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The effects of aeration on growth and toxicity of *Prymnesium parvum* grown with and without algal prey

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ABSTRACT

We investigated the effects of aeration on growth and toxicity of the haptophyte *Prymnesium parvum* in the presence and absence of the algal prey *Rhodomonas salina*. Batch monocultures of P-limited *P. parvum* and N and P sufficient *R. salina* and mixed cultures of the two microalgae were grown with no, low (20) and high (100) ml min^{-1} aeration for 18 days. Cell growth of *P. parvum* and *R. salina* and cell toxicity of *P. parvum* were studied over the experimental period. The highest specific growth rates of *P. parvum* were found at low aeration rates. *R. salina* in monocultures showed typical growth patterns, while *R. salina* numbers declined rapidly in the mixed cultures. Of the initial cell densities, 98–100% of the *R. salina* cells were lysed or ingested within 24 h of mixing with *P. parvum* cells. The maxima *P. parvum* biomasses were significantly higher in the mixed cultures than in the monocultures. Cell toxicity of *P. parvum* increased significantly in response to aeration rates and the highest levels were found in the high aeration condition. Availability of prey and resupply of inorganic nutrients decreased *P. parvum* cell toxicity. Our study suggests that *P. parvum* is tolerant and is able to grow over a broad range of aeration and associated turbulence effects though low aeration represents an optimal condition for growth. As *P. parvum* toxicity was higher in the high aeration treatment we suggest that the higher concentrations of oxygen cause more toxins to be produced, as these are oxygen rich compounds. We suggest that oxygenation and turbulence of surface waters caused by mixing may be involved in promoting high toxic *P. parvum* blooms in shallow lakes and coastal waters.

1. Introduction

The haptophyte *Prymnesium parvum* (Carter, 1937) is a unicellular bi-flagellated microalga which occurs in marine, estuarine and inland waters worldwide (Kaartvedt et al., 1991; Lindholm et al., 1999; Edvardsen and Imai, 2006; Granéli et al., 2012). It is known to produce persistent near-monospecific, high biomass algal blooms that disrupt or degrade ecosystem structure and function (Sunda et al., 2006). Massive fish kills and extensive economic and recreational losses are some of the clearly evident impacts of *P. parvum* blooms (Kaartvedt et al., 1991; Lindholm et al., 1999; Brooks et al., 2011). The incidences of *P. parvum* blooms have increased dramatically over recent decades and this represents a significant ecological threat in many regions; e.g. the Baltic Sea, Black Sea, China, Israel, North America, and Australia (Edvardsen and Paasche, 1998; Brooks et al., 2011; Roelke et al., 2011; Granéli et al., 2012).

Prymnesium parvum can tolerate large variations in temperature (12–30 °C) and salinity (from 0.8 to 45) which allows them to survive and bloom in a wide range of environmental conditions (Edvardsen and Imai, 2006; Sunda et al., 2006; Brooks et al., 2011; Roelke et al., 2011). *P. parvum* is a mixotrophic organism, which can obtain energy both through phototrophy and through heterotrophic nutritional modes such as phagotrophy, via ingestion of prey items such as bacteria and other algae, and through osmotrophy, via uptake of dissolved organic substances (Legrand et al., 2001; Fistarol et al., 2003; Tillmann, 2003; Lindehoff et al., 2011). This alga however, shows heterotrophic life strategies more prevalent than autotrophic (Flynn et al., 2013; Granéli et al., 2012).

Further, *Prymnesium parvum* produces a set of highly potent toxins called prymnesins which have cytotoxic, ichthyotoxic and neurotoxic properties (Igarashi et al., 1995, 1996; Edvardsen and Imai, 2006). These toxins and other secondary metabolites produced by *P. parvum* might act as allelochemicals; those that

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inhibit the growth and/or kill co-occurring phytoplankton species, and act as grazer deterrents (Fistarol et al., 2003; Tillmann, 2003; Granéli et al., 2012). Collectively, all of these eco-physiological traits make *P. parvum* successful in a wide range of aquatic ecosystems, contributing to their competitive advantage over co-occurring algal species, and as such are likely contributors to bloom formation. Effects of *P. parvum* on aquatic life are strain- and site-specific because its growth, toxicity and allelopathy are largely influenced by a complex set of physical, chemical and biological factors such as the availability and ratios of inorganic nutrients (nitrogen; N and phosphorus; P), temperature, salinity, pH, etc. (Granéli and Flynn, 2006; Granéli and Hansen, 2006; reviewed in Graneli and Salomon, 2010; Granéli et al., 2012; Prosser et al., 2012). In most instances increase of toxicity and/or allelopathy are associated with the disturbed physiology caused under stress conditions not optimal for growth (Granéli and Flynn, 2006; Granéli and Hansen, 2006). Therefore, if we are to understand the potential effects of *P. parvum* on an aquatic system and to implement management measures a thorough understanding of the factors that influence their growth and toxicity is needed.

Possible effects of aeration and turbulence on *Prymnesium parvum* growth and toxicity are not extensively studied so far. In a previous study, Igarashi et al. (1995) found that vigorous aeration (100 ml s^{-1}) significantly increased *P. parvum* biomass and toxicity compared to cultures grown without aeration. It is not known if this increase in toxicity is a stress response or if the increased oxygen level in the medium favored the growth of *P. parvum* and the production of oxygen rich toxic compounds (e.g. prymnesins). Despite the cause, this increase of cell toxicity might also induce allelopathy of *P. parvum*, which results in severe effects on the co-occurring phytoplankton species (c.f. Fistarol et al., 2003). On the other hand, presence of prey organisms might reshape the effects of aeration and associated turbulence on the growth and toxicity dynamics of *P. parvum*. Aiming to clarify which of these factors would have the highest impact on *P. parvum* growth and toxicity we have performed the present study. *P. parvum* cultures were exposed to different aeration conditions in the presence and absence of cryptophyte prey (*Rhodomonas salina*) to check their effects on growth and toxicity of *P. parvum*. Although phagotrophy in *P. parvum* is common even when nutrient concentrations are high, they rely more on this mode of nutrition when nutrients become limiting (Carvalho and Granéli, 2010). The production of toxins is also induced when nutrients (N and particularly P) are limiting (Johansson and Granéli, 1999). Therefore, in this study we used *P. parvum* cultures grown in P-limiting medium. Furthermore, when mixing the two microalgae, *P. parvum*: *R. salina* ratio of 2:1 was chosen as it has been shown that allelopathy is higher at low prey densities in relation to the algal predator densities (Tillmann, 2003; reviewed in Granéli et al., 2012). Therefore, the specific objectives of this study were to determine: (1) if aeration influences *P. parvum* growth in the presence and absence of an algal prey: (2) if *P. parvum* cell toxicity increases with the increase of aeration rates, and: (3) if the availability of prey and/or increase of inorganic nutrient concentration could decrease or inhibit such an aeration-mediated cell toxicity increase.

2. Materials and methods

2.1. Experimental conditions

Non-axenic cultures of *Prymnesium parvum* and *Rhodomonas salina* (KAC 39 and KAC 30, Kalmar Algae Collection, Linnaeus University, Kalmar, Sweden) were selected for this study. Cells were first grown as batch cultures in 10 l bottles. *P. parvum* cells were grown in P-limiting f/10 medium (N = 116 μM and P = 5.8 μM) with N:P ratio of 20:1 and *R. salina* cells, which were used as prey, were grown in N and P sufficient f/2 medium (N = 580 μM and P = 36.3 μM) with N:P ratio of 16:1 (Guillard and Ryther, 1962). Trace metals, iron, vitamins (B12, biotin and thiamine) and EDTA were added to the culture media at levels corresponding to f/10 or f/2 media. Culture media were prepared with filtered (Whatman GF/C glass-fiber filters, 1.2 μm mesh size) and autoclaved natural seawater (salinity 7) collected from the Baltic Sea. Both *P. parvum* and *R. salina* cultures were grown at 20 °C and on a 16:8 h light:dark (L:D) photoperiod with an irradiance of 100 μmol photons m^{-2} s^{-1}.

The experiment was started when the exponentially growing stock cultures reached high cell densities (*Prymnesium parvum*: 6.38×10^5 cells ml^{-1}, and *Rhodomonas salina*: 7.33×10^5 cells ml^{-1}). The experimental set-up included mixed cultures of *P. parvum* and *R. salina* as well as monocultures of the two microalgae (Fig. 1). Mixed cultures were prepared by mixing 210 ml of *P. parvum* culture and 90 ml of *R. salina* culture to have an initial *P. parvum*:*R. salina* ratio of 2:1. The monocultures were prepared by diluting 210 ml of *P. parvum* culture in 90 ml of P limited f/10 medium and 90 ml of *R. salina* culture in 210 ml of f/2 medium to get approximately the same initial cell densities of each algal species as in the mixed cultures. Thus, the total initial culture volumes were 300 ml inoculated into 500 ml glass bottles (Schott Duran), and grown under the laboratory conditions mentioned above. Each culture treatment was performed in triplicate and set up in 3 sets, representing 3 different aeration rates. One set of mixed cultures and monocultures was grown without aeration as a control and the other two sets were grown with aeration rates of 20 ml min^{-1} (low aeration) and 100 ml min^{-1} (high aeration) (Fig. 1). Airflow rate was measured and adjusted to the desired levels using a flow meter (Cole Parmer PMRI-010792) and kept constant throughout the experimental period. Air inflow was filtered through 0.2 μm sterile filters before supplied to culture bottles. Aerated cultures were monitored against the non-aerated cultures and adjusted daily by adding sterile MQ water. This was done in order to account for changes in the culture volume caused by evaporation. The pH in each culture bottle was recorded daily using a pH meter (inoLab pH Level 1) with an electrode with 2-point calibration (VWR 662-9237). The pH levels in the non- and low-aerated cultures were adjusted to the same pH range as in high-aerated cultures using sterile filtered (0.2 μm) 0.1 M HCl and/or 0.1 M NaOH.

All the culture treatments were sampled daily for cell counts and every fourth day (i.e. day 4, 8, 12, 16 and 18) for analyses of hemolytic activity. Cultures were homogenized manually before sampling. The experiment lasted a total of 18 days.

2.2. Re-feeding of experimental cultures

Cell densities of *Rhodomonas salina* in the mixed cultures decreased rapidly in the first 1–2 days and no *R. salina* cells were found in any of the mixed cultures by day 4 (see Section 3.1). Therefore, *P. parvum* in the mixed cultures were re-fed on day 4 (after daily sampling was performed) with 95 ml of an exponentially growing *R. salina* culture. This addition increased *R. salina* cell densities in all the mixed cultures to the level of 2.12×10^5 cells ml^{-1} which was equivalent to the initial cell densities of *R. salina* in these cultures. Correspondingly, monocultures of *Prymnesium parvum* and *R. salina* were provided with the same volumes (95 ml) of fresh growth media (P-limiting f/10 medium or N and P sufficient f/2 medium, respectively).

2.3. Estimation of biomass accumulation, specific growth rates and ingestion rates

Sub-samples of 1 ml were taken daily from all the bottles and cell densities were quantified using flow cytometry (FACSCalibur flow cytometer, Becton Dickinson). Signals were detected as forward and side scattered light (FSC and SSC), chlorophyll *a*