Can increases in temperature stimulate blooms of the toxic benthic dinoflagellate *Ostreopsis ovata*?

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**ABSTRACT**

*Ostreopsis ovata* Fukuyo is an epiphytic, toxic dinoflagellate, inhabiting tropical and sub-tropical waters worldwide and also in certain temperate waters such as the Mediterranean Sea. Toxic blooms of *O. ovata* have been reported in SE Brazil in 1998/99 and 2001/02 and the French–Italian Riviera in 2005 and 2006. These blooms had negative effects on human health and aquatic life. Chemical analyses have indicated that *O. ovata* cells produce palytoxin, a very strong toxin, only second in toxicity to botulism. Increase in water temperature by several degrees has been suggested as the reason for triggering these blooms. Four laboratory experiments were performed with *O. ovata* isolated from Tyrrhenian Sea, Italy to determine the effects of water temperature and co-occurring algae on the cell growth and/or the toxicity of *O. ovata*. The cells were grown under different temperatures ranging from 16 °C to 30 °C, and cell densities, growth rates and the cell toxicities were studied. Results indicated high water temperatures (26–30 °C) increased the growth rate and biomass accumulation of *O. ovata*. In mixed cultures of *O. ovata* with other co-occurring algae, biomass decreased due to grazing by ciliates. Cell toxicity on the other hand was highest at lower temperatures, i.e., between 20 and 22 °C. The present study suggests that sea surface temperature increases resulted by global warming could play a crucial role inducing the geographical expansion and biomass accumulation by blooms of *O. ovata*.

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1. Introduction

Climate-induced changes and other anthropogenic activities have been attributed to be the major driving forces behind the stimulation, distribution and the intensification of harmful algal blooms on the last decades (Van Dolah, 2000; Anderson et al., 2002; Hallegraeff, 2003; Granéli, 2004; Glibert et al., 2005; Cloern et al., 2005). The ecological and biogeochemical significance of blooms depend strongly on the species composition of the growing community, which is shaped by changing physical dynamics over multiple time scale (Cloern et al., 2005). These physical dynamics may vary from the local winds and heat flux that result in a stratification inside a small bay, to the ocean – basin scale atmospheric processes that produce variability in wind-driven circulation patterns (Cloern et al., 2005).

There is near unanimous scientific consensus that the world may be entering a period of global warming in response to atmospheric greenhouse gas accumulation generated due to human activities (Van Dolah, 2000). According to the Intergovernmental Panel on Climate Change, continued emissions will lead to increase of world average temperature by 1.8–4 °C over the 21st century (IPCC, 2007). Since the oceans are core components of the climate system, these changes have a particular significance on the structure and function of the marine ecosystems. The direct and indirect impacts of the increases in greenhouse gas concentrations on the ocean will include increasing sea surface temperatures, acidification, changes to the density structure of the upper ocean which will alter vertical mixing of waters, intensification/weakening of upwelling winds and changes in the timing and volume of freshwater runoff into coastal marine waters (Moore et al., 2008).

The processes that select a particular algal species to increase in cell numbers ultimately forming a bloom are still unresolved questions (Cloern et al., 2005). It is however, well understood that the growth and toxicity of many toxic dinoflagellates are influenced by physical factors such as temperature, salinity, light and by the amount of inorganic nutrients available, which play a significant role on the bloom formation (Morton et al., 1992; Granéli and Flynn, 2006).

Warmer temperatures may result in expanded ranges of warm water Harmful algal species such as Gambierdiscus toxicus, which was evident by the high abundance and extended distribution of *G. toxicus* in higher latitudes in response to elevated sea surface...
temperature during the warm phases of ENSO cycle (Hales et al., 1999; Chateau-Degat et al., 2005). Low temperature has been reported to decrease the growth rate and to increase the toxin concentration of PSP (Paralytic Shellfish Poisoning) producing dinoflagellates such as, _Alexandrium catenella_, _A. cohorteula_ and _Gymnodinium catenatum_ (Ogata et al., 1989; Granéli and Flyn, 2006). Indirect impacts of increased temperature may also favor algal blooms, e.g., coral bleaching events free up space for macroalgae to colonize making available more habitats for benthic epiphytic toxic dinoflagellates to explore (Moore et al., 2008).

Interactions of toxin producing algal species with non-toxic algal species can be a factor of ultimate importance, on the structuring of the plankton communities. Specially if the toxins are the same as allelopathic substances, which are released into the water, enabling the harmful species to out-compete co-occurring phytoplankton species (Granéli and Johansson, 2003b; Legrand structuring of the plankton communities. Specially if the toxins are epiphytic toxic dinoflagellates to explore (Moore et al., 2008).

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Many less information is available in the international scientific literature regarding interactions concerning benthic harmful dinoflagellates. The existing knowledge on species distribution and factors involved in the production of toxins are on the benthic dinoflagellates producing ciguatera (Morton et al., 1992; Van Dolah, 2000; Lewis, 2001; Chateau-Degat et al., 2005; Yasumoto et al., 1977).

*Ostreopsis ovata* Fukuyo is a toxic benthic dinoflagellate, inhabiting tropical and sub-tropical waters worldwide. *Ostreopsis* species are often found in epiphytic association with red and brown macroalgae (Faust et al., 1996; Granéli et al., 2002). Recent occurrence of *O. ovata* blooms has been also recorded from many temperate regions (Shears and Ross, 2009). *O. ovata* has attracted attention during the past years due to its unusual blooms and production of palytoxin, which is one of the most potent known existing toxin. *O. ovata* and *O. lenticularis* have been shown to produce palytoxin and its analogues (e.g. ostreosin-D), that can cause human fatalities by accumulating higher up in marine food webs (Granéli et al., 2002; Rhodes et al., 2002; Ashton et al., 2003; Taniyama et al., 2003; Ciminiello et al., 2006; Monti et al., 2007). Observation of toxic compounds found both in *Ostreopsis* cells and shellfish tissues collected in Greek coastal waters (Aligiazaki et al., 2008) also in *Ostreopsis* cells and parrot fish, collected from Tokushima Prefecture, Japan (Taniyama et al., 2003) indicate that the toxins produced by *Ostreopsis* cells can be transferred to higher trophic levels via food chains. Aerosol containing toxins from the bloom of *O. ovata* in Genova, Italy in 2005 and 2006 have caused respiratory problems for more than 150 people, who had to be hospitalized (Brescianini et al., 2006; Durando et al., 2007). In Cabo, Frio, SE Brazil in summer of 1998/99 and 2001/02, sea urchins (*Echinometra lucunter*) started to loose their spines, finally facing death due to necrosis after ingestion of *O. ovata* cells, epiphytic on the seaweed species. During the time of the blooms the water temperatures have been around 22–26 °C (Granéli et al., 2002; Ciminiello et al., 2006). Even though the blooms of *O. ovata* have been recorded at high water temperatures and atmospheric pressures and at low water turbulence (Brescianini et al., 2006), no documentation exists on the environmental conditions and factors promoting palytoxin production in *O. ovata* cells.

The aims of the present study were: (1) to determine the optimum temperature for the growth of *O. ovata*, isolated from the Tyrrenian Sea; (2) to determine whether the optimum temperature to growth would be the same for the highest production of toxin and (3) to find whether *O. ovata* cells produce allelochemicals at high temperatures, which allow the species to out-compete the other co-existing algae. To answer these questions we performed four laboratory experiments with *O. ovata* and its co-occurring species under different temperatures.

### 2. Materials and methods

Four experiments were performed at the Linnaeus University, Sweden. Seawater from the Genova Bay and cultures of *O. ovata*, Kalmar Algae Collection (KAC 85) which was used for the experiments were shipped to Sweden. *O. ovata* cells were isolated from seaweeds collected from the Tyrrenian Sea, Italy in 2007 during a bloom of *O. ovata*. Prior to commencement of the experiments, *O. ovata* cells were grown in f/10 media (Guillard and Ryther, 1962), prepared using water brought from Italy (filtered through Whatman GF/C glass-fibre filters and autoclaved). These cultures were maintained at a salinity of 38 psu, temperature of 25 °C and illuminated under 140 μE cm⁻² s⁻¹ by cool-white Osram tubes and exposed to 16:8 hr light: dark cycle. Algal species found in natural community assemblages were collected from Monte Argentario, Tyrrenian Sea, Italy and brought to Sweden.

#### 2.1. Experiment 1

The aims of the experiment 1 were to determine the optimum temperature for growth of *O. ovata* and effect of temperature on exudation of allelochemicals.

Three treatments were used in this experiment, viz. *O. ovata* monocultures, a natural community assemblage and a mixture of the two. Polystyrene 8 ml Petri dishes (3.5 cm in diameter) were used for the experiment. Stock cultures for controls were prepared by diluting 100 ml of *O. ovata* monoculture and 100 ml of natural community assemblage with 150 ml of f/10 medium and the mixed treatment was prepared by mixing 100 ml of *O. ovata* monoculture and 100 ml of natural community assemblage diluted with 50 ml of f/10 medium to obtain the same initial cell concentrations for each treatment. Five millilitres from each of the three stock solutions were poured into the petri dishes and tightly closed using paraffin. Each treatment was exposed to 6 temperatures (16 °C, 20 °C, 24 °C, 26 °C, 28 °C, 30 °C) in triplicates.

The *Petri* dishes were kept floating on water in 13 L polyethylene aquaria exposed to the temperatures given above, and photoperiod as mentioned above. Temperatures in the aquaria were maintained by using aquarium heaters. Whenever droplets of water were formed inside the lids of the *Petri* dishes, due to evaporation, they were gently shaken to avoid salinity increases in the culture media. Cell densities of *O. ovata* and/or the two most dominant co-occurring algae (Sp. 1 and 2) in each chamber were estimated every second day, by directly counting the live cells after placing the Petri dishes on an inverted microscope (Olympus CKX 41). Cell sizes of *O. ovata*, other dominant co-occurring algal species and ciliates were measured. The experiment was continued for 13 days. Samples from each treatment were subjected to epifluorescence microscopy (Olympus BX50). The specific growth rate (μ) for each culture was calculated during the exponential growth phases at each temperature using the following formula.

$$
\mu = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}
$$

$N_1$ and $N_2$ are the average values of cell numbers at time $t_1$ and $t_2$. 

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E. Granéli et al./Harmful Algae 10 (2011) 165–172
2.2. Experiment 2

Experiment 2 was performed to determine whether the optimum temperature for the growth of O. ovata is same as the temperature for highest toxicity.

Monocultures of O. ovata were used for this experiment and same experimental conditions used for experiment 1 were applied. Samples for analyses of toxin were obtained when cell densities could be visually detected as brownish filaments inside the Petri dishes. Cells were collected by concentrating the cultured cells on 25-mm glass-fiber filters (Whatman GF/C) and they were placed in 2 ml eppendorf tubes. Cells and the cell free filtrates were kept frozen, to be used for the hemolytic test (details are given below). 2.0 ml from each replicate was preserved with acid Lugol’s solution and cells were counted as in experiment 1. One-way ANOVA test was used to determine whether there is a variation in final cell counts in cultures maintained under different treatments (i.e. different temperatures) and Tukey’s pairwise comparison test was performed whenever significant differences were observed.

2.3. Experiment 3

Observation of highest toxicity in O. ovata cultures maintained between 16 and 24 °C in the experiment 2 led to perform the experiment 3 and it was carried out with the objective of determining the most critical temperature for the toxicity of O. ovata, as the interval for temperatures used in experiment 2 were of 4 °C. Experimental design was similar to experiment 2 but the cells were exposed to 17 °C, 18 °C, 19 °C, 20 °C, 21 °C, 22 °C and 23 °C and instead of using Petri dishes, 250 ml Erlenmeyer flasks containing 200 ml of cultures were used. The cell growth was determined by manually counting the number of cells in sub-samples drawn from the cultures every second day and the experiment was continued for 20 days. 60.0 ml of the culture from each replicate was harvested at day 14 and the rest were harvested at the end of the experiment for analyses of toxin concentrations in the cultures. The cell densities and the toxin concentrations were statistically compared in cultures maintained under different temperatures using one-way ANOVA and Tukey’s pairwise comparison test.

2.4. Experiment 4

Experiment 4 was performed to further clarify the pattern of cell growth vs. cellular toxin levels of O. ovata under different temperatures. Cells were grown at the temperatures where the highest (20 °C and 22 °C) and lowest (30 °C) cellular toxin levels were observed in the previous experiments.

Monocultures of O. ovata in f/10 media were grown at above three temperatures and other experimental conditions were maintained as in experiment 3. Except toxin analyses were performed on three occasions representing different growth stages. The experiment was carried out until few days after commencing the declining phase of their growth and therefore the final harvest was taken on day 38. The results were statistically analyzed using one-way ANOVA and repeated measures ANOVA.

2.5. Extraction of toxic substances

Hemolytic tests were performed on sub-samples drawn from the cultures on different occasions (depending on the experiment). Cells in each sample were filtered onto 25-mm glass-fiber filters (Whatman GF/C) and cells retained on the filters were extracted in 1.5 ml methanol for 30 min in the dark. The extracts were used immediately for analyses or stored at −20 °C prior to analyses.

2.6. Hemolytic procedure

The tests were carried out in duplicates. A standard hemolytic curve based on concentrations of saponin (Sigma S-2149) in an isotonic phosphate buffer was used as the reference, and the hemolytic activity of the cells was determined as saponin equivalents. The results were expressed as saponin nano-equivalent per cell (ng SnE cell−1). Hemolytic activity in methanol was also determined, following the same procedure, to exclude the possible toxic effect of methanol that could be interpreted as the toxin itself.

The hemolytic test for cultures in experiment 2 was performed on 5% horse blood using the method adopted by Stolte et al. (2002) and the hemolytic activity was measured with a plate reader (BMG FLUOstar) after an incubation period of 4 h, by measuring the absorbance at 540 nm.

In experiments 3 and 4 the same method was adopted but with some modifications according to Eshbach et al. (2001). The horse blood concentration used was 1% and the absorbance was measured after 4 h at 405 nm instead of 540 nm using the same equipment as in experiment 2.

The HE50 of the algae cells was calculated according to Simonsen and Moestrup (1997) using the following equation:

\[ \text{HE}_{50} \times \frac{C_{50}}{S} \text{ cells ml}^{-1} \]

Where C is the concentration of cells (cells ml−1), S is saponin equivalents (mg ml−1), and K50 is the concentration of saponin (mg ml−1) causing 50% hemolysis of the horse blood cells.

2.7. Cell carbon analysis

The cell carbon contents of O. ovata were analysed in cells retained on 25 mm pre-observed (450 °C, 2 h) Whatman GF/C glass-fiber filters after filtering 30 ml of cultures grown at the same conditions as above, but at 25 °C. The filters were dried at 65 °C for 24 h, and analysed for particulate carbon (POC) with a CHN elemental analyzer (Fisons Instruments model NA 1500). Cell carbon contents of Nitzschia closterium (Sp.1) and Navicula sp. (Sp. 2) were calculated following the stereometric formulas explained by Edler (1979).

3. Results

3.1. Experiment 1

3.1.1. Cell densities

O. ovata cell densities increased at all the temperatures except 16 °C, following a typical growth pattern (Fig. 1(a)). Maximum cell densities (5.67 ± 0.4 × 10^5 cells ml−1) were obtained at 26 °C on day 9 followed by 5.41 ± 0.4 and 5.13 ± 0.4 × 10^5 cells ml−1, observed on the day 13, at 28 °C and 30 °C respectively. On the last day of the experiment the highest cell counts were recorded at 30 °C while the lowest were at 16 °C. The highest specific growth rates were recorded at 30 °C (0.74 ± 0.1), which was followed by that of 28 °C (0.59 ± 0.1) and 26 °C (0.53 ± 0.1), and the lowest were recorded at 16 °C (0.1 ± 0.1).

In the natural community assemblage and in the mixed treatments, Nitzschia closterium and Navicula sp. (25 μm in length and 7 μm in width) were identified as sp. 1 and 2, the two most dominant co-occurring algal species. In the mixed treatments, O. ovata cells decreased to very low levels from day 2 while the cell densities of diatom N. closterium increased. At the end of the experiment, O. ovata cells were not observed in any of the cultures maintained at different temperatures (Fig. 1b). At higher temperatures (28–30 °C) no cells of O. ovata were observed after day 4.
The cell densities of *N. closterium* in the mixed treatment were significantly higher than that of the control treatment of the natural algal community. Even though the cell numbers of *Navicula* sp. in mixed treatments and in control treatments did not show a significant difference, it was fairly high in mixed treatments when compared to the controls. The cell densities of sp. 1 and 2 were higher at lower temperatures than at higher temperatures.

To compensate for the differences in cell sizes in *O. ovata*, *N. closterium* and *Navicula* sp., two most dominant co-occurring algae in the mixed treatment, the biomass accumulation was expressed in terms of carbon content (Fig. 2). *O. ovata* cell carbon (3439.1 ± 563.4 pg cell⁻¹) was much higher than the other two diatoms (13 pg cell⁻¹ and 2.42 pg cell⁻¹ for *N. closterium* and *Navicula* sp. respectively). Total cell carbon contents (µg C l⁻¹) of *N. closterium* and *Navicula* sp. in the mixed treatments were significantly lower than that of *O. ovata* and remained in the same low levels throughout the whole experimental period at all the temperatures except 16 °C. Slight increase in total cell carbon content of *N. closterium* in the mixed treatments at 16 °C was evident which decreased after day 10.

In the mixed treatments at all the temperatures different species of ciliates, in the size range of 90–160 µm (length) and 25–45 µm (width), e.g. *Euplotes* sp. (95 µm in length and 45 µm in width) were observed. Some heliozoans and heterotrophic dinoflagellate, *Oxyrrhis marina* were also present in the mixed treatments.

3.2. Experiment 2

3.2.1. Cell densities

In this experiment 2, *O. ovata* cell densities increased with temperature i.e. the higher the temperature the higher cell densities (Fig. 3). Statistically significant differences were found between the average final cell numbers at different temperatures (ANOVA, *F* = 10.96, *p* < 0.05). Cell densities at 30 °C were significantly higher than those maintained at 16 °C, 20 °C, 24 °C, and 26 °C (Tukey’s, *p* < 0.028). Also cell densities at 28 °C were significantly higher than those maintained at 16 °C, 20 °C (Tukey’s, *p* < 0.016).

3.2.2. Hemolytic activity

Hemolytic activity was found at all temperatures. In contrast to the cell growth, a significantly higher toxicity was observed at 20 °C, which was 18.1 ng SnE cell⁻¹. The second highest value was found for cells grown at 16 °C and it decreased gradually towards the higher temperatures. Thus toxicity of *O. ovata* grown at lower temperatures produces higher amounts of toxins per cell than when grown at high temperatures, where maximum cell numbers were observed (Fig. 3).
3.3. Experiment 3

3.3.1. Cell densities

As in experiments 1 and 2 higher cell densities were found at increasing temperatures (Fig. 4). A significant difference in final cell densities among the treatments was observed (ANOVA, $F = 15.570$, $p < 0.05$). The highest final cell densities were recorded at 23°C, which was followed by the cultures in 21°C and 22°C. The cell densities were $3.05 \pm 0.6$, $2.56 \pm 0.4$ and $2.51 \pm 0.1 \times 10^3$ cells ml$^{-1}$ respectively. The lowest cell densities, $1.08 \pm 0.2 \times 10^3$ cells ml$^{-1}$, were recorded at 17°C.

3.3.2. Hemolytic activity

In all treatments the cells produced hemolytic activity (HA). However, HA on day 14, were lower than that of day 20, except for the cultures growing at 23°C. The highest toxicities, $11.57 \pm 1.9$ ng SnE cell$^{-1}$, were recorded at 22°C while the lowest, $1.93 \pm 0.8$ ng SnE cell$^{-1}$, at 23°C (Fig. 5). Toxicity increased from 17°C to 22°C, with a rapid drop with further increases in temperature. The toxin levels in the cells grown at 22°C were significantly higher than in those grown under other treatments and it was six fold higher compared to the levels observed in the cells grown at 23°C (ANOVA, $F = 10.685$, $p < 0.05$). The results from both experiments 2 and 3 showed that the most critical temperature range for high toxicity of O. ovata was observed between 20°C and 22°C.

3.4. Experiment 4

3.4.1. Cell densities

As in the previous experiments O. ovata had significantly higher cell densities at 30°C than at 22°C and 20°C (Fig. 6) during the stationary phase of the growth curve (ANOVA, $F = 25.424$, $p < 0.05$). The cell numbers in all the treatments did not differ significantly during the declining phase. The maximum cell densities recorded during the experiment were $2.68 \pm 0.3 \times 10^3$ cells ml$^{-1}$, which were recorded on 18th day at 30°C. The cell densities in the cultures grown at 20°C were comparatively low during the whole experimental period.

3.4.2. Hemolytic activity

Although hemolytic activity was found for all treatments, there was a variation in activity along O. ovata growth cycle. Even though the difference in toxin concentrations in the cells harvested at different phases of the growth was not statistically significant, comparatively higher toxin concentrations were detected in the cells harvested at day 31, where the cell growth was in the declining phase. Significantly higher cellular toxin levels were detected during the declining phase in the cells grown at 20°C and
22 °C when compared to those grown at 30 °C (ANOVA, \( F = 107.093, p < 0.05 \)). The maximum toxin contents per cell recorded were 5.06 ± 0.2 ng SnE cell⁻¹, followed by 5.02 ± 0.2 ng SnE cell⁻¹ and they were observed in the samples harvested at day 31, from the cultures in 20 °C and 22 °C respectively (Fig. 7). The lowest toxin concentrations were observed in 30 °C at day 24 (1.86 ± 0.7 ng SnE cell⁻¹). A slight difference in the cellular toxicity levels among treatments was evident at day 24 (ANOVA, \( F = 10.578, p < 0.05 \)) whereas the difference was not significant at day 38.

4. Discussion

During the present study stimulation of O. ovata cell growth was observed at high temperatures (26–30 °C) while highest cellular toxicities were observed at low temperatures (20–22 °C).

4.1. Effects of temperature on the growth of O. ovata

Dinoflagellate species as well as all other phytoplankton groups/species depend on the optimum sea surface temperature to grow at their highest rates (Morton et al., 1992; Hales et al., 1999; Chateau-Degat et al., 2005; Granelli and Flynn, 2006; Navarro et al., 2006). The results from all the experiments presented here clearly demonstrate that the growth of O. ovata is strongly stimulated under high temperatures and it is consistent with the previous findings from field observations during the blooms in Genova, Italy (Ciminiello et al., 2006), Brazil (Granelli et al., 2002), Tosa Bay, Japan (Adachi et al., 2008) and on temperate reefs in New Zealand (Shears and Ross, 2009). Nevertheless, the present study also shows that O. ovata can grow at a wide range of temperatures (16–30 °C). The optimum temperatures for O. ovata growth were found to be between 26 and 30 °C during this study. Similar observations have been made by Mangialajo et al. (2008) who recorded the highest abundance of O. ovata in Genova when water temperature exceeded 26 °C. These evidence suggest that there is a pronounced effect of relatively high temperature on the growth of O. ovata. Therefore it can be speculated that the rising in temperatures have stimulated the growth of O. ovata during the blooms in both Brazil and Italy.

The growth of some other benthic and planktonic dinoflagellates such as Prorocentrum donghaeense (Xu et al., 2010), G. toxicus and Coolia monotis (Morton et al., 1992), A. tamiyavanichii and A. minutum (Lim et al., 2006) and the raphidophyte Heterosigma akashiwo (Fu et al., 2008) have also been shown to be enhanced by high seawater temperatures. The optimum temperature range for P. minimum growth has been recorded between 18 and 26 °C with a rapid decrease above 26.5 °C and a rapid increase between 13 and 18 °C (Grzebyk and Berland, 1996). On the contrary, a different temporal pattern of O. ovata bloom formation has been observed in northern Adriatic Sea where the maximum cell abundance was found for decreasing temperatures from 26 °C to 16 °C (Totti et al., 2010). Also the growth of O. heptagona and O. siamensis were found to be optimal at temperatures less than 26 °C (Morton et al., 1992), on the other hand, no significant correlation was observed between epiphytic dinoflagellate assemblages and water temperature in the Mediterranean Sea (Vila et al., 2001). Similarly, in southwestern Puerto Rico, the seasonality of dominant epiphytic dinoflagellates O. lenticularis and G. toxicus were not strongly correlated with temperature (Ballantine et al., 1988). Thus, the importance of high temperature as a triggering factor for O. ovata cell growth could be strain specific.

4.2. Effects of co-occurring algae on the growth of O. ovata and vice-versa

It has long been argued that many toxic dinoflagellates produce allelochemicals in order to compete with other co-occurring algae under unfavorable environmental conditions for growth (Granelli and Johansson, 2003b; Granelli and Hansen, 2006). Several authors have observed that Ostreopsis species are able to produce and release hemolytic compounds and polysaccharides (mucilage) into the water (Yasumoto et al., 1987; Vila et al., 2001; Ashton et al., 2003; Taniyama et al., 2003; Lenoir et al., 2004). During the blooms in Brazil in 1998/99 and 2001/22, microscopic investigations have revealed that, all seaweeds (most calcarean ones such as Jania sp.), were covered with a 0.5 cm thick layer of mono-specific O. ovata cells in gelatinous mass and no other microalgae were found among them (Granelli et al., 2002; Granelli, pers. observation). This indicates that either the polysaccharides or the hemolytic compounds produced by O. ovata cells have been acting as allelochemicals. However laboratory studies have not yet been conducted on production of allelochemicals and/or their allelopathic effects on other microalgae. During the present study, in the mixed cultures growth of O. ovata was not observed, but N. closterium and Navicula sp. were observed at all the experimental temperatures. However, when transforming the cell number to level of carbon, the results show that O. ovata was not out-competed either by N. closterium or Navicula sp. in mixed treatments. Since O. ovata grew well in monocultures in all the temperatures, the decline of the cells after some days in all the mixed treatments can be explained by the impact of grazing by ciliates that increased quite dramatically during the course of the experiment. These ciliates were present among the microalgal community in the natural assemblages from the beginning of the experiment. The epiflorescence microscopy on the samples from mixed treatments showed that Euplotes sp. contained photosynthetic cells similar to O. ovata cells in size. The ciliates were
selectively grazing *O. ovata* cells, which are more size-suitable to them than the smaller *N. closterium*. This might explain why *O. ovata* disappeared during the course of the experiment in the mixed cultures, as a result of grazing and not due to competition with the other algal species.

The ciliate preferences for *O. ovata* cells as a food item also explain the comparatively high cell densities of *N. closterium* and *Navicula* sp. in the mixed treatments when compared to the controls. At the beginning of the experiment, grazing pressure might be high on *O. ovata* cells, than on other smaller co-occurring algae. In order to elucidate whether *O. ovata* toxins and/or polysaccharides have allelopathic significance, experiments similar to those conducted by Granéli and Johansson (2003b) and Fistaro et al. (2004) should be carried out. In this case only monocultures of the co-occurring algae will be used and grazing by ciliates should be avoided.

4.3. Effects of temperature on the toxicity of *O. ovata*

In general changes in toxin content are associated with stress caused under disturbed physiology (Johansson and Granéli, 1999; Granéli and Flynn, 2006) by several factors such as pH (Hwang and Lu, 2000), temperature (Ogata et al., 1989; Ashton et al., 2003), salinity (Parkhill and Cembella, 1999; Gedaria et al., 2007), illumination (Hwang and Lu, 2000), and nutrient deficiency (Granéli and Johansson, 2003b).

Present study shows that toxicity was higher in *O. ovata* cells grown between 16 and 22 °C, which was maximized at 20 °C/22 °C where comparatively low *O. ovata* cell densities were observed. Contrary to this observation, Ashton et al. (2003) reported, an enhanced toxicity for *Ostreopsis lenticularis* at 29.5 °C, than at 25 °C. But, as *O. ovata* and *Lenticularis* are two different species, the environmental conditions that enhance toxicity in them can be completely different from one another.

Similar to the observation made during the present study for *O. ovata*, an inverse correlation between temperature and toxin concentration have been observed for *A. catenella* (Navarro et al., 2000). Contrary to *O. ovata*, the optimum growth for *Protoceratium reticulatum* has been reported to be between 16 and 20 °C, while the highest toxicity has been at 26 °C, at which the growth inhibition has been evident (Guerrini et al., 2007). Similar inverse correlations between optimum growth conditions and toxicity have been found for some other dinoflagellate species, viz. *A. cohoritcula* (Ogata et al., 1989); *Pyrodinium bahamense* var. *compressum* (Gedaria et al., 2007) and Heterosigma akashiwo (Ono et al., 2000). Growth rates and the toxin production rates of *A. minutum* and *A. tamiyavanichii* have been found to be increased with elevated temperatures (Lim et al., 2006). As it is often reported, factors stimulating toxin production in one algal species/group may have a different impact on another (Granéli and Flynn, 2006).

Results from the experiment 4 (Fig. 7) clearly confirmed that toxin concentration per cell vary with the growth phase and was highest during the declining phase. Although the cell densities were not significantly different among different treatments during this phase, the cellular toxin levels were twice as higher in the cultures grown at 20 °C and 22 °C compared to those grown at 30 °C. Toxic concentrations of *O. ovata* strains isolated from Tyrrhenian and Adriatic seas have also been found to be high at the end of stationary phase than at the end of exponential phase (Guerrini et al., 2008). An enhanced toxin concentration at the declining stage, compared to the other phases of growth cycle has also been demonstrated in *A. minutum* (Hwang and Lu, 2000). However the toxin content of *Pyrodinium bahamense* var. *compressum* during the exponential phase has been the highest and a rapid decline has been observed at late exponential and stationary phases (Gedaria et al., 2007). Decrease in cellular toxin content of *O. ovata* cells after day 31 (declining phase) could be probably due to the increased release of toxins to the medium.

Results of the present study, confirm the findings for other toxic species (see e.g. Granéli and Flynn, 2006), i.e., that the level of toxicity per cell increase at temperatures when the cell growth is sub-optimal. Even if this is the case, at high temperatures, which are favourable for the cell growth and toxicity per cell is low, high cell densities can result in same or higher levels of toxins, that can be transported through food chain or aerosols, with a capacity strong enough to cause considerable negative effects on the human health and the environment. This seems to be the case during the blooms of *O. ovata* in Genova, Italy in 2005/06 and in Brazil in 1998/99 and 2001/02. This indicates that if a bloom is triggered under lower temperatures (at higher latitudes), it could result in higher toxin levels and higher impacts on the environment.

5. Conclusions

The optimum temperatures for growth and toxicity of *O. ovata* were found to be inversely related. High water temperatures (26–30 °C) stimulated *O. ovata* cells growth rate and biomass accumulation and low toxicities while lower temperatures (20–22 °C) induced higher toxicity per cell and lower cell numbers.-Based on the results of the present experiments it can be suggested that increased sea surface temperature, which can result from global warming may play a crucial role inducing the geographical expansion and biomass increase, blooms of *O. ovata* in future.

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E. Granéli et al. / Harmful Algae 10 (2011) 165–172


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