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The Effect of pH and Salinity on the Toxicity and Growth of the Golden Alga, *Prymnesium parvum*

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Bioassays using cultures of the toxic haptophyte *Prymnesium parvum* and the ciliate *Cyclidium* sp. as prey were conducted to test the effect of pH (range = 6.5 – 8.5), salinity (range = 1.50 – 7.50‰), and a combination of pH and salinity on the toxicity of *P. parvum*. pH had a significant effect on *P. parvum* toxicity. Toxicity was rapidly (within 24 hr) induced by increasing pH of the medium, or reduced by lowering pH. Conversely, lowering salinity reduced toxicity, albeit less effectively compared to pH, and *P. parvum* cells remained toxic at the lowest values tested (1.50‰ at pH 7.5). An additional effect between pH and salinity was also observed: low salinity combined with low pH led to not only decreased toxicity, but also resulted in lower *P. parvum* growth rates. Such effects of pH and salinity on *P. parvum* growth and toxicity provide insight into the environmental factors supporting community dominance and toxic blooms of the alga.

Key words: *Prymnesium parvum*; golden alga; toxicity; bioassay; pH; salinity.

Introduction

Prymnesium parvum, often referred to as the 'Golden Alga', is a mixotrophic haptophyte that consumes a wide variety of planktonic microorganisms. Numerous studies have demonstrated that the capture and consumption of microscopic prey contribute directly to carbon and/or nutrient acquisition by the alga (Brutemark and Granéli 2011; Carpenter et al. 2018; Carvalho and Granéli 2010; Granéli and Johansson 2003a; Lindehoff et al. 2010; Tillmann 2003). Additionally, toxicity resulting in the death of presumably non-target species has also been commonly observed during massive blooms of the alga. Significant among these latter effects are fish kills that have been reported for decades in conjunction with *P. parvum* blooms (Holdway et al. 1978; Johnsen et al. 2010;

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Otterstrøm and Steeman Nielsen 1940). The wide spectrum of trophic levels that the alga can affect has resulted in the description of *P. parvum* blooms as 'Ecosystem Disruptive Algal Blooms'; EDABs (Brooks et al. 2011; Jones et al. 2018; Michaloudi et al. 2008).

Prymnesins are well known toxins produced by *P. parvum* (Manning and La Claire 2010). Although other toxic compounds produced by this species exist, they are still poorly characterized (Taylor et al. 2020). At least some of these compounds aid prey capture through cell-to-cell contact, a mechanism that also explains presumably non-target mortality of zooplankton and fish (Remmel and Hambright 2012; Sopanen et al. 2006; Tillmann 2003). Allelopathic compounds have also been attributed to *P. parvum* as well as the possibility that they may serve as deterrents to grazing (Blossom et al. 2014b; Fistarol et al. 2003; Granéli and Salomon 2010; Granéli et al. 2012;). The concentrations and/or types of toxic compounds appear to vary among strains of *P. parvum* (Rasmussen et al. 2016; Weissbach and Legrand 2012) but additionally are affected by a range of environmental and physiological factors. Parameters studied for their effects on the alga's toxicity have included nitrogen and phosphorus sufficiency and limitation, salinity, temperature and pH (Baker et al. 2007; Carvalho and Granéli 2010; Hambright et al. 2014; Larsen et al. 1998). The findings of these various studies have yielded some generalities but also some conflicting results, in part due to variability in the inherent toxin composition of different strains of the alga (Binzer et al. 2019), and the type of assay employed. Hemolytic, neurotoxic, cytotoxic and ichthyotoxic effects have been examined and confirmed using an array of model systems and bioassays (Manning and La Claire 2010).

P. parvum is globally distributed, and appears to have a rather wide tolerance for environmental conditions. The species is euryhaline and has been reported growing in full strength seawater but also in ecosystems with salinity as low as 0.5‰ (Larsen et al. 1998; Roelke et al. 2016). The presence of *P. parvum* has been reported in nearly half of the continental U.S. states (Roelke et al. 2016). This expansive distribution is believed to be due, in part, to salinization of inland waters which has made some ecosystems more conducive to its proliferation and success. Natural and anthropogenic nutrient enrichment of freshwater bodies also contribute to the wide occurrence of *P. parvum* (Hambright et al. 2014). However, community dominance and resulting fish kills are a relatively new phenomenon across the southwestern U.S.. Fish-killing blooms of the alga were first documented in Texas state waters in the mid-1980s (Prosser et al. 2012; Roelke et al. 2011; Southard et al. 2010), and fish kills attributable to *P. parvum* in that state alone have affected numerous fish species and resulted in fish mortalities numbering in the tens of millions. Since the first documentation of fish kills in Texas, the list of southwestern U.S. states that have experienced these events has increased, resulting in the characterization of the alga as an invasive species (Hambright et al. 2014).

Lake Mission Viejo in Mission Viejo, Orange Country, California, USA is an approximately 51 hectare (125 acres) man-made lake whose construction began in 1974. The lake is a prized recreational/residential waterbody, renowned for its recreational fishing. Lake Mission Viejo is bermed around its shore, preventing urban and yard runoff into the lake in an effort to maintain good water quality. Water level has been controlled via the addition of several water sources to address seepage and evaporative losses. Nitrogen and phosphorus concentrations in the lake have remained relatively low for nearly half a century due to effective water management (presently, phosphate concentrations are typically $\sim 0.11\ \mu M$ and ammonium + nitrate concentrations are typically $\sim 0.81\ \mu M$ (Roohk 2019), while pH has remained relatively stable albeit somewhat high, generally 8–8.5 (Roohk 2019)). In contrast, electrical conductivity (a proxy for salinity) has increased nearly linearly throughout the ~50-year history of the lake from ~0.65 to ~2.40‰ in 2020. The gradual increase in salt concentration is a result of water replenishment combined with evaporative losses of water (Roohk 2019).

A minor bloom of *P. parvum* in November 2014 decimated the fish population of Lake Mission Viejo, and the alga has sporadically continued to cause fish kills since that time. Microscopical assessments at the time and since the initial fish kill revealed the presence of *P. parvum*, but at abundances (Roohk 2019) that were much lower than algal abundances that have led to fish kills in other regions. While most ecosystems have experienced fish kills caused by *P. parvum* at abundances $\geq 10^4$ cells ml^{-1} (Roelke et al. 2011; VanLandeghem et al. 2015), Lake Mission Viejo has experienced repeated mortality events at abundances of 100 s to 1,000 s of cells ml^{-1}. Nonetheless, repeated fish kills have

implicated *P. parvum* as the causative factor in these mortality events in Lake Mission Viejo, although the specific environmental factors explaining the extreme toxicity of the alga in the lake have remained unclear.

Knowledge of how specific environmental factors affect the toxicity of *P. parvum* in Lake Mission Viejo can improve our understanding of toxin production by the strain of this haptophyte inhabiting the lake. Toward this end, we developed a toxicity bioassay using a *P. parvum* culture isolated from the lake, and *Cyclidium* sp., a ciliate that is readily attacked and killed by the alga. A range of pH values, salinities and combinations of various pH and salinity values that bracketed historical and present-day values of these parameters in the lake were investigated using the newly developed bioassay. The results of this study demonstrated that high pH and high salinity (comparable to present-day conditions in Lake Mission Viejo) both yielded more toxic *P. parvum*, although the effect of pH was more pronounced. Conversely, low pH and low salinity resulted in the lowest toxicity of the alga. Additionally, the growth rate of the alga was reduced at combined low pH and low salinity relative to higher values of these parameters. The results from these bioassays provide information that explain the high toxicity of *P. parvum* presently observed in Lake Mission Viejo, and may help guide future lake management aimed at reducing community dominance and toxicity of the toxic haptophyte.

Results

The bioassay setup was designed to compare mortality in cultures of the ciliate, *Cyclidium* sp., in the presence (Experimental treatments) and absence (Control treatments) of *P. parvum* incubated at various pH and salinity values. That is, *Cyclidium* sp. mortality was used as a proxy for comparing *P. parvum* toxicity. Bioassays were conducted for a range of pH values at a constant salinity, a range of salinity values at a constant pH, and a combination of various pH and salinity values. Only recently has Lake Mission Viejo experienced toxic *P. parvum* blooms, and it is unclear if these occurrences are a consequence of recent invasion by the haptophyte or changing pH and salinity conditions in the lake. These parameters have varied independently since the lake was created, although previous studies of the toxicity of *P. parvum* have indicated that they may work in concert. Therefore, we first tested them separately, and then in combination.

Effect of pH on *P. parvum* Toxicity

Bioassays included testing a range of pH values (6.5–8.5) at a constant salinity of 2.50‰ in order to assess the response of *P. parvum* toxicity to different pH values. This range encompasses the present average pH of the lake (8.3), and lower values that are target values for reducing lake pH through existing management strategies. The toxicity of *P. parvum* towards the ciliate *Cyclidium* sp. was substantially reduced in bioassays conducted at low pH relative to bioassays conducted at high pH across the range of pH values examined (Fig. 1). Changes in the abundance of the ciliate in the pH 6.5 treatment were similar in the Experimental (gray bars in Fig. 1a) and Control treatments (white bars in Fig. 1a), indicating no significant toxic effect of the alga on the ciliate at that pH. In contrast, ciliate abundances in treatments at pH 7.0, 7.5, 8.0 and 8.5 exhibited differences between Experimental and Control treatments (gray bars vs white bars, respectively, in Fig. 1b-e), with *Cyclidium* sp. abundances decreasing more significantly in the presence of *P. parvum* compared to flasks without the alga by day 3 (two-way ANOVA, $p < 0.001$). Such difference between the Experimental and Control treatments reflect *Cyclidium* sp. mortality due to *P. parvum* toxicity. Linear regression analysis confirmed a significant effect of pH on *Cyclidium* sp. mortality in Experimental treatments ($p < 0.001$) whereby higher pH led to greater decreases in *Cyclidium* sp. abundance. Linear regression did not reveal any significant effect of pH on changes in *Cyclidium* sp. abundance among the Control treatments ($p > 0.05$).

Ciliate abundance in the Experimental treatment at pH 6.5 increased by 260 % relative to starting abundance over the course of the bioassay. That change was significantly greater than values from Experimental treatments at all other pH values tested ($\geq$pH 7.0; two-way ANOVA, $p < 0.001$). Ciliate abundance in the Experimental treatment at pH 7.0 also increased by day 3 (25 % relative to initial abundance). That change was also significantly greater than values from Experimental treatments conducted at pH 7.5, 8.0 and 8.5 (two-way ANOVA, $p < 0.001$). Changes in ciliate abundance in the Experimental treatments at pH 7.5, 8.0 and 8.5 were all negative (i.e. there were decreases in ciliate abundances relative to the start of the bioassay) and there were no significant differences in ciliate abundances among these three treatments (two-

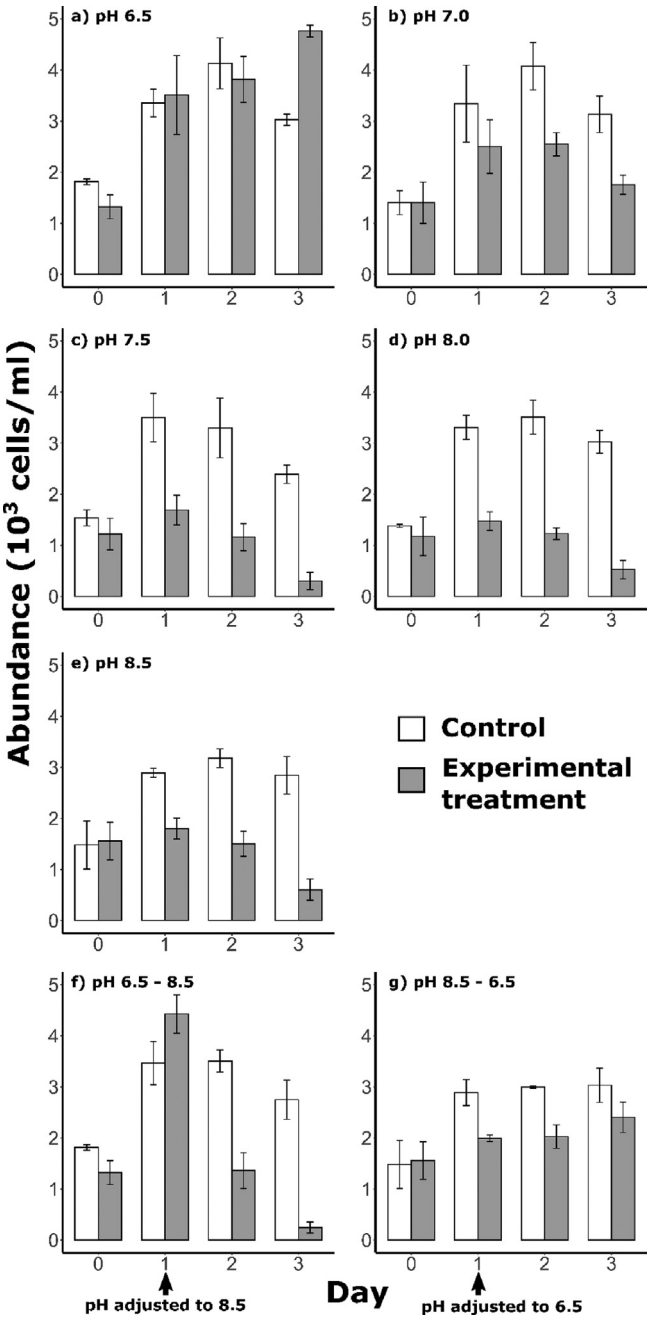

Figure 1. Daily average abundances (error bars: standard deviation; n = 3) of ciliate prey (*Cyclidium* sp.) in pH bioassays conducted for three days. Experimental treatments were exposed to *P. parvum* (gray bars) while Control treatments (white bars) had no algae present. The 'pH 6.5–8.5′ bioassay began at pH 6.5, but pH of the medium was adjusted to 8.5 after one day (arrow in panel), as noted in the Methods. The 'pH 8.5–6.5′ bioassay began at pH 8.5, but pH of the medium was adjusted to 6.5 after one day. The salinity of all treatments was 2.5‰.

way ANOVA, $p > 0.05$). That is, toxicity of the alga between pH 7.5 and 8.5 was indistinguishable in the bioassays. Changes in ciliate abundances were similar and positive (i.e. positive net growth) in all Control flasks during the three-day bioassays, indicating no apparent direct effect of pH on the ciliate (white bars in Fig. 1). Ciliate abundances in all Controls (i.e. the absence of *P. parvum*) increased during the first 48 h and then decreased slightly presumably due to changes in the availability of

residual bacterial prey carried over from the stock cultures.

Rapid Changes in *P. parvum* Toxicity Following Changes in pH

The effects of pH on *Cyclidium* sp. mortality in the presence of *P. parvum* occurred rapidly (within one day) with changes in the pH of the cultures (Fig. 1f,g). Therefore, two bioassays were conducted at constant salinity (2.50‰) to characterize the degree to which *P. parvum* toxicity was altered by a rapid shift in pH. The 6.5–8.5 and 8.5–6.5 treatments had pH 6.5 or 8.5, respectively, at the beginning of the bioassay (between day 0 – 1), and the pH of the culture medium was then adjusted to the other extreme (8.5 or 6.5, respectively) after sampling on day 1. Toxicity of the *P. parvum* responded rapidly and reversibly to changes in pH. That is, toxicity in cultures initiated at low pH became more toxic when the pH was raised, while toxicity in cultures at high pH became less toxic when the pH was lowered. *Cyclidium* sp. abundance on day 1 in the Experimental treatment initiated at pH 6.5 (Fig. 1f) was significantly greater than abundance in their respective Controls (two-way ANOVA, $p < 0.001$), indicating that *Cyclidium* sp. abundances did not decrease due to the presence of *P. parvum*. However, increasing the pH in this treatment from 6.5 to 8.5 after sampling on day 1 was followed by a rapid and substantial decrease in ciliate abundance (~70 %) in the Experimental treatment from day 1 to day 2, and a further decrease from day 2 to day 3 (gray bars in Fig. 1f; pH 6.5–8.5 bioassay). Mortality of *Cyclidium* sp. in the Experimental treatment of the pH 6.5–8.5 bioassay was significantly greater compared to the Control treatment by day 3 of the bioassay (gray vs white bars in Fig. 1f; two-way ANOVA, $p < 0.001$).

In contrast to the results of the pH 6.5–8.5 bioassay, *Cyclidium* sp. abundance in the Experimental treatment of the bioassay initiated at pH 8.5 (Fig. 1g) was significantly lower than ciliate abundance in the respective Controls on day 1 (gray vs white bars in Fig. 1g). Adjustment of pH from 8.5 to 6.5 after sampling on day 1 in the pH 8.5–6.5 bioassay (Fig. 1g) resulted in no further decreases in ciliate abundance for the remainder of the three-day bioassay, while ciliate abundance in the treatment kept at pH 8.5 for the entire bioassay (Fig. 1e) continued to decrease on day 2 and 3. That is, a rapid decrease in the toxicity of *P. parvum* occurred with the adjustment of the pH of the medium from 8.5 to 6.5 after day 1 in the pH 8.5–6.5 bioassay.

Effect of Salinity on *P. parvum* Toxicity

Bioassays included testing a range of salinity values (1.50–7.50‰) at a constant pH of 7.5 in order to assess the response of *P. parvum* toxicity to different salinities. This range encompasses the present average salinity of the lake (≈2.50‰), and lower values that are target values for reducing lake salinity through existing management strategies. Overall, the effect of salinity on *P. parvum* toxicity was less pronounced compared to the effect of pH over the ranges of the two variables examined. *Cyclidium* sp. abundances (relative to starting abundances) in all Experimental treatments (Fig. 2a – d) were significantly lower than ciliate abundances in their respective Controls at day 3 (two-way ANOVA, $p < 0.001$), implying higher mortality of *Cyclidium* sp. in the presence of *P. parvum* due to algal toxicity at all salinities tested at pH 7.5. Nonetheless, ciliate abundances at the lowest salinity tested (1.50‰) increased initially from day 0 to day 1, and also exhibited the smallest decrease over the three-day bioassays (50 % decrease vs > 79 % decrease at other salinities test; gray bars in Fig. 2a vs gray bars in Fig. 2b,c,d). *Cyclidium* sp. abundance (relative to the starting abundance) in the 1.50‰ salinity Experimental treatment was significantly greater than the Experimental treatments at the other salinities (two-way ANOVA, $p < 0.05$) except for the Experimental treatment at 5.00‰ (two-way ANOVA, $p > 0.05$). Despite the higher survival of *Cyclidium* sp. in the Experimental treatment with the lowest salinity, linear regression analysis indicated that there was no significant trend for changes in ciliate abundances with salinity in the Experimental treatments at day 3 ($p > 0.05$).

Effect of Salinity × pH on *P. parvum* Toxicity

A set of bioassays was also conducted to characterize the interaction between pH and salinity on *P. parvum* toxicity. A total of six combinations of pH and salinity were examined based on results of the pH bioassays and salinity bioassays described above that spanned the least toxic conditions (lowest pH and lowest salinity tested independently) and the most toxic conditions (highest pH and highest salinity tested independently). In agreement with the

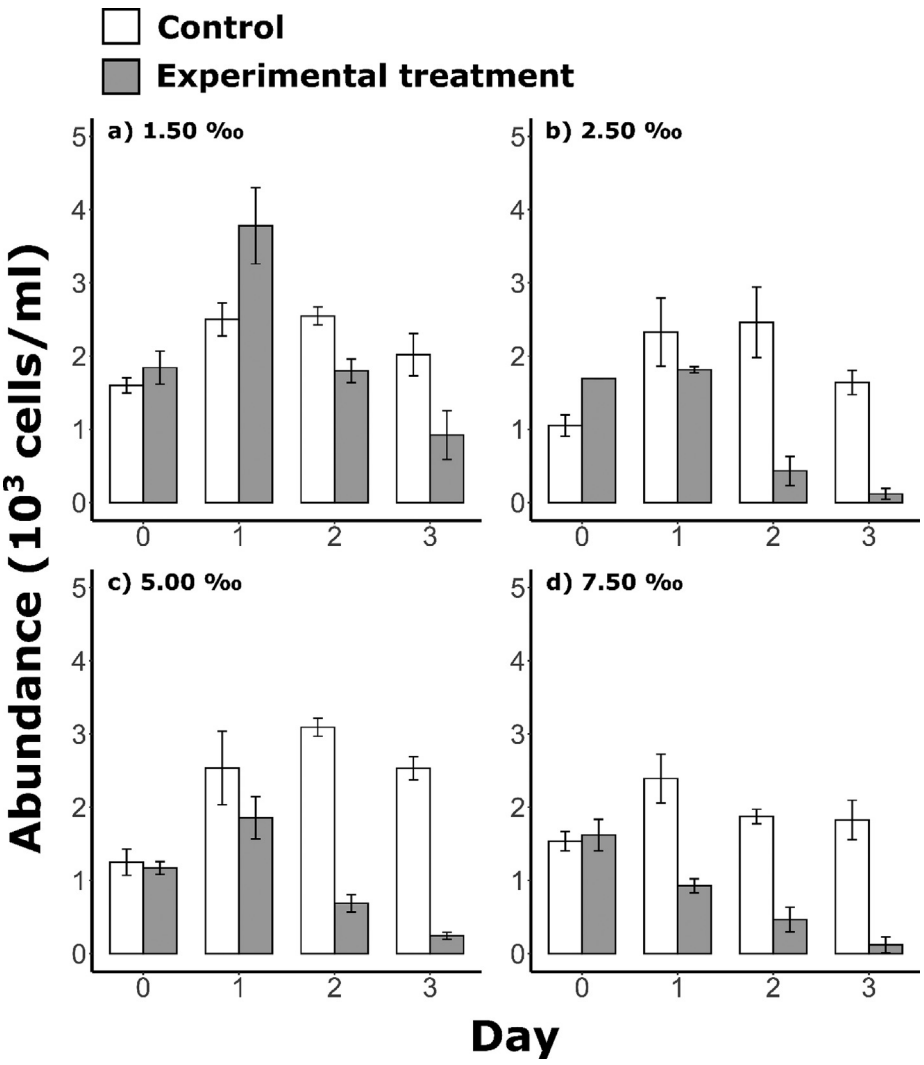

Figure 2. Daily average abundances (error bars: standard deviation; n = 3) of ciliate prey (*Cyclidium* sp.) in salinity bioassays conducted for three days. Experimental treatments were exposed to *P. parvum* (gray bars) while Control treatments (white bars) had no algae present. The pH was maintained at 7.5 throughout the bioassay.

bioassays conducted independently on pH and salinity, the results of the matrix experiment indicated that pH was more influential on *Cyclidium* sp. abundances. Abundances of *Cyclidium* sp. increased in the two Experimental treatments examining pH 6.5 (salinity at 1.50‰ or 2.50‰), and were not significantly different than changes in *Cyclidium* sp. abundance in Controls conducted at pH 6.5 on day 3 (i.e. no observed mortality due to the presence of *P. parvum*; Fig. 3a,c; three-way ANOVA, $p > 0.05$). *Cyclidium* sp. abundances at all other pH (7.5 and 8.5) and salinity (1.50‰, 2.50‰ and 7.50‰) values tested (Fig. 3b,d-f) decreased significantly in the Experimental treatments compared to Controls (three-way ANOVA, $p < 0.01$). Multiple regression and relative importance analysis confirmed that pH was more important than salinity in affecting ciliate abundances. pH, salinity, and the interaction of pH and salinity all had significant effects on the abundances of *Cyclidium* sp. (multiple regression, $p < 0.001$), but the relative importance analysis indicated that pH accounted for 58 % of the effect on ciliate abundances while salinity and the interaction of pH and salinity accounted for 36 % and 6 % respectively.

Effect of pH and Salinity on *P. parvum* Growth

Changes in the abundances of *P. parvum* were measured during the three-day bioassays in order

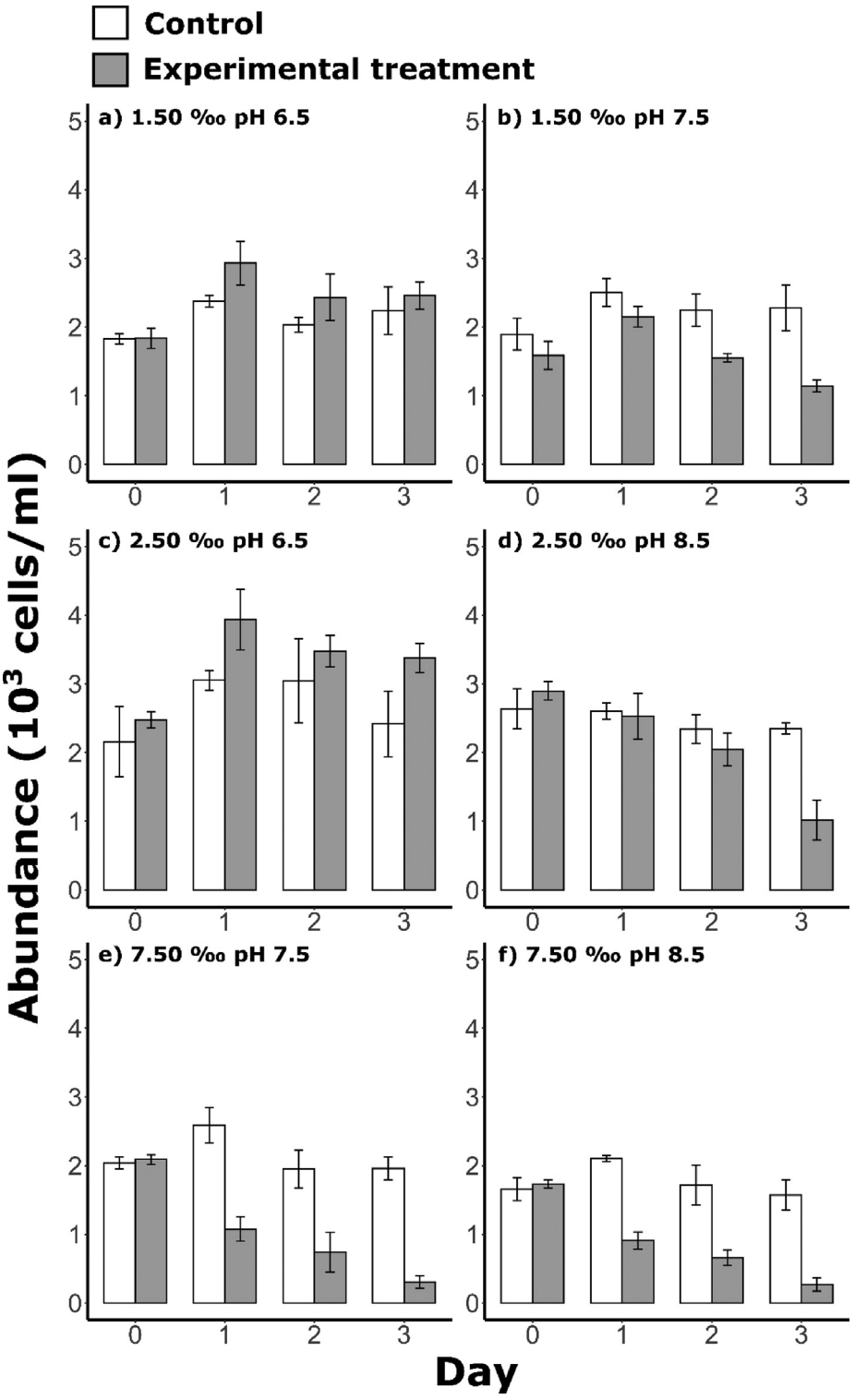

Figure 3. Daily average abundances (error bars: standard deviation; n = 3) of ciliate prey (*Cyclidium* sp.) in salinity × pH bioassays conducted for three days. Experimental treatments were exposed to *P. parvum* (gray bars) while Control treatments (white bars) had no algae present.

to examine the response of algal growth to pH, salinity, and salinity × pH (Supplementary Material Fig. S1). Abundances of *P. parvum* in the bioassay examining the combination of low pH and low salinity (1.50‰ at 6.5) decreased by the end of the three-day bioassay (i.e. net algal mortality, with growth rate (μ) = −0.44 d^{-1}; Fig. S1c, purple line). The bioassay conducted at pH 6.5 and 2.50‰ in the pH × salinity bioassay also yielded a lower growth rate compared to other treatments (Supplementary Material Fig. S1c; red dotted line). Beyond that, overall differences among μ calculated for treatments in the three-day bioassays did not differ greatly (overall range of 0.51–0.78 d^{-1} among 15 bioassays).

Based on growth responses of the alga during the three-day bioassays (Supplementary Material Fig. S1), a longer term (twelve-day) experiment was conducted to further investigate the effect of pH and salinity on *P. parvum* growth rate (Fig. 4). The alga was grown without prey (i.e. dependent on photosynthetic ability, as affected by salinity and pH). Consistent with observations in the three-day bioassays, pH and salinity had a demonstrable effect on *P. parvum* growth. Overall, low pH combined with low salinity resulted in slower growth of *P. parvum*. Also consistent with the alga's response observed in the three-day bioassays, the combination of lowest pH (6.5) and lowest salinity (1.50‰) resulted in no growth of *P. parvum*. Exponential growth rates of the alga were calculated over the first four days of the twelve-day incubations to maintain relative consistency with growth rates calculated for the three-day bioassays. Growth rate in the salinity 1.50‰ x pH 6.5 treatment was −0.02 d^{-1} (Fig. 4, solid purple line). The highest growth rates were observed for the treatments combining high pH and high salinity (7.50‰ at pH 7.5, and 7.50‰ at pH 8.5; μ = 0.65 d^{-1} and 0.64 d^{-1}, respectively). Growth rates for the other treatments were intermediate to these extremes (μ = 0.35–0.61 d^{-1}). Multiple regression analysis indicated that there were significant effects of pH ($p < 0.001$), salinity ($p < 0.01$), and the interaction of pH and salinity ($p < 0.01$) on *P. parvum* growth rate, but pH accounted for 62 % of the effect, while salinity and interaction accounted for 25 % and 13 % respectively.

Discussion

Our strain of *Prymnesium parvum* cultured from Lake Mission Viejo appears to be a particularly toxic one as it has repeatedly caused fish kills in Lake Mission Viejo at abundances of 100 s to 1,000 s of cells ml^{-1} since its first detection (Roohk 2019). Such abundances are well below those that have caused fish kills in other ecosystems or mortality of prey in laboratory settings (generally > 10^4 cells ml^{-1}) (Acosta et al. 2015; Blossom et al. 2014b; Brooks et al. 2010; Johnsen et al. 2010; Lundgren et al. 2016; Roelke et al. 2011; Qin et al. 2020). Our study was designed to examine two of the environmental factors that might give rise to high toxicity of our strain, and help shed light on approaches that might be taken to mitigate its impact on the biota of Lake Mission Viejo.

Genetic and/or physiological aspects of *P. parvum* may explain some of the high toxicity of our strain. Suites of prymnesins, a major class of toxins

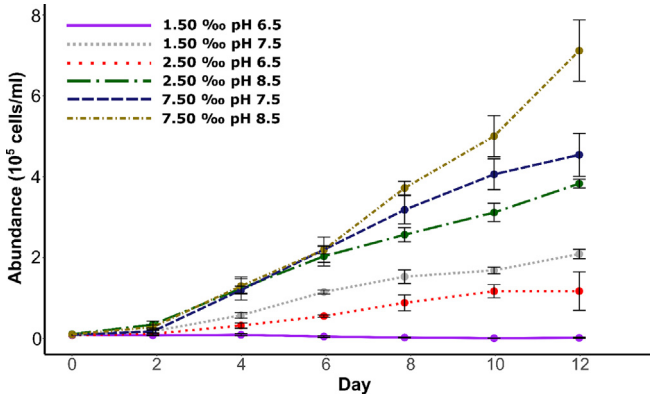

Figure 4. Daily average abundances (error bars: standard deviation; n = 3) of *P. parvum* in a 12-day growth experiment conducted for acclimated cultures of the alga grown at six different combinations of pH and salinity (different lines and symbols noted in insert).

produced by *P. parvum*, have been shown to vary among different strains of the species (Binzer et al. 2019), a situation that presumably explains some of the variability in toxicity that has been observed (Blossom et al. 2014a). The degree of toxicity of the alga is also strongly affected by its nutritional status. Toxicity in the mixotrophic alga *P. parvum* is believed to be a mechanism for capturing prey for the purpose of obtaining nutrients to supplement photosynthetic activity. Numerous studies demonstrating differences in the toxic nature of *P. parvum* under nutrient-stressed or nutrient-replete conditions support this hypothesis (Carvalho and Granéli 2010; Granéli and Johansson 2003a, b; Lundgren et al. 2016). More recent information using stable isotope analysis and gene expression data have further implicated a role for mixotrophic activity in *P. parvum* for nutrient acquisition, although carbon and energy demands appear to be met largely via photosynthesis (Brutemark and Granéli 2011; Carpenter et al. 2018; Liu et al. 2015a, b). Additional support for those laboratory studies has been provided by experimental studies employing nutrient enrichment of enclosed natural communities that have demonstrated reduced toxicity of a natural population of *P. parvum* following nutrient enrichment (Roelke et al. 2007).

These genetic and physiological considerations aside, many studies including this one have focused on various environmental (chemical and physical) variables as factors that might directly affect the toxicity and/or growth of *P. parvum* cells. In this regard, our results clearly demonstrated an important role for pH of the environment, with the lowest pH tested in this study (6.5) resulting in a rapid, dramatic and reversible decrease in toxicity of the alga towards its prey. Lowering salinity resulted in a significant albeit less dramatic impact on toxicity. Varying these two parameters together affected not only toxicity (lowest pH and lowest salinity yielded the least toxic conditions) but also decreased growth rate of the alga (Fig. 4).

Influence of Salinity on *Prymnesium parvum*

The toxicity of *P. parvum* towards its ciliate prey in the present study exhibited a significant but modest response to the salinity of the culture medium to which the algal was acclimated (Fig. 2). *P. parvum* toxicity at the lowest salinity examined in this study (1.50‰) was less compared to algal toxicity at higher salinities in bioassays conducted at the same pH.

Growth and toxicity of *P. parvum* at different salinities have been examined in several experimental studies and meta-analyses. These studies have sometimes yielded conflicting results, but often the ranges examined have been extreme rather than narrowly defined by the regions where the alga forms blooms and/or causes fish kills. Israël et al. (2014), for example, noted an inverse relationship between abundance of the alga across a range of natural ecosystems with specific conductance values ranging from 4,408 to 73,786 μS/cm (roughly 2.3–39‰), while Baker et al. (2007) noted greatest toxicity at the lowest and highest salinities tested over a roughly similar salinity range. That range, however, was wide and generally much greater than salinities at which toxic blooms of *P. parvum* have been reported, although as noted previously this euryhaline species can be found across a wide range of salinities (Rashel and Patiño 2017; Richardson and Patiño 2021). In contrast to the findings of Israël et al. (2014), it has been reported that growth and toxicity of the alga in laboratory cultures scaled positively with salinity, although other conditions (e.g. nutritional sufficiency) also played important roles in toxigenicity (Hambright et al. 2014). Still other studies have reported that growth and toxicity varied with salinity but the relationship between them was not systematic (Larsen et al. 1998). Nonetheless, recent studies characterizing toxins (Taylor et al. 2020) and gene expression analyses (Talarski et al. 2016) have observed different toxin profiles at different salinities, implying a role for salinity in affecting toxicity of *P. parvum*.

The reconciliation of these apparently contradictory findings may be explained by the observation that *P. parvum* tends to form blooms and toxic situations at intermediate salinities rather than at its extreme tolerances for this parameter (Roelke et al. 2016). Roelke et al. proposed a relationship whereby very low salinity creates a highly stressful situation for the alga where blooms and community dominance are not supported, while high salinity is not sufficiently stressful to result in toxin production and therefore ecosystem dominance. According to the hypothesis, intermediate salinity creates sufficient stress to induce toxin production but does not substantively impede growth, resulting in situations where *P. parvum* can dominate the planktonic community. Our findings are, at least in part, consistent with that hypothesis in that very low salinity (1.50‰) tended to reduce the level of toxicity observed at higher salinities. However, the highest

salinities employed in our bioassays (7.50‰) apparently were not sufficiently high to demonstrate a lessening in toxicity predicted by the hypothesis, at least in comparison to pH which had an overriding effect on toxicity (note similar decreases in ciliate abundances among the Experimental treatments of Fig. 2; gray bars).

Our study also revealed that low salinity led to decreased growth rates of *P. parvum* (compare growth curves at different salinities but the same pH in Fig. 4). This finding is consistent with a number of laboratory experiments that have demonstrated a positive relationship between salinity and *P. parvum* growth rates (Baker et al. 2007; Hambright et al. 2014; Hill et al. 2020; Padillo 1970). In addition to growth rates of the alga, Rashel and Patiño (2017) noted that an increase in salinity increased the maximum carrying capacity of *P. parvum*. These results appear to corroborate field observations documenting the expansion of *P. parvum* in inland waters throughout the southern USA (Brooks et al. 2011; Hambright et al. 2015; Roelke et al. 2011), as an increasing number of freshwater bodies are salinized through natural (e.g. evaporation) or anthropogenic processes (e.g. agriculture and resource extraction (Cañedo-Argüelles et al. 2016; Dugan et al. 2017; Herbert et al. 2015)). Salinization appears to create suitable conditions for *P. parvum* growth and community dominance.

Influence of pH on *Prymnesium parvum*

Toxicity in *P. parvum* is known to be responsive to pH. Evidence of enhanced toxicity at high pH (or inhibition of toxicity at low pH) has been reported at least as early as 1953 (Shilo and Aschner 1953), and has been since supported by other studies (Igarashi et al. 1998; Kim and Padilla 1977; Prosser et al. 2012; Shilo and Aschner 1953; Valenti et al. 2010). The specific mechanism for the effect of pH has been debated. Valenti et al. (2010) proposed a model attempting to explain the direct effect of pH on prymnesins-1,2. The authors suggested that the toxins are weak bases (pKa = 8.9) and that a larger proportion of prymnesins will be ionized at pH 6.5 (compared to 8.5), resulting in a lower propensity of toxins to cross cell membranes at low pH and cause toxicity. pH of the culture medium has been shown to affect cytoskeletal modeling and cell function in at least some protists (Vanegas-Villa et al. 2022). Therefore it appears possible that pH has the potential to modify toxins in *P. parvum*, although a pH-dependent hypothesis for reduced toxicity at pH 6.5 has been questioned (Cichewicz and Hambright 2010). The exact mechanism for the effect of external pH on the toxicity of *P. parvum* will probably not be clearly understood without further studies on the responsiveness of prymnesins to pH, and indeed until all potentially toxic compounds produced by the species are identified and their responsiveness to pH characterized.

Nonetheless, our study empirically demonstrated a strong effect of pH on the toxicity of the Lake Mission Viejo isolate of *P. parvum* over the range of values examined using our newly developed bioassay (Figs 1, 3). High pH of the culture medium ($\geq$7.5) resulted in significantly higher mortality of the ciliate prey than lower pH. Shilo and Aschner (1953) showed that toxicity of *P. parvum* plateaued at pH 7.5, a finding that is consistent with the results of our pH bioassays showing no significant differences in changes in *Cyclidium* sp. abundances (i.e. toxicity) between Experimental treatments with pH 7.5 or above (Fig. 1; two-way ANOVA, $p > 0.05$).

Results contradictory to those observed in this study examining the effect of pH on *P. parvum* toxicity have been reported in the literature, although the comparability of the results of those studies to the present one are confounded by the fact that they have generally examined ichthyotoxic and hemolytic activity, and/or used different bioassay organisms (Binford et al. 1973; Padillo 1970). For example, increasing pH was shown to decrease the ichthyotoxicity of prymnesin-2, although it enhanced its hemolysis effect (Igarashi et al. 1998). These contrasting results may indicate differences in how pH affects the interaction between target cells and toxins, or even differences in the hemolytic, cytoxic and ichthyoxic properties of different toxins produced by *P. parvum*.

Our results also demonstrated that the effect of pH on toxicity of *P. parvum* occurred rapidly and reversibly. This finding is consistent with the results of Shilo and Aschner (1953), who showed that *P. parvum* toxins can be repeatedly activated and inactivated by changing the pH between 6 and 7. Prosser et al. (2012) also found complete elimination of *P. parvum* toxicity when the pH in their samples was lowered from 7.5 or 8.5 to 7. However, the authors found no toxicity in a reverse assay in which the pH of *P. parvum* samples was increased from 7 to 8.5, as opposed to the results of the pH 6.5–8.5 bioassay in our study (Fig. 1f) which exhibited a

rapid decline of *Cyclidium* abundance after the pH of the culture was increased from 6.5 to 8.5. Prosser et al. suggested that the *P. parvum* in their pH 7 sample did not have enough toxins to be toxic, thus implying that low pH not only reduces the potency of prymnesins (as discussed in previous paragraphs), but also the production of toxins. Their results do not necessarily contradict ours, as the *P. parvum* abundance in their sample (~5,000 cells/ml) was 4 X lower than the starting abundance in our bioassays (>20,000 cells/ml), and it is possible that toxin concentration in our pH 6.5–8.5 bioassay was high enough (due to the higher abundance of *P. parvum* present) for the culture to become toxic after the pH was increased from 6.5 to 8.5.

In addition to lowering *P. parvum* toxicity, low pH also decreased the growth rate of *P. parvum* (Fig. 4; Supplementary Material Fig. S1). Studies of the effect of pH on *P. parvum* growth rate are scarce compared to other factors that have been studied. Prosser et al. (2012) conducted mesocosm experiments and found that pH 7 led to lowering of growth of a natural population of *P. parvum* and prevented bloom formation. Indeed, the prevalence, if not relegation, of toxic blooms of *P. parvum* in water bodies with pH > 8 may indicate the impact of pH on both the alga's growth rate as well as cellular toxicity.

Interaction Between Salinity and pH

Studies examining the interaction of chemical/physical parameters such as temperature, salinity and pH on *P. parvum* growth and toxicity have been carried out, but generally these studies examined the factors that might limit the geographical distribution of the species (Baker et al. 2007) rather than investigating the effect of these parameters over ranges pertinent to blooms formed by this species. The results of the present study demonstrate that, within the narrow ranges of pH and salinity examined, there was a direct, additive relationship between pH and salinity on the toxicity of *P. parvum* (Figs 1, 2, 3), although the effect of pH appeared to be dominant. For example, the dominance of pH in establishing toxicity was shown by the absence of toxicity in all treatments conducted at pH 6.5 (Figs 1a, 3a, 3c). Toxicity observed at intermediate pH (7.5) was moderated by salinity; i.e. toxicity at 1.5‰ was generally lower compared to toxicity at higher salinities (Fig. 2).

The twelve-day growth experiment to examine the effect of pH and salinity on the growth rate of *P. parvum* clearly indicated that these parameters affected algal growth in the absence of ciliate prey in a similar manner as they affected toxicity; i.e., an overall positive interaction between growth rate, pH and salinity (Fig. 4). Similar findings were observed in the three-day bioassays where prey were present (Supplementary Material Fig. S1). The overall range of growth rates (0.51–0.78 d^{-1} among 15 bioassays, with the exception of the lowest pH and salinity values tested) indicated reasonable but not excessively high population growth rates. This finding was somewhat surprising given field observations and the hypothesis that *P. parvum* blooms often form under conditions that are not conducive to high growth rate, and that stress is often considered an inducer for toxin production (Roelke et al. 2016). However, the growth rates observed in this study under nutrient replete conditions were less than the maximal growth rates that have been reported for the alga (Baker et al. 2007; Larsen et al. 1998; Rashel and Patiño 2017) and therefore may still represent a moderately stressful condition for the alga.

Interestingly, the growth rates of *P. parvum* in the absence of ciliate prey at particular pH and salinity values in the twelve-day growth experiment (Fig. 4) were overall consistent with the growth rates of the alga observed at similar pH and salinity values when prey were present in the three-day bioassays (Supplementary Material Fig. S1c). This finding indicates that pH and salinity, more than the availability of prey, affected growth rate of the alga in our study. That result is consistent with previous studies that have largely characterized the alga as an obligate phototroph, despite its highly developed ability to attack and kill a wide spectrum of prey (Brutemark and Granéli 2011; Carpenter et al. 2018; Liu et al. 2015a, b). Also, our studies were conducted using nutrient-enriched medium, so one might expect that sufficient inorganic nutrients were present to support algal growth with little need for mixotrophic (predatory) supplementation. In that case, however, one might also have expected low toxicity of the alga in our study, if toxins are primarily used for capturing prey to supplement nutrient uptake. It is unclear if our *P. parvum* strain would have been even more toxic than demonstrated in this study if it were nutrient-starved, but nonetheless its toxicity remained strongly affected by pH and salinity of the external medium used in our study.

One issue addressed by our study was whether high pH and high salinity enables community dom-

inance by *P. parvum* through its effect on the alga's growth rate, or whether these environmental parameters act through their effect on cellular toxicity. Based on our results, both factors appear to contribute to the alga's success in nature. High pH and brackish conditions alone (i.e. without considering predation-enabled mixotrophy by the alga) could provide conditions conducive to expansion of a *P. parvum* population in nature by their positive effect on growth rate. However, increased toxicity was also observed at high pH and high salinity, and various combinations of these two conditions (Figs 1–3). Moreover, the responses to changes in pH were rapid (i.e. within 24 h, faster than the population growth response of the alga) and reversible (Fig. 1f,g). Therefore, it would appear that the effects of increasing salinity and/or pH act to increase the growth rate of the *P. parvum* as well as increasing toxicity, creating ideal conditions for population growth and community dominance. These findings may help explain the extreme toxicity of the *P. parvum* population in Lake Mission Viejo. Moreover, since low pH and low salinity act to constrain both population growth of the alga and its toxicity, this information may provide guidance on possible mitigative approaches for combatting fish kills attributable to the Golden Alga.

Conclusions

A bioassay to determine the toxicity of the haptophyte *Prymnesium parvum* was developed using a ciliated protist as prey, and used to examine the effect of varying pH and salinity on the toxicity of an isolated strain of the alga from Lake Mission Viejo, Mission Viejo, CA, USA. Toxicity was substantially reduced at lower pH over the range investigated (6.5–8.5) in three-day bioassays. Toxicity of *P. parvum* cells was also rapidly (within one day) and reversibly altered in response to abrupt increases or decreases of pH of the culture medium. Toxicity was reduced, but to a lesser degree, at lower salinities over the range investigated (1.50–7.50‰), and additive effects were apparent as decreased toxicity at the lowest pH and salinity values examined. The growth rate of *P. parvum* was directly related to salinity and pH over the ranges examined, and growth was not supported (net mortality) at the lowest salinities and pH combined (salinity 1.50‰ at pH 6.5).

Methods

Isolation and maintenance of cultures: A monoclonal strain of *Prymnesium parvum* was isolated by micropipetting single cells from a sample collected from Lake Mission Viejo in February 2015. The monoclonal culture was subsequently grown in enriched phytoplankton media (DY-V algal medium) at ~23 °C on a 12:12 h light/dark cycle at ~250 μEinstein m^{-2} s^{-1} for use in all bioassays. A monoclonal culture of the ciliate *Cyclidium* sp. (strain Gcycl-1; obtained from Dr. Robert Sanders) was used as prey for *P. parvum* in all bioassays. Both cultures (alga and ciliate) contained an uncharacterized, mixed bacterial flora. The algal medium (Andersen 2005) was used to culture both *P. parvum* and maintain *Cyclidium* sp. (the latter survived on the attendant bacteria; see below). *Cyclidium* sp. cultures were fed live bacteria (the attendant bacteria were induced to grow by the addition of ~0.05% sterile yeast extract five days prior to the bioassays). Culture media of various salinities were prepared by adding 0.2 μm filtered seawater (obtained at the University of Southern California San Pedro Ocean Time-Series station and aged for > 3 months in the dark) to ultrapure water (Barnstead Ultrapure; Thermo Scientific; Waltham, MA, USA). Electrical conductivity was measured using a Health-Metric conductivity meter (Cheyenne, WY, USA) and converted to salinity (specific conductivity (μS/cm) at 25 °C × conversion factor of 0.67). HCl or NaOH (0.5 M) were used to adjust the pH of the media. The pH of the cultures and various bioassay treatments were measured using an Orion Star A211 pH meter (Thermo Scientific, Waltham, MA, USA).

Culturing conditions for bioassays: *P. parvum* was acclimated to each treatment condition (i.e. various salinities and pH values) for more than two weeks prior to the bioassays. Culture conditions were the same as noted above for both *P. parvum* and *Cyclidium* sp.. All stock cultures were placed on an orbital shaker with slow shaking (~40 rpm) to promote gentle mixing. Ciliate abundances were concentrated immediately prior to the start of the bioassays by gravity filtration through 5 μm polycarbonate filters (Whatman, Maidstone, UK) to retain ciliates but allow most of the culture medium and bacteria to pass through the filters.

Bioassays were carried out in 50 ml culture flasks (Falcon, Corning, USA) with each treatment conducted in triplicate. Experimental treatments included both *Cyclidium* sp. and *P. parvum,* while Controls included only *Cyclidium* sp. (i.e. no *P. parvum* predation). All bioassays were started with a target abundance of 20,000 *P. parvum* ml^{-1} and 2,000 *Cyclidium* sp. ml^{-1} at a total volume of ~45 ml. All flasks were placed in the same incubator on an orbital shaker, as employed for the stock cultures. Samples were collected daily (days 1, 2 and 3) and the abundance of both *P. parvum* and *Cyclidium* sp. were quantified by light microscopy.

Bioassays with only one variable (i.e. various pH values with salinity held constant, or various salinities with pH held constant) were first conducted to avoid interference by the other variable that would otherwise confound interpretation of the results.

pH bioassay: Bioassays were conducted for three days in order to examine a range of pertinent pH values on the toxicity of *P. parvum* towards ciliate prey. These bioassays consisted of treatments of the following pH values: 6.5, 7.0, 7.5, 8.0, 8.5. This range of pH values brackets the present pH recorded at Lake Mission Viejo (8.0 – 8.5) and lower pH values that may be effective in reducing *P. parvum* toxicity as suggested by past studies (Igarashi et al. 1998; Kim and Padilla 1977; Prosser et al. 2012; Shilo and Aschner 1953; Valenti et al. 2010). Additionally, two treatments were conducted that investigated whether toxicity of *P. parvum* could be altered rapidly by adjusting the pH of the medium. These treatments were initiated at pH 6.5 or 8.5 and sampled after one day of incubation. Following

sampling on day one, the treatment at pH 6.5 was adjusted to 8.5, while the treatment at pH 8.5 was adjusted to 6.5. Both treatments were sampled for two more days. Small volumes (<40 μl) of HCl or NaOH (0.5 M) were used to adjust the pH of the media in these treatments. The pH of all treatments was measured and adjusted daily to ensure that they remained at the target pH values. The salinity of all treatments in the pH bioassays was 2.50‰, the current average salinity of Lake Mission Viejo.

Salinity bioassay: Bioassays were conducted for three days in order to examine a range of pertinent salinities on the toxicity of *P. parvum* towards ciliate prey. These bioassays consisted of four treatments: 1.50, 2.50, 5.00, and 7.50‰ prepared as noted above by the addition of filter-sterilized natural seawater to the DY-V medium to achieve each salinity. This salinity range includes historical (1.50‰) and present (2.50‰) salinity values recorded at Lake Mission Viejo, as well as 'worst-case scenarios' of future projected salinity (Roohk 2019; 5.00 and 7.50‰). The pH of all treatments in the salinity bioassay was checked daily and maintained at 7.5 using 0.5 M HCl or NaOH. That pH value represents a median of the range of pH values used in the pH bioassay, and a management goal for Lake Mission Viejo.

Salinity × pH bioassay: Bioassays were conducted for three days in order to examine a matrix of salinity and pH values on the toxicity of *P. parvum* towards ciliate prey. These bioassays examined the effects of different salinities and pH values at three salinities (1.50, 2.50, 7.50‰) and three pH values (pH 6.5, 7.5, 8.5). Treatments with the lowest and highest pH values tested in the pH bioassay, as well as those with the lowest and highest salinities in the salinity bioassay were included. Two additional treatments were also included, one with the lowest pH and salinity and one with the highest salinity and pH for a total of six experimental treatments: 1.50‰ at pH 6.5; 1.50‰ at pH 7.5; 2.50‰ at pH 6.5; 2.50‰ at pH 8.5; 7.50‰ at pH 7.5; 7.50‰ at pH 8.5.

Effect of pH and salinity on *P. parvum* growth over a twelve-day period: The growth rate of *P. parvum* at three salinities and three pH values was examined for the same conditions studied in the salinity × pH bioassay. This set of treatments was conducted to determine algal growth rate (but not necessarily toxicity) at constant light intensity, temperature and nutritionally replete conditions. Cultures of *P. parvum* were grown in the same 50 ml culture flasks and under the same conditions as the salinity × pH bioassay. No ciliate prey was provided to the algae and no bacterial enrichment was performed, although a low abundance of attendant bacterial flora was present. Samples were collected every-two days and abundances of the alga determined by light microscopy.

Sample processing and data analysis: Aliquots (5 ml) of each flask were collected at the time of each sampling and preserved with Lugol's solution (3% final concentration). Samples were stored at 4 ℃ until processed. Cell abundances were determined using a Sedgewick-Rafter counting chamber at 200 times magnification on a compound light microscope (BX51; Olympus, Waltham, USA). Growth rates of *P. parvum* were determined from the best fit slopes of plots of the natural log of cell abundance versus time.

Data obtained from the bioassays were used to test (1) if ciliate abundances were different between Experimental and Control treatments, and (2) if ciliate abundances were different between Experimental treatments within the same set of bioassays (e.g. pH, salinity or salinity × pH bioassays; statistical analysis for the 6.5–8.5 and 8.5–6.5 treatments was performed separately from the rest of the treatments in the pH bioassay). *Cyclidium* sp. mortality is a proxy for *P. parvum* toxicity, therefore these tests identified (1) under which environmental conditions *P. parvum* was most toxic, and (2) trends between *P. parvum* toxicity and pH or salinity. Because the initial ciliate abundances were slightly different among the bioassays, percent changes in ciliate abundances relative to their starting abundances were calculated and used in all statistical tests:

$$\text{Percent abundance change} = \frac{\text{Abundance}_{\text{Day 3}} - \text{Abundance}_{\text{Day 0}}}{\text{Abundance}_{\text{Day 0}}} \times 100\%$$

Percent abundance changes for Experimental treatments and their respective Controls were generated separately. Repeated two-way ANOVA with Tukey post-hoc analyses (presence/absence of *P. parvum* as a variable, pH or salinity as the second variable) were performed for results from each day of the three-day incubation experiment, but focus was placed on statistical results from day 3 when the differences between Experimental treatments and their respective Controls, and between different Experimental treatments were most apparent. Similarly, three-way ANOVA with Tukey post-hoc analysis (presence/absence of *P. parvum* as a variable, pH as the second variable, salinity as the third variable) was performed using results from the salinity × pH bioassay. Linear regression was used to fit changes in *Cyclidium* sp. abundances in Experimental treatments against either pH or salinity values for the pH or salinity bioassays, respectively. Multiple regression analysis was used when both pH and salinity were varied (i.e. the salinity × pH bioassay). A post-hoc analysis of relative importance (from the R relaimpo package) was used to obtain percentage contributions of pH, salinity, or interactive effects of salinity × pH from the multiple regression analysis results. All statistical tests were performed in R.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary Material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.protis.2022.125927.

References

Acosta F, Zamor R, Najar FZ, Roe BA, Hambright KD (2015) Dynamics of an experimental microbial invasion. Proc Natl Acad Sci USA **112**:11594–11599

Andersen RA (2005) Algal Culturing Techniques. Academic Press; Burlington, MA, 592p

Baker JW, Grover JP, Brooks BW, Ureña-Boeck F, Roelke DL, Errera R, Kiesling RL (2007) Growth and toxicity of *Prymnesium parvum* (Haptophyta) as a function of salinity, light, and temperature. J Phycol **43**:219–227

Binford JS, Martin DF, Padilla GM (1973) Hemolysis induced by *Prymnesium parvum* toxin calorimetric studies. Biochim Biophys Acta (BBA). Biomembranes **291**:156–164

Binzer SB, Svenssen DK, Daugbjerg N, Alves-de-Souza C, Pinto E, Hansen PJ, Larsen TO, Varga E (2019) A-, B- and C-type prymnesins are clade specific compounds and chemotaxonomic markers in *Prymnesium parvum*. Harmful Algae **81**:10–17

Blossom HE, Andersen NG, Rasmussen SA, Hansen PJ (2014a) Stability of the intra- and extracellular toxins of *Prymnesium parvum* using a microalgal bioassay. Harmful Algae **32**:11–21

Blossom HE, Rasmussen SA, Andersen NG, Larsen TO, Nielsen KF, Hansen PJ (2014b) *Prymnesium parvum* revisited: relationship between allelopathy, ichthyotoxicity, and chemical profiles in 5 strains. Aquat Toxicol **157**:159–166

Brooks BW, James SV, Valenti JTW, Urena-Boeck F, Serrano C, Berninger JP, Schwierzke L, Mydlarz LD, Grover JP, Roelke DL (2010) Comparative toxicity of *Prymnesium parvum* in Inland waters. J Am Water Resour Assoc **46**:45–62

Brooks BW, Grover JP, Roelke DL (2011) *Prymnesium parvum*: An emerging threat to inland waters. Environ Toxicol Chem **30**:1955–1964

Brutemark A, Granéli E (2011) Role of mixotrophy and light for growth and survival of the toxic haptophyte *Prymnesium parvum*. Harmful Algae **10**:388–394

Cañedo-Argüelles M, Hawkins CP, Kefford BJ, Schäfer RB, Dyack BJ, Brucet S, Buchwalter D, Dunlop J, Frör O, Lazorchak J, Coring E, Fernandez HR, Goodfellow W, Achem ALG, Hatfield-Dodds S, Karimov BK, Mensah P, Olson JR, Piscart C, Prat N, Ponsá S, Schulz C-J, Timpano AJ (2016) Saving freshwater from salts. Science **351**:914–916

Carpenter KJ, Bose M, Polerecky L, Lie AAY, Heidelberg KB, Caron DA (2018) Single-cell view of carbon and nitrogen acquisition in the mixotrophic alga *Prymnesium parvum* (Haptophyta) inferred from stable isotope tracers and NanoSIMS. Frontiers Mar Sci **5**:157

Carvalho WF, Granéli E (2010) Contribution of phagotrophy versus autotrophy to *Prymnesium parvum* growth under nitrogen and phosphorus sufficiency and deficiency. Harmful Algae **9**:105–115

Cichewicz RH, Hambright KD (2010) A revised amino group pKa for prymnesins does not provide decisive evidence for a pH-dependent mechanism of *Prymnesium parvum*'s toxicity. Toxicon **55**:1035–1037

Dugan HA, Bartlett SL, Burke SM, Doubek JP, Krivak-Tetley FE, Skaff NK, Summers JC, Farrell KJ, McCullough IM, Morales-Williams AM, Roberts DC, Ouyang Z, Scordo F, Hanson PC, Weathers KC (2017) Salting our freshwater lakes. Proc Nat Acad Sci USA **114**:4453–4458

Fistarol GO, Legrand C, Granéli E (2003) Allelopathic effect of *Prymnesium parvum* on a natural plankton community. Mar Ecol Prog Ser **255**:115–125

Granéli E, Edvardsen B, Roelke DL, Hagström JA (2012) The ecophysiology and bloom dynamics of *Prymnesium* spp. Harmful Algae **14**:260–270

Granéli E, Johansson N (2003a) Effects of the toxic haptophyte *Prymnesium parvum* on the survival and feeding of a ciliate: the influence of different nutrient conditions. Mar Ecol Prog Ser **254**:49–56

Granéli E, Johansson N (2003b) Increase in the production of allelopathic substances by *Prymnesium parvum* cells grown under N- or P-deficient conditions. Harmful Algae **2**:135–145

Granéli E, Salomon PS (2010) Factors influencing allelopathy and toxicity in *Prymnesium parvum*. J Am Water Resour Assoc **46**:108–120

Hambright DK, Beyer JE, Easton JD, Zamor RM, Easton AC, Hallidayschult TC (2015) The niche of an invasive marine microbe in a subtropical freshwater impoundment. ISME J **9**:256–264

Hambright KD, Easton JD, Zamor RM, Beyer J, Easton AC, Allison B (2014) Regulation of growth and toxicity of a mixotrophic microbe: implications for understanding range expansion in *Prymnesium parvum*. Freshw Sci **33**:745–754

Herbert ER, Boon P, Burgin AJ, Neubauer SC, Franklin RB, Ardón M, Hopfensperger KN, Lamers LPM, Gell P (2015) A global perspective on wetland salinization: ecological consequences of a growing threat to freshwater wetlands. Ecosphere **6**:1–43

Hill BN, Saari GN, Steele WB, Corrales J, Brooks BW (2020) Nutrients and salinity influence *Prymnesium parvum* (UTEX LB 2797) elicited sublethal toxicity in *Pimephales promelas* and *Danio rerio*. Harmful Algae **93**:101795

Holdway PA, Watson RA, Moss B (1978) Aspects of the ecology of *Prymnesium parvum* (Haptophyta) and water chemistry in the Norfolk Broads, England. Freshwater Biol **8**:295–311

Igarashi T, Aritake S, Yasumoto T (1998) Biological activities of prymnesin-2 isolated from a red tide alga *Prymnesium parvum*. Natural Toxins **6**:35–41

Israël NMD, VanLandeghem MM, Denny S, Ingle J, Patiño R (2014) Golden alga presence and abundance are inversely related to salinity in a high-salinity river ecosystem, Pecos River, USA. Harmful Algae **39**:81–91

Johnsen TM, Eikrem W, Olseng CD, Tollefsen KE, Bjerknes V (2010) *Prymnesium parvum*: the Norwegian experience. J Am Water Resour Assoc **46**:6–13

Jones AC, Hambright KD, Caron DA (2018) Ecological patterns among bacteria and microbial eukaryotes derived from network analyses in a low-salinity lake. Microb Ecol **75**:917–929

Kim YS, Padilla GM (1977) Hemolytically active components from *P. parvum* and *G. breve* toxins. Life Sci **21**:1287–1292

Larsen A, Bryant S, Bámstedt U (1998) Growth rate and toxicity of *Prymnesium parvum* and *Prymnesium patelliferum* (haptophyta) in response to changes in salinity, light and temperature. Sarsia **83**:409–418

Lindehoff E, Granéli E, Glibert PM (2010) Influence of prey and nutritional status on the rate of nitrogen uptake by *Prymnesium parvum* (haptophyte)1. J Am Water Resour Assoc **46**:121–132

Liu Z, Jones AC, Campbell V, Hambright KD, Heidelberg KB, Caron DA (2015a) Gene expression in the mixotrophic prymnesiophyte, *Prymnesium parvum*, responds to prey availability. Frontiers Microbiol **6**:1–12

Liu Z, Koid AE, Terrado R, Campbell V, Caron DA, Heidelberg B (2015b) Changes in gene expression of *Prymnesium parvum* due to nitrogen and phosphorus limitation. Frontiers Microbiol **6**:631

Lundgren VM, Glibert PM, Granéli E, Vidyarathna NK, Fiori E, Ou L, Flynn KJ, Mitra A, Stoecker DK, Hansen PJ (2016) Metabolic and physiological changes in *Prymnesium parvum* when grown under, and grazing on prey of, variable nitrogen:phosphorus stoichiometry. Harmful Algae **55**:1–12

Manning SR, La Claire JW (2010) Prymnesins: Toxic metabolites of the Golden Alga, *Prymnesium parvum* Carter (Haptophyta). Marine Drugs **8**:678–704

Michaloudi E, Moustaka-Gouni M, Gkelis S, Pantelidakis K (2008) Plankton community structure during an ecosystem disruptive algal bloom of *Prymnesium parvum*. J Plankton Res **31**:301–309

Otterstrøm CV, Steeman Nielsen E (1940) Two cases of extensive mortality in fishes caused by the flagellate *Prymnesium parvum*, Carter. Rept Danish Biol Station **44**:1–24

Padillo GM (1970) Growth and toxigenesis of the chrysomonad *Prymnesium parvum* as a function of salinity. J Protozool **17**:456–462

Prosser KN, Valenti TW, Hayden NJ, Neisch MT, Hewitt NC, Umphres GD, Gable GM, Grover JP, Roelke DL, Brooks BW (2012) Low pH preempts bloom development of a toxic haptophyte. Harmful Algae **20**:156–164

Qin J, Hu Z, Zhang Q, Xu N, Yang Y (2020) Toxic effects and mechanisms of *Prymnesium parvum* (Haptophyta) isolated from the Pearl River Estuary. China. Harmful Algae **96**:101844

Rashel RH, Patiño R (2017) Influence of genetic background, salinity, and inoculum size on growth of the ichthyotoxic golden alga (*Prymnesium parvum*). Harmful Algae **66**:97–104

Rasmussen SA, Meier S, Andersen NG, Blossom HE, Duus JØ, Nielsen KF, Hansen PJ, Larsen TO (2016) Chemodiversity of ladder-frame prymnesin polyethers in *Prymnesium parvum*. J Nat Prod **79**:2250–2256

Remmel EJ, Hambright KD (2012) Toxin-assisted micropredation: experimental evidence shows that contact micropredation rather than exotoxicity is the role of *Prymnesium* toxins. Ecol Lett **15**:126–132

Richardson ET, Patiño R (2021) Growth of the harmful alga, *Prymnesium parvum* (Prymnesiophyceae), after gradual and abrupt increases in salinity. J Phycol **57**:1335–1344

Roelke DL, Errera RM, Kiesling R, Brooks BW, Grover JP, Schwierzke L, Urena-Boeck F, Baker J, Pinckney JL (2007) Effects of nutrient enrichment on *Prymnesium parvum* population dynamics and toxicity: results from field experiments, Lake Possum Kingdom, USA. Aquat Microb Ecol **46**:125–140

Roelke DL, Grover JP, Brooks BW, Glass J, Buzan D, Southard GM, Fries L, Gable GM, Schwierzke-Wade L, Byrd M, Nelson J (2011) A decade of fish-killing *Prymnesium parvum* blooms in Texas: roles of inflow and salinity. J Plankton Res **33**:243–253

Roelke DL, Barkoh A, Brooks BW, Grover JP, Hambright KD, LaClaire JW, Moeller PDR, Patino R (2016) A chronicle of a killer alga in the west: ecology, assessment, and management of *Prymnesium parvum* blooms. Hydrobiologia **764**:29–50

Roohk DL (2019) Lake Mission Viejo annual water monitoring evaluation. In, Laguna Niguel, CA

Shilo M, Aschner M (1953) Factors governing the toxicity of cultures containing the phytoflagellate *Prymnesium parvum* Carter. Microbiology **8**:333–343

Sopanen S, Koski M, Kuuppo P, Uronen P, Legrand C, Tamminen T (2006) Toxic haptophyte *Prymnesium parvum* affects grazing, survival, egestion and egg production of the calanoid copepods *Eurytemora affinis* and *Acartia bifilosa*. Mar Ecol Prog Ser **327**:223–232

Southard GM, Fries LT, Barkoh A (2010) *Prymnesium parvum*: The Texas experience. J Am Water Resour Assoc **46**:14–23

Talarski A, Manning SR, La Claire II JW (2016) Transcriptome analysis of the euryhaline alga, *Prymnesium parvum* (Prymnesiophyceae): effects of salinity on differential gene expression. Phycologia **55**:33–44

Taylor RB, Hill BN, Bobbitt JM, Hering AS, Brooks BW, Chambliss CK (2020) Suspect and non-target screening of acutely toxic *Prymnesium parvum*. Sci Total Environ **715**:136835

Tillmann U (2003) Kill and eat your predator: a winning strategy of the planktonic flagellate *Prymnesium parvum*. Aquat Microb Ecol **32**:73–84

Valenti TW, James SV, Lahousse MJ, Schug KA, Roelke DL, Grover JP, Brooks BW (2010) A mechanistic

explanation for pH-dependent ambient aquatic toxicity of *Prymnesium parvum* Carter. Toxicon **55**:990–998

Vanegas-Villa SC, Torres-Cifuentes DM, Baylon-Pacheco L, Espíritu-Gordillo P, Durán-Díaz Á, Rosales-Encina JL, Omaña-Molina M (2022) External pH variations modify proliferation, erythrophagocytosis, cytoskeleton remodeling, and cell morphology of *Entamoeba histolytica* trophozoites. Protist **173**:125857

VanLandeghem MM, Denny S, Patiño R (2015) Predicting the risk of toxic blooms of golden alga from cell abundance and environmental covariates. Limnol Oceanogr Methods **13**:568–586

Weissbach A, Legrand C (2012) Effect of different salinities on growth and intra- and extracellular toxicity of four strains of the haptophyte *Prymnesium parvum*. Aquat Microb Ecol **67**:139–149