenoid.	North Arabia Sea	Baig et al (2006)
Amphidinium carterae	18-28	13-18	nd	Oval from the ventral side and flattened dorsoventrally, the epitheca is asymmetric and directed to the left. The cell contains one large, multilobed chloroplast with a central pyrenoid structure.	Gulf of California	Garate-Lizarraga (2012)
Amphidinium carterae	10-17	7-13	1.2-1.6	Cells oval, dorsoventrally flatned. Epicone crescent-shaped, clearly deflected towards the left. Chloroplast, greenish-yellow, probably single with multiple lobes, pyrenoid central.	nd	Hoppenrath et al (2014)
Amphidinium carterae	11-17	9-13	1.2-1.6	The epicone is crescent or tongue shaped in ventral view and much smaller than the hypocone. Chloroplast, greenish-yellow, probably single with multiple lobes pyrenoid central.	South-eastern Australian waters	Murray & Patterson (2002)
Amphidinium cf. *carterae*	12.7-14.6	9.0-10.5	1.3-1.5	Oval in shape, dorsoventrally flatned, epicone deflected to the left in dorsal view, the epicone was tongue shaped in ventral view, presence of chloroplast, probably single with multiple lobes in the cell.	Cape Ghir Atlantic Morocco coast	Present study

nd-no data available.

In this study, L1 medium associated with a temperature of 20°C and an irradiance of 130 µmol m^{-2} s^{-1} gave the highest growth rate of 0.88 day^{-1} and a cell density of 0.96×106 cell mL^{-1}. Dixon & Syrett (1988) showed that a 2 to 8 fold increase in the normal nutrient concentration could increase the population of *A.* cf. *carterae* by more than 50%, but with no effect on the exponential growth rates. In contrast to our study the cell densities with variations in nutrient concentrations are almost similar but like stated by the latter authors, there was no effect on the exponential growth rates. On the other hand, the Moroccan coast is one of the five known areas in the world influenced by the upwelling phenomenon, and the Cape Ghir region is characterized by a seasonal upwelling activity (Ismail et al 2017). This activity brings deep, cold, nutrient-rich water to the surface, which promotes photosynthesis (Makaoui et al 2005) and thus phytoplankton blooms. However, according to our results, the *A.* cf. *carterae* of Cape Ghir seem to evolve independently of nutrient inputs, which may explain the non-detection of their bloom during the upwelling period.

In this study, we limited ourselves to the effect of different nutrient concentrations on the growth rate of the species. However, Aquino-Cruz & Okolodkov (2016) showed that increasing the temperature from 5 to 25°C resulted in high nutrient consumption during the exponential growth phase. The highest consumption occurred between 20 and 25°C, while a higher temperature (30°C) caused the lowest nutrient consumption, although still within the optimal growth range (25-30°C); the irradiance was 35-70 µmol m^{-2} s^{-1}. The study performed by Valenzuela-Espinoza et al (2011) revealed that the irradiance associated with the best growth rate of *A.* cf. *carterae* Hulburt is not the one that allows the best nutrient uptake, in a high nutrient medium and that as the medium became rich in nutrient, the nutrient uptake decreased. This could explain the decreased growth rates of the crops observed in our study during high nutrient inputs. Regarding the limiting factor in crops, Aquino-Cruz & Okolodkov (2016) have shown that phosphates are absorbed more rapidly and they are the limiting factor during the stationary growth phase. In contrast, the studies published by Dixon & Syrett (1988) and Valenzuela-Espinoza et al (2011) have shown that nitrates are the limiting factors in cultures. In this study, the N/P ratio was kept constant in the growth experiments, which does not allow to specify the limiting nutrient.

A. cf. *carterae* in our Cape Ghir area severely impacted the vitality of *A. salina* with an LC_{50} of 2.4×10^6 cell mL^{-1}. This is in agreement with the studies conducted by Pagliara & Caroppo (2012) in the northern Ionian Sea, which shows that *A. carterae* lysate highly affects the vitality of *A. salina*, although their tested LC_{50} of 3.67 mg. mL^{-1} (equivalent to 6.62×10^6 cell mL^{-1}) was much higher than ours. Moreover, they determined that such a concentration of *A.* cf. *carterae* also has an effect on the development of the sea urchin embryo. On the other hand, for Baig et al (2006), in the northern Arabian Sea in Pakistan, the culture filtrate of *A. carterae* did not appear to show significant toxicity to *A. salina*: the highest mortality observed was 13% at the highest dose 2.5×10^5 cell mL^{-1}. However, in albino mice, temporary effects were observed, such as: muscle contractions in the lower back, increased respiration, immobility and paralysis of the hind legs, at the concentration of 4×10^4 cell mL^{-1}. In this study, the lethal effect resulting from the bioassay of *A. salina* challenged with *A* cf. *carterae* is consistent with the presence of toxin and harmful bioactive compounds in *Amphidinium* species. Indeed, benthic dinoflagellates can produce potent secondary bioactive metabolites, especially phycotoxins (Kobayashi 2008). *Amphidinium* species produce many bioactive substances such as Amphidinols, Amphidinolides. These compounds present various toxicological impacts, including hemolytic, cytotoxic, ichthyotoxic, antifungal and antibacterial effects (Lassus 2016; Rhodes et al 2010; Nuzzo et al 2014; Vicente et al 2006). On the contrary, some authors have indicated *A.* cf. *carterae* as a non-toxic species (Aligizaki et al 2009; Mohammad-Noor et al 2007). This could be explained by genotypic variability within the species (Murray et al 2004), as well as by insufficient monitoring of potential adverse effects, thus suggesting that both toxic and non-toxic strains of *A. carterae* exist. However, Vasconcelos et al (2010) stated that marine toxins impact the early life stages of invertebrate and vertebrate species; the same is true in our study.

Conclusions. This is the first study to evaluate the impact of temperature, nutrient concentration and light intensity on the growth of *A.* cf. *carterae* in the Moroccan Atlantic coast and to characterize its potential toxicity by a bioassay. Our optimal growth conditions of the potentially toxic species *A.* cf. *carterae* in-vitro will allow us to determine the periods and areas at risk of such a proliferation along our Atlantic coast. Also, the bioassay on *A. salina* allowed us to reveal the potential toxic effects linked to the bioactive compounds produced by *A. carterae* and their impact on the first life stage of invertebrates. Monitoring of *A. carterae* blooms would be precautionary, given the effect it could have on the ecology of marine organisms and the adverse effects in the fisheries and aquaculture sector. Therefore, complementary studies should be carried out in order to enrich the database necessary for the prevention and management of eventual blooms of this dinoflagellate.

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