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RESEARCH ARTICLE

Grazer-induced bioluminescence and toxicity in marine dinoflagellates

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Abstract

Marine copepods are the most abundant multicellular zooplankton in the global oceans. They imprint their surrounding waters with a unique bouquet of chemical compounds, including polar lipids such as copepodamides. Prey organisms can detect copepodamides and respond by inducing defensive traits including bioluminescence, toxin production, changes in colony size, and structural modifications. This mechanism has been suggested to contribute to harmful algal bloom formation, but to date only a limited number of species and strains have been experimentally exposed to copepodamides. Here, we quantify bioluminescence and toxin content in response to increasing concentrations of copepodamides in three harmful algal bloom-forming species of marine dinoflagellates: *Alexandrium catenella*, *Protoceratium reticulatum*, and *Gymnodinium catenatum*. All three species up-regulated their defensive traits in response to copepodamide exposure, including the first example of copepodamide-induced GC-toxin production. Neither bioluminescence nor toxin production was associated with measurable costs in terms of reduced growth rates. The results support the role of copepodamides as general alarm cues in marine phytoplankton. Moreover, the expression of simultaneous defensive traits may confound studies addressing the costs and benefits of these co-varying traits.

Phytoplankton are the principal primary producers in the marine food webs (Field et al. 1998; Frederiksen et al. 2006; Pershing et al. 2015). The majority of the phytoplankton production is consumed by zooplankton, which play a multifaceted role in nutrient cycling (Sailley et al. 2015; Meunier et al. 2016), transfer of energy to higher trophic levels (Turner 2004; Heneghan et al. 2016), and the structuring of phytoplankton communities (Bergquist et al. 1985). Copepods often dominate the metazoan zooplankton biomass (Froneman 2001; Pane et al. 2004) and are key grazers on microzooplankton and larger phytoplankton. In addition to direct grazing effects, copepods exude a bouquet of chemical compounds that induce putative defensive traits in prey organisms (Selander et al. 2006, 2019). The active compounds have been identified as a group of polar lipids named copepodamides (Selander et al. 2015). Copepodamides all have one of two closely related molecular scaffolds, separated only by the presence of a methyl or methylene group in position C3, with a variable fatty acid moiety attached to the scaffold by an ester linkage (Selander et al. 2015; Grebner et al. 2019). To date, 41 unique copepodamide structures have been described (Selander et al. 2015; Grebner et al. 2019; Arnoldt et al. 2024). All calanoid and cyclopoid copepods tested so far, from both limnic and marine environments, produce copepodamides (Selander et al. 2015; Grebner et al. 2019; Arnoldt et al. 2024), with the possible exception of carnivorous species (Lundholm et al. 2018). Although the physiological role of copepodamides in copepods is still unknown, similar compounds have been suggested to function as emulsifiers facilitating lipid uptake in the ciliate *Tetrahymena* (Kunimitsu and Keiko 1986).

Some phytoplankton sense naturally occurring (femto- to picomolar) concentrations of copepodamides and respond by launching traits associated with increased resistance to grazing. These include bioluminescence (Lindström et al. 2017;

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Prevett et al. 2019), amnesic shellfish toxin (AST) production (Selander et al. 2015; Olesen et al. 2022), paralytic shellfish toxin (PST) production (Selander et al. 2015; Ryderheim et al. 2021), colony size plasticity (Rigby and Selander 2021) and silicification in diatoms (Grønning and Kiørboe 2020). Exposure to phycotoxins such as PSTs, brevotoxins, okadaic acid group toxins, and dinophysistoxins has variable effects on copepod grazers. Some are seemingly unaffected, while others show a range of adverse effects (Turner 2006). These include increased mortality (Huntley et al. 1986; Abdulhussain et al. 2020; Ladds et al. 2024), reduced egg production and viability (Bagoien et al. 1996; Frangópulos et al. 2000; Guisande et al. 2002) and alterations in swimming behavior (Han et al. 2021; Kang et al. 2022). Behavioral rejections of toxic prey have also been observed in copepods (Schultz and Kiørboe 2009; Abdulhussain et al. 2020, 2021), which together with the grazer-induced up-regulation, supports a defensive role of PSTs. The grazer deterrent effect of phycotoxins is, however, based on correlative evidence, and adverse effects may also result from correlated traits. The toxic dinoflagellates *Alexandrium catenella* and *Protoceratium reticulatum* are for example also bioluminescent, a trait also associated with efficient grazer deterrence (Prevett et al. 2019). The grazer deterrent effect of bioluminescence is not fully understood. Three competing hypotheses have been put forward. The first suggests that bioluminescent flashes trigger a startling response in the copepod that allows the dinoflagellate to escape (Esaias and Curl 1972), the second that bioluminescence serves as a "burglar alarm," where the light attracts the grazer's own predators (Burkenroad 1943), and the third that bioluminescence serves as an aposematic warning, signaling its toxicity to the predator (Hanley and Widder 2017). From this perspective it is notable that at least 11 common bioluminescent bloom-forming species in the genera *Alexandrium*, *Gonyaulax*, *Lingulaulax*, and *Protoceratium* are known to be toxic (Cusick and Widder 2020). The co-occurrence of bioluminescence and toxicity raises the question of whether these defensive traits are regulated independently or in concert when exposed to copepodamides.

Copepod-induced production of harmful algal toxins has been suggested to contribute to harmful algal bloom (HAB) formation. In addition to the increased toxin production copepods selectively reject more toxic (Huntley et al. 1986; Teegarden 1999; Schultz and Kiørboe 2009; Olesen et al. 2022) or more bioluminescent cells (Esaias and Curl 1972; Prevett et al. 2019), thereby increasing their relative abundance in the phytoplankton community (Cusick and Widder 2020). This grazer-mediated competitive edge could explain the success of defended dinoflagellates despite their slow growth rates and poor abilities to compete for nutrients (Banse 1982; Smayda 1997; Litchman et al. 2007). However, the generality and relative importance of this mechanism are poorly understood.

While copepodamides have been suggested to be general defense inducers in phytoplankton, with the capacity to structure plankton communities (Rigby et al. 2024) only a limited subset of species has been experimentally exposed. In addition these include taxa that do not show a marked response to copepodamides, for example, the diarrhetic shellfish toxin producer genus *Dinophysis* spp. (Pourdanandeh et al. 2025). To better understand the role of grazer-induced bioluminescence and toxin production in HAB formation a more comprehensive coverage of exposed species and the associated costs and benefits is needed.

Among the common HAB-forming dinoflagellates that have not yet been experimentally exposed to copepodamides we find *P. reticulatum*, and *Gymnodinium catenatum*. Both of which, together with *A. catenella*, are responsible for recurrent and economically significant HABs worldwide (Koike et al. 2006; Bravo et al. 2008; Álvarez et al. 2011; Ribeiro et al. 2012; Condie et al. 2019; Anderson et al. 2021). Copepodamide-induced bioluminescence has been demonstrated in *A. catenella* (formerly *Alexandrium tamarense*; John et al. 2014; Lindström et al. 2017). However, the simultaneous onset of bioluminescence and toxin production in response to copepodamide cues has not been explored. Moreover, *A. catenella* and *G. catenatum* produce PSTs in response to copepod presence, but it is not known if the response is mediated by copepodamides or other copepod-derived compounds (Selander et al. 2012; Selander et al. 2016; Griffin et al. 2019; Park et al. 2024).

Theory predicts that the induction of defensive traits should entail fitness costs, often assumed to manifest as a reduced growth rate for microalgae cells (Pančić and Kiørboe 2018). Without costs, microalgae should evolve to a constant defended state, which does not appear to be the case. The literature on ecological costs associated with defensive traits in phytoplankton is, however, highly inconsistent and includes direct costs in terms of reduced growth rates (Olesen et al. 2022; Rigby et al. 2022; Park et al. 2023) as well as undetectable, or even negative costs (faster growth of better defended cells, Pančić and Kiørboe 2018; Ryderheim et al. 2021).

Here we exposed the three dinoflagellates *A. catenella*, *P. reticulatum*, and *G. catenatum* to copepodamides and hypothesized that copepodamides would induce toxin production in *G. catenatum*, bioluminescence in *P. reticulatum*, and both toxin production and bioluminescence in *A. catenella*. We further predicted that copepodamide-induced stimulation of toxin and bioluminescence would be accompanied by a direct allocation cost that would manifest as reduced growth rates, with the greatest cost incurred by species that simultaneously upregulate both phycotoxins and bioluminescent capacity.

Materials and methods

Cell cultures

Gymnodinium catenatum (IO13-27-02, isolated from Cascais, Portugal, September 2018), *P. reticulatum* (109, isolated from

Washington USA, in 1999), and *A. catenella* (130, unknown isolation location and date), were obtained from the Gothenburg University marine algae collection (GUMACC). *Gymnodinium catenatum* was grown in a dark : light cycle of 12 : 12 h and at ~ 19°C. *Alexandrium catenella* and *P. reticulatum* were grown in a reversed dark : light cycle of 10 : 14 h at ~ 16°C to allow daytime sampling of dark-adapted cultures, as bioluminescence is largely absent during the light phase. All three were cultured under a photon flux of 135 μmol $m^2 \cdot s^{-1}$ from fluorescent tubes. The cultures were re-inoculated (~ 1:2, inoculum: media) every 7–14 d with fresh L1 medium (Guillard and Hargraves 1993) with a salinity of 26 $g \cdot kg^{-1}$. Experiment cultures were prepared the week before the experiment and kept under the above-mentioned conditions to ascertain exponentially growing starting materials. All handling of *P. reticulatum* and *A. catenella* was performed during the light phase to avoid exhausting the bioluminescence (Valiadi and Iglesias-Rodriguez 2013).

Dose–response experiments

Experimental set-up

Dose response experiments were carried out in glass test tubes (12 × 75 mm) coated with copepodamides (CA) from *Calanus finmarchicus* dissolved in 1–2 μL methanol (Selander et al. 2015). The composition of the copepodamide extracts used can be seen in Supporting Information Table S1. *Alexandrium catenella* and *P. reticulatum* were exposed to 0, 0.5, 1 and 5 nM ($n = 4$ replicates) and *G. catenatum* to 0, 0.1, 0.2, 0.5 and 1 nM of copepodamides ($n = 5$ replicates). Lower copepodamide concentrations were used for *G. catenatum* based on pilot experiments (data not shown). Controls received the same amount of methanol without copepodamides. The solvent was evaporated in the fume hood and 1–2 mL of cell cultures were added (1 mL for bioluminescence, and 2 mL for toxin assays). Copepodamides degrade rapidly in sea water (Selander et al. 2019), so cell cultures were transferred to freshly coated glass tubes every 48 h to maintain exposure.

Bioluminescence measurements

Bioluminescent capacity was measured on Days 0, 1, 3, and 5 using a Berthold FB12 luminometer (Titrek-Berthold, Berthold Detection Systems GmbH, Pforzheim, Germany). Measurements were taken 3 h into the dark phase, when cells are dark adapted and bioluminescent (Biggley et al. 1969). Each tube was carefully transferred to the luminometer, and the total bioluminescent capacity was triggered by the addition of 1 : 1 volumes of 1 M acetic acid (aq). A well-mixed aliquot (100–200 μL) of each sample was added onto a 96-well plate, fixed with Lugol's solution for cell enumeration at 10x magnification (Olympus CK40) or through imaging (COE-200-M-USB-080-IR-C Opto Engineering Camera, Olympus CKX41 fitted to the camera port of the stereomicroscope) and automated cell counting with Fiji ImageJ (Schindelin et al. 2012).

Toxin measurements

Gymnodinium catenatum and *A. catenella* were harvested at the start of the experiment and after 4 and 8 d by centrifuging the samples for 10 min at 560 RCF (Relative Centrifugal Force) at 4°C for *G. catenatum* (Allegra X-30R Centrifuge, Beckman Coulter) and 11,900 RCF at room temperature for *A. catenatum* (Heraeus Biofuge Pico). The supernatant was gently removed, and the pellets stored frozen (−20°C) until analysis. *Alexandrium catenella* formed fragile pellets and the cells lost when removing the supernatant were manually counted (Olympus CK40, 10× magnification) and accounted for when calculating cell-specific toxicity. Samples were freeze-dried (Heto LyoLab 3000 lyophilizer) before extraction through 3 consecutive freeze–thaw cycles in 300 μL 0.05 M acetic acid. The extracts were centrifuged as above, filtered through a low-volume glass fiber filter (GF/F, Whatman) and transferred to HPLC vials. For the toxin analysis of PSTs, water, acetonitrile and methanol (LC–MS grade) were acquired from Carlo Erba Reagents (Milan, Italy). Acetic acid, formic acid, ammonium hydroxide solution 25%, and ammonium formate (LC–MS grade) were purchased from Sigma-Aldrich (Darmstadt, Germany). Certified reference materials (CRMs): N-sulfocarbamoyl gonyautoxin-2 (C1), 40.1 ± 2.4 $\mu g \cdot g^{-1}$; N-sulfocarbamoyl gonyautoxin-3 (C2), 11.5 ± 0.9 $\mu g \cdot g^{-1}$; N-sulfocarbamoyl gonyautoxin-1 (C3), 12.6 ± 0.9 $\mu g \cdot g^{-1}$; N-sulfocarbamoyl gonyautoxin-4 (C4), 3.4 ± 0.3 $\mu g \cdot g^{-1}$; Gonyautoxin-1 (GTX1), 27.3 ± 1.6 $\mu g \cdot g^{-1}$; Gonyautoxin-2 (GTX2), 22.2 ± 1.5 $\mu g \cdot g^{-1}$; Gonyautoxin-3 (GTX3), 8.2 ± 0.6 $\mu g \cdot g^{-1}$; Gonyautoxin-4 (GTX4), 7.3 ± 0.6 $\mu g \cdot g^{-1}$; Gonyautoxin-5 (GTX5), 18.1 ± 1.2 $\mu g \cdot g^{-1}$; Gonyautoxin-6 (GTX6), 10.0 ± 0.5 $\mu g \cdot g^{-1}$; Decarbamoylgonyautoxin-2 (dcGTX2), 35.1 ± 1.9 $\mu g \cdot g^{-1}$; Decarbamoylgonyautoxin-3 (dcGTX3), 8.0 ± 0.9 $\mu g \cdot g^{-1}$; Neosaxitoxin dihydrochloride (NEO), 20.3 ± 1.2 $\mu g \cdot g^{-1}$; Saxitoxin dihydrochloride (STX), 20.3 ± 1.3 $\mu g \cdot g^{-1}$; Decarbamoylneosaxitoxin dihydrochloride (dcNEO), 9.1 ± 0.5 $\mu g \cdot g^{-1}$; and Decarbamoylsaxitoxin dihydrochloride (dcSTX), 19.5 ± 1.7 $\mu g \cdot g^{-1}$, were purchased from CIFGA Laboratories S.A. (Lugo, Spain).

LC–HRMS analysis. Liquid chromatography–high resolution mass spectrometry (LC–HRMS) analysis was performed according to Lage et al. (2022). Samples were analyzed with an UltiMate 3000 UHPLC coupled on an Orbitrap Elite mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a heated electrospray ionization source (HESI-II). The PST analogues were separated using an ACQUITY Premier BEH Amide (2.1 × 100 mm, 1.7 μm, Waters, Milford, MA, USA) at 35°C. Samples were held in the autosampler at 4°C. The mobile phase consisted of water with 0.1% formic acid (*v/v*) and 10 mM ammonium formate (*w/v*) (A) and acetonitrile with 0.1% formic acid (*v/v*) and 2% 10 mM ammonium formate solution (*w/v*) (B). The gradient (in *v/v* %) started with 5% of A and increased linearly to 95% in 11 min. This composition was maintained for 1 min and then returned to 5% of A in 1 min

and maintained at this composition for 2 min before the next run. The flow rate was 0.3 mL min^{-1} and the injection volume was 10 μL. Data were acquired under positive (ESI+) and negative (ESI−) polarity using the following ionization parameters: spray voltage, 3.8 kV; sheath gas, 40 arbitrary units; auxiliary gas, 10 arbitrary units; heater temperature, 300°C; capillary temperature, 325°C; and S-Lenses RF level, 69.06%. The LC–HRMS acquisition was performed under full-scan mode with the *m/z* ranging between 100 and 500.

The LC–HRMS quantification was performed by generating accurate mass-extracted ion chromatograms (AM-XIC) obtained from full-scan ESI+ and ESI− profiles using the exact mass (*m/z*) of each PST analogue and a mass extraction window of ± 5 ppm (Lage et al. 2022). Quantification of the classical PSTs was performed by preparing a calibration curve with the CRMs mixture, with five concentration points. Quantification of STX, NEO, dcSTX, and dcNEO was performed under ESI+ mode and quantification of GTX1 to GTX6, dcGTX2 and dcGTX3, and C1 to C4 was performed under negative ESI− mode. Both positive and negative profiles were assessed for the presence of all PST analogues.

Certified reference materials for the emerging PST analogues, namely *G. catenatum* toxins (GC-toxins) are not commercially available. Therefore, their annotation was based on exact mass, retention time, and fragmentation patterns obtained via Collision-Induced Dissociation (CID) and compared with data reported in the literature (Negri et al. 2003; Costa et al. 2015). Collision-Induced Dissociation spectra of the GC-toxins were acquired using product ion scans across the full chromatographic separation (LC–$HRMS^2$), targeting the $[M + H]^+$ ion of each analogue (i.e., *m/z* 473.109; 377.157; 489.103; and 393.152 for GC1/GC2, GC3, GC4/GC5, and GC6, respectively) and detecting fragments in the 100–500 *m/z* range with a collision energy of 35 arbitrary units. Fragmentation was performed using a mixture of the five *G. catenatum* samples corresponding to the 0.5 nM treatment on Day 8.

Signals corresponding to GC1/GC2, GC3, GC4/GC5, and GC6 were detected. However, only GC6 ($[M + H]^+$ at *m/z* 393.152) could be confidently annotated, as the other GC-toxins exhibited weak signals, and their fragmentation was not possible. The fragmentation spectra of GC6 displayed a dominant fragment at *m/z* 375, indicative of water loss, along with additional characteristic fragments at *m/z* 238, 225, and 220 (Supporting Information Fig. S1) (Costa et al. 2015). Quantification of GC6 was performed using a calibration curve of the structurally closest PST analogue, that is, NEO.

The limits of detection (LOD) and quantification (LOQ) were calculated from the standard deviations (SDs) obtained after five injections of the calibration curve second-lowest concentration (3 × SD and 10 × SD, respectively).

Calculations, statistical analysis, and visualization

Bioluminescence

Total bioluminescence capacity was extracted by integrating the luminometer readout from just before the addition of acetic acid until the light intensity returned to background levels. Bioluminescence per cell was calculated by dividing the light measurements by the number of cells in each tube. The data were expressed as percentage increases relative to the controls and fitted to the Michaelis–Menten equation as:

$$\text{Response variable increase} = \frac{V_{max} * [\text{copepodamides}]}{K_m + [\text{copepodamides}]} \quad (1)$$

where $V_{\max}$ corresponds to the maximum increase in bioluminescence, and K_m the concentration of copepodamides needed to reach half of the maximum increase, together characterizing both the reaction norm and the relevant concentration needed to trigger the response.

Growth rates and net toxin/bioluminescence production rate

Specific growth rates were calculated as:

$$\mu = \frac{(\ln N_t - \ln\langle N_{t-1}\rangle)}{t_t - t_{t-1}} \quad (2)$$

where N_t and N_{t-1} are the cell concentrations (cells mL^{-1}) at time t and the previous sampling occasion ($t-1$).

The production rate per cell and day, R, was calculated for both bioluminescence (R_{biolum}) and toxins (R_{tox}) as:

$$R = \frac{(BT_t - BT_{t-1})}{(\overline{N})(\Delta t)} \quad (3)$$

where BT is the bioluminescence or toxin content per mL at time t and the previous sampling occasion ($t-1$), $\overline{N}$ is the average concentration of cells, and Δt is the elapsed time (Anderson et al. 1990).

$\overline{N}$ is calculated as:

$$\overline{N} = \frac{N_t - N_{t-1}}{lnN_t - lnN_{t-1}} \quad (4)$$

Statistical analyses

Bioluminescence and toxin induction experiments in response to CA treatments were analyzed using a priori planned contrast variance analysis (Ruxton and Beauchamp 2008; Quinn and Keough 2023a) comparing treatment groups against controls. These were conducted separately for each sampling day (Days 1, 3, and 5 for bioluminescence, Days 4 and 8 for toxins) after confirming that there were no interaction effects between sampling day and treatment using two-factor ANOVAs. The assumption of equal variance of errors (homoscedasticity) for all linear models was assessed using residuals-vs.-fitted plots. Data was Log_{10}-transformed if deviations from the expected null relationship were observed. The assumption of heteroscedasticity and normality was not formally tested, as ANOVAs in balanced experimental designs are generally robust to violations of their assumptions (Glass et al. 1972; Harwell

et al. 1992; Lix et al. 1996), especially to non-normality (Gelman and Hill 2006; Quinn and Keough 2023b). The 5 nM treatment in the *A. catenella* experiment was excluded from the final statistical analysis, as its induction effects were comparable to those of the 0.5 and 1 nM treatment groups across both sampling days but also contributed to severely unequal error variances (Supporting Information Fig. S2). Potential allocation costs of bioluminescence or toxin induction were assessed visually and with correlation analyses.

The level of significance was set to $\alpha = 0.05$ for all statistical tests. Unless stated otherwise, summary statistics are presented as mean ± 95% CI, mean (95% CI range), or (mean, 95% CI). Effect sizes were calculated as log response ratios (LRR; Hedges et al. 1999) and reported as mean percentage increases with 95% CI ranges, for example, as mean (lower–upper CI) or as ranges of mean percentage increases for multiple groups.

All statistical analyses and visualizations were performed in R v.4.4.1 (R Core team, 2024) using RStudio v.2024.4.1.748 (Posit team) and packages: *readxl* (Wickham and Bryan 2023), *car* (Fox and Weisberg 2019), *drc* (Ritz et al. 2015), *DescTools* (Signorell 2025), *afex* (Singmann et al. 2024), *broom* (Robinson et al. 2024), *tidyverse* (Wickham et al. 2019), *Rmisc* (Hope 2022), *ggtext* (Wilke and Wiernik 2022), *ggpubr* (Kassambara 2023), kableExtra (Zhu 2024), *patchwork* (Pedersen 2024), *cowplot* (Wilke 2024), *ggplot2* (Wickham 2016), *wesanderson* (Ram and Wickham 2023), *grid* (R Core Team 2024), *ggimage* (Yu 2023).

The analysis code with all output, and the datasets it uses to perform all statistical analyses and produce visualizations is openly accessible at https://doi.org/10.5281/zenodo.14883074.

Results

Bioluminescence

Both bioluminescent dinoflagellates, *P. reticulatum* and *A. catenella*, responded to copepodamides with increased bioluminescent capacity (Fig. 1a,b). The response developed over time and doubled from 31%–37% on Day 1 to 53%–83% after 5 d of exposure relative to controls (Table 1). Half saturation values (K_m) were generally lower for *A. catenella* (mean 0.17 nM) than *P. reticulatum* (mean 0.51 nM; Table 2), indicating that *A. catenella* is more sensitive to copepodamides than *P. reticulatum*. Growth rates averaged 0.14 ± 0.06 d^{-1} and 0.11 ± 0.10 d^{-1} (mean ± SD) for *A. catenella* (Fig. 1c) and *P. reticulatum* (Fig. 1d) respectively. Net bioluminescence production rate (RLU cell^{-1} d^{-1}) was not significantly correlated with growth rate for *A. catenella* ($r = -0.01$, $p = 0.92$; Fig. 1e) or *P. reticulatum* ($r = -0.17$, $p = 0.24$; Fig. 1f).

Paralytic shellfish toxins

The toxin profile of *G. catenatum* predominantly contained GC6, C2 at lower concentrations, and congeners with trace amounts of GTX3, NEO, STX, dcSTX, C4 and B2 (Fig. 2a), whereas *A. catenella* was dominated by neosaxitoxin (NEO), GTX4 and saxitoxin (STX), with trace amounts of decarbamoyl derivatives (dcSTX, dcGTX4, dcGTX3) and N-sulfocarbamoyl toxin C2 and GTX3 (Fig. 2b). *Gymnodinium catenatum* toxins increased significantly by the first sampling day, and peaked on day four with up to a 165% increase in cell-specific toxin content compared to the controls ($p \geq 0.001$; Table 3; Fig. 2a). On Day 8, the increase was 81% in the 0.5 nM treatment compared to the controls ($p = 0.23$; Table 3; Fig. 2a). *Alexandrium catenella* showed a similar, although marginally non-significant, trend with increasing toxin content in copepodamide-exposed cultures (Fig. 2b). Cell-specific toxin content averaged 63% higher ($p = 0.12$–0.16; Table 3) than controls after 4 d and 103%–104% after 8 d ($p = 0.09$ and 0.05 for 0.5 and 1 nM, respectively; Table 3).

Growth rates averaged 0.12 ± 0.05 d^{-1} and 0.08 ± 0.09 d^{-1} (mean ± SD) for *G. catenatum* (Fig. 2c) and *A. catenella* (Fig. 2d) respectively. Net toxin production rate (fmol cell^{-1} d^{-1}) was not significantly correlated with the growth rate of *G. catenatum* ($r = -0.23$, $p = 0.1$; Fig. 2e) or *A. catenella* ($r = -0.04$, $p = 0.8$; Fig. 2f).

Discussion

All three species of dinoflagellates in this study responded to copepodamides by increasing toxin content and/or bioluminescence, corroborating the role of copepodamides as a general inducer of putative defenses in microphytoplankton. *Protoceratium reticulatum* increased its bioluminescent intensity, *G. catenatum* enhanced its PST content and *A. catenella* increased bioluminescence and simultaneously showed a strong trend toward up-regulating PSTs ($p = 0.05$–0.16; Table 3). Moreover, this is the first time that a GC toxin has been shown to be induced by copepodamide cues in *G. catenatum* (Fig. 2a). In contrast to the other three groups of hydrophilic PST analogues, GC toxins are uniquely hydrophobic, a property linked to the presence of a hydroxybenzoate group (Negri et al. 2003; Costa et al. 2015). Thus far, GC toxin production has been observed exclusively in *G. catenatum*, with the majority of the strains generating high levels of these toxins, and some strains only producing GC toxins (Negri et al. 2007). As a result, the risk of paralytic shellfish poisoning linked to *G. catenatum* may be largely attributed to GC toxins, even though the toxicological properties of these analogues have not yet been fully elucidated (Llewellyn et al. 2004).

The effective copepodamide concentrations are the combined result of a slow desorption from the coated culture vessel and the degradation of copepodamides in the culture media over time. The effective CA (copepodamide) concentrations in a similar experimental set-up were measured and averaged to approximately 1% of the nominal concentrations over 48 h after exposure (Selander et al. 2019; Supporting Information). The half saturation constants (K_m) for the bioluminescence induction experiments varied between nominal CA

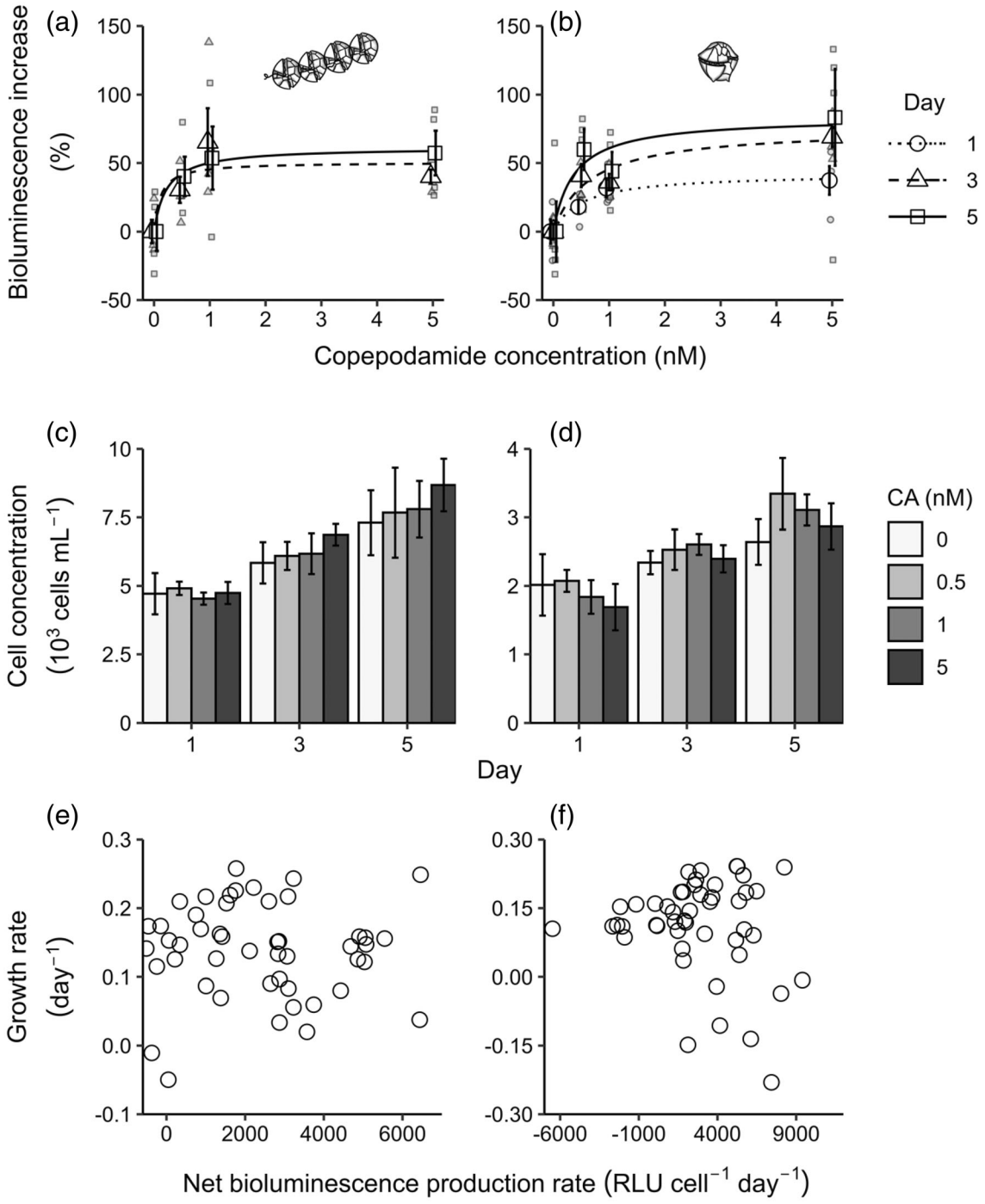

Fig. 1. (**a**, **b**) Michaelis–Menten curve fit for dose–response experiment as percentage increase in bioluminescence relative to controls in response to increasing copepodamide concentrations for (a) *Alexandrium catenella* and, (b) *Protoceratium reticulatum* after 1, 3 and 5 d of copepodamide exposure. Small filled geometric shapes are individual replicate values, large hollow shapes are mean values of $n = 4$ replicates, and error bars denote 95% confidence intervals. (**c**, **d**) Cell concentrations for each copepodamide treatment after 1, 3 and 5 d for (c) *A. catenella* and (d) *P. reticulatum*. Bars are mean values of $n = 4$ replicates and the error bars denote 95% confidence intervals. (**e**, **f**) Scatter plots of growth rates and net bioluminescence production rates for (e) *A. catenella* and (f) *P. reticulatum*.

concentrations of 0.11–0.22 nM in *A. catenella* and 0.37–0.65 nM in *P. reticulatum* (Table 2). This corresponds to average effective copepodamide concentrations of 1.1–2.2 pM and 3.7–6.5 pM, respectively, which is on par with the concentrations of copepodamides found in nature (40 fM–2 pM), corresponding to copepod densities (copepodites and adult) of < 1 to 51 L^{-1}

Table 1. Summary statistics of copepodamide-induced bioluminescence experiments for *Protoceratium reticulatum* and *Alexandrium catenella*. CA (nM) denotes nominal copepodamide concentration in the treatment groups, effect size of mean bioluminescence increase compared to controls and its 95% CI are derived from the log response ratio. Treatment groups significantly different from their controls ($p < 0.05$) in a planned-contrast variance analysis are denoted in bold.

Species	Day	CA (nM)	Effect size (%)	95 CI (%)	*p* value
P. reticulatum	1	0.5	18	−13 to 61	0.136
		1	**31**	−4 to 81	**0.015**
		5	**37**	−5 to 98	**0.006**
	3	0.5	**41**	8 to 83	**0.001**
		1	**36**	8 to 71	**0.003**
		5	**69**	35 to 112	**<0.001**
	5	0.5	60	−25 to 241	0.092
		1	44	−33 to 209	0.203
		5	**83**	−27 to 363	**0.025**
A. catenella	1	0.5	**37**	9 to 71	**0.001**
		1	**35**	10 to 66	**0.002**
		5	**36**	3 to 80	**0.001**
	3	0.5	30	−9 to 86	0.055
		1	**65**	−4 to 185	**0.002**
		5	**40**	5 to 87	**0.018**
	5	0.5	40	−19 to 143	0.126
		1	**53**	−20 to 195	**0.049**
		5	**57**	−10 to 174	**0.037**

(Selander et al. 2019). In situ concentrations of copepodamides follow copepod biomass and thus provide a reliable proxy of the copepod densities for the responding algae. Moreover, copepods can sometimes reach densities of hundreds per liter (Hamner and Carleton 1979; Ambler et al. 1991) and a single copepod can exude up to 120 pmol of copepodamides per day (Selander et al. 2015), suggesting that even the higher copepodamide concentrations of our experiments may be ecologically relevant. The copepodamides used in this study were purified from freeze-dried *Calanus finmarchicus* and contain a lower proportion of dihydro-copepodamides (methyl-containing copepodamides) compared to natural samples from both limnic and temperate marine copepods (Arnoldt et al. 2024). Given that dihydro-copepodamides may be more potent toxin inducers than their methyl-containing counterparts (Selander et al. 2015), our observed effects may be conservative compared to the exposure of the copepodamide profiles found in the natural environment of the responders.

Trade-offs in phytoplankton defenses often manifest as a reduced grazer-induced mortality associated with lower growth rates (Pančić and Kiørboe 2018). A quantitative understanding of this trade-off is necessary to predict the outcome of this predator–prey relationship, yet this inquiry is rarely covered, and results are inconsistent (Pančić and Kiørboe 2018). *Alexandrium catenella* has, based on correlation with *cyc* gene (a genetic growth marker), been suggested to grow slower when toxin production increased in response to copepod grazers (Park et al. 2023). Likewise, growth rates decreased with increased grazer-induced domoic acid production in *Pseudo-nitzschia* sp. (Lundholm et al. 2018). In contrast, copepod-mediated toxin induction had no effect on the growth of *A. catenella* (Selander et al. 2012), then *A. tamarense* (John et al. 2014), and resulted in a counter intuitive increase in growth rate for *Alexandrium minutum* (Ryderheim et al. 2021). None of the grazer-induced defenses observed here correlated with changes in growth rates (Figs. 1e,f, 2e,f). However, such defenses may incur indirect ecological costs not captured in simplified laboratory conditions (Strauss et al. 2002), such as altered swimming behavior (Selander et al. 2011) or sinking rates (Lürling & Van Donk, 2000), which could negatively affect fitness in situ. The experimental conditions—continuous exponential growth, abundant nutrient availability, and lack of interspecific resource competition—are arguably a poor mimic of the conditions found in the ocean (Bristow et al. 2017). Under these near-optimal conditions, compromising growth to support defensive traits may not be necessary. It is possible, however, that a longer experimental duration might have revealed such trade-offs, although this would require careful monitoring of nutrient dynamics over time.

There was a weak trend toward lower growth rates in the most bioluminescent species, *P. reticulatum*, which could indicate that bioluminescence may be costly in this species. This is supported by the circadian regulation of bioluminescence capacity observed in some dinoflagellates. Some species are known to daily degrade and re-synthesize the scintillons and its bioluminescent machinery, producing light only during

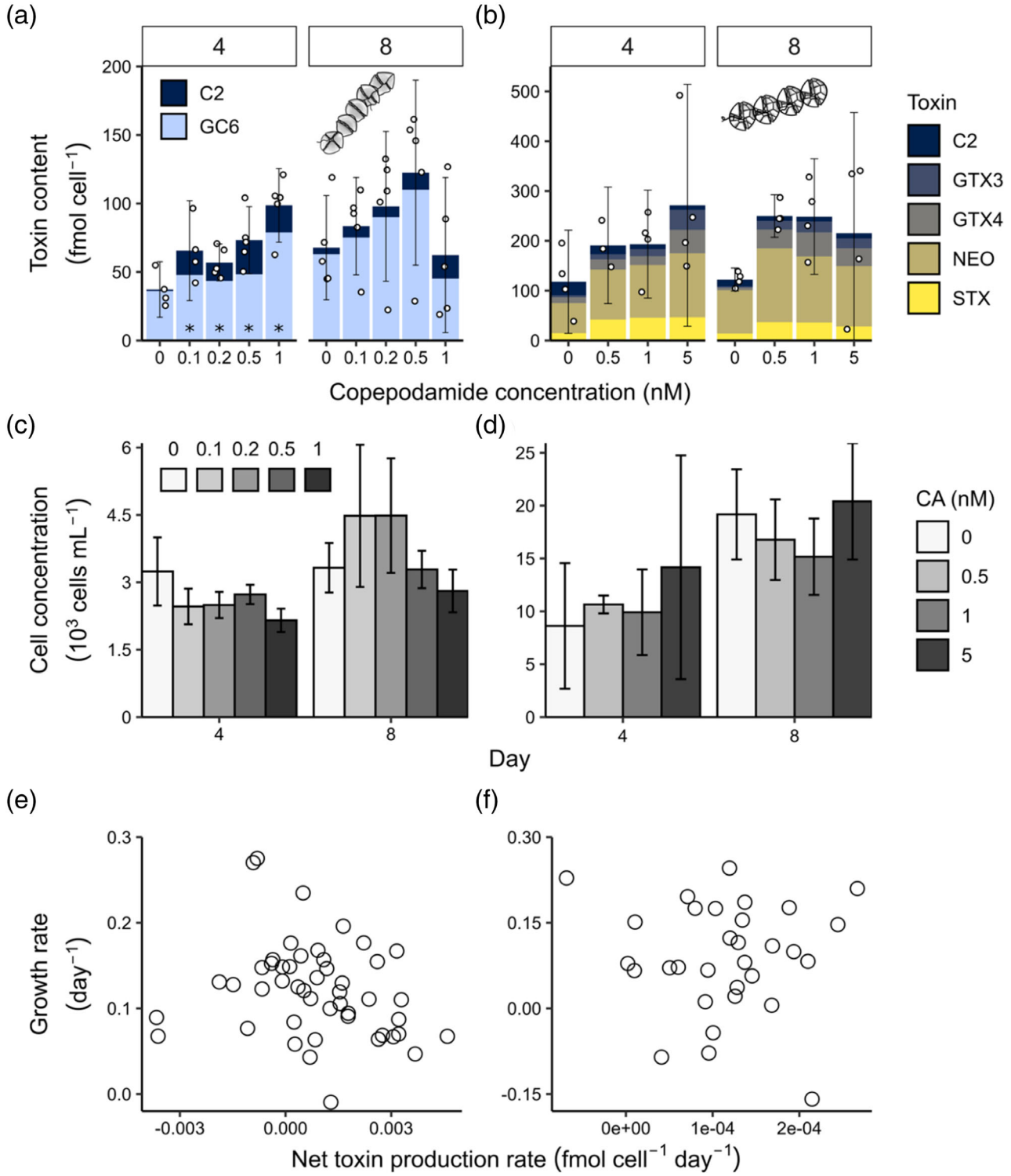

Fig. 2. (**a**, **b**) Paralytic shellfish toxin (PST) amounts and composition (GC6: hydroxyl-benzoyl analogue 6, C2: N-Sulfocarbamoyl-gonyautoxin-2, GTX3: Gonyautoxin-3, GTX4: Gonyautoxin-4, NEO: Neosaxitoxin, STX: Saxitoxin) for (a) *Gymnodinium catenatum* and (b) *Alexandrium catenella* across copepodamide treatments and days (4–8). Colored bars are means of each toxin congener based on $n = 4$ replicates for *A. catenella* and $n = 5$ replicates for *G. catenatum*, error bars denote 95% confidence intervals of pooled toxins, and asterisks (*) denote statistically significant differences in total toxins compared to control (Table 3). An outlier replicate in CA treatment 0.5 nM on Day 8 is not visible in (b). (**c**, **d**) Cell concentrations for each copepodamide treatment after 4 and 8 d for (c) *G. catenatum* and (d) *A. catenella*. Bars are mean values of $n = 4$ and 5 replicates, respectively, and error bars denote 95% confidence intervals. (**e**, **f**) Scatter plots of growth rates and net toxin production rates for (e) *G. catenatum* and (f) *A. catenella*.

the dark hours of the day (Dunlap and Hastings 1981) whereas bioluminescence is conserved in other species by relocating scintillons within the cell during the light phases of the cycle (Colepicolo et al. 1993). Both mechanisms are expected to incur energetic costs: the first through the daily production of luciferin-related components (binding protein, enzymes, and substrate mRNA) and their subsequent degradation (Hastings 2013), and the latter through the maintenance and relocation of the bioluminescent machinery within the cell (Valiadi and Iglesias-Rodriguez 2013). Moreover, it is possible that *P. reticulatum* produced yessotoxins in response to copepodamides, which should be addressed in future studies. The trend toward lower growth rates in induced cultures may consequently reflect investment in more defensive traits than the observed bioluminescence.

The simultaneous increase in bioluminescence and toxin production in *A. catenella* is one of few examples of phytoplankton expressing multiple defenses in response to predator cues. Selander et al. (2011) and Lindström et al. (2017) found that the same strain of *A. catenella* (no. 3, GUMACC, previously *A. tamarense*) induced both bioluminescence and changes in chain length in response to copepod grazer cues. Similarly, Selander et al. (2012) observed chain length shortening in concert with increased toxin production for two additional strains of *A. catenella* (no. 1 and no. 9, GUMACC, previously *A. tamarense*). Combined with our findings here, this suggests that *A. catenella* is capable of simultaneously upregulating three (or more, hitherto unknown) defensive traits in response to copepod cues. Multiple defense strategies may provide more robust protection against grazers and may also fine-tune the composition of defensive traits to the composition of the grazer community (Smayda and Reynolds 2003; Long et al. 2007). Moreover, the presence of multiple defense strategies within a single organism is an important factor to consider when resolving the costs and benefits of defensive traits. Cost–benefit analyses are typically performed on single traits and both costs and benefits may consequently be confounded by the simultaneous onset of additional, non-monitored traits. In Park et al. (2023) toxin production in *A. catenella* was associated with reduced growth rates through correlation with genetic growth markers. Here, toxin induction was less pronounced and we saw no significant reduction in growth rate. The simultaneous onset of increased bioluminescence, however, suggests that the cost in Park and colleagues' study may also encompass the cost of bioluminescence.

The community structure and composition of phytoplankton is regulated both by a bottom-up control of resource availability (Manzi Marinho and de Moraes Huszar 2002; Moschonas et al. 2017; Burson et al. 2018) and via top-down grazing pressure from zooplankton (McCauley and Briand 1979; Kenitz et al. 2017). Phytoplankton may consequently increase their fitness both through competition and by resisting

Table 2. Constants from the Michaelis–Menten curve describing copepodamide-induced bioluminescence increase of *Alexandrium catenella* and *Protoceratium reticulatum*. The half saturation constant (K_m) shows the concentration needed to trigger half the maximum bioluminescence increase (V_{max}) relative to control.

Sp.	Day	K_m (nM)	V_{max} (%)
A. catenella	3	0.11	50.6
	5	0.22	61.3
P. reticulatum	1	0.51	42.3
	3	0.65	75.3
	5	0.37	83.3

Table 3. Summary statistics of the copepodamide-induced toxin experiments for *Alexandrium catenella* and *Gymnodinium catenatum*. CA (nM) denotes nominal copepodamide concentration in the treatment groups, effect size of mean toxin increase compared to controls and its 95% CI are derived from the log response ratio. Treatment groups significantly different from their controls ($p < 0.05$) in a planned-contrast variance analysis are denoted in bold.

Species	Day	CA (nM)	Effect size (%)	95 CI (%)	*p* value
A. catenella	4	0.5	62	−40 to 336	0.163
		1	64	−42.1 to 365	0.124
	8	0.5	104	58 to 163	0.09
		1	103	23 to 236	0.053
G. catenatum	4	0.1	**76**	−19 to 283	**0.009**
		0.2	**53**	−17 to 181	**0.024**
		0.5	**97**	1 to 283	**0.001**
		1	**165**	41 to 396	**>0.001**
	8	0.1	23	−45 to 176	0.617
		0.2	44	−42 to 258	0.508
		0.5	81	−27 to 346	0.233
		1	−8	−73 to 213	0.532

predation. Dinoflagellates are generally poor competitors under nutrient-limited conditions compared to, for example, diatoms and non-toxic flagellates (Riegman et al. 1996; Yamamoto and Tarutani 1999). In contrast, they are overrepresented among the harmful algal bloom (HAB) producing taxa (Smayda 2002), which suggests that dinoflagellates may depend on defensive traits to compete with faster growing competitors.

Although we did not perform any feeding preference experiments with our induced cells, previous studies have shown that copepods tend to feed preferentially on less defended cells. Copepods have notably been shown to selectively feed on non-toxic cells even in studies where no direct effects of toxins were measured (Abdulhussain et al. 2020, 2021: Schultz and Kiørboe 2009), suggesting that avoidance behavior may be driven by sublethal cues or evolved recognition of harmful prey (Xu and Kiørboe 2018). Similarly, Prevett et al. (2019) illustrated how bioluminescent *Lingulaulax polyedra* (previously *Lingulodinium polyedra*) went from being the preferred prey of the copepod *Acartia tonsa* to completely rejected when up-regulating bioluminescent capacity in response to copepod cues. Multiple studies have indicated this feeding preference of copepods toward non-bioluminescent cells vs. bioluminescent ones (Esaias and Curl 1972; White 1979). Likewise, strong evidence supports the importance of defense traits for enabling large dinoflagellates to compete with smaller and faster-growing phytoplankton (Guisande et al. 2002; Ryderheim et al. 2021).

In conclusion, this study adds two new species to the list of phytoplanktonic species capable of sensing and reacting to copepodamides. Given the array of phylogenetically distant microalgae demonstrating the ability to detect these alarm cues (Selander et al. 2015; Lindström et al. 2017; Grebner et al. 2019; Olesen et al. 2022), our findings support a widespread distribution of these predator recognition mechanisms. Moreover, induced bioluminescence and toxin production correlate with efficient deterrence of copepod grazers (Guisande et al. 2002; Prevett et al. 2019; Ryderheim et al. 2021) and hence may contribute to the success of the studied species. Grazing pressure is redirected to the non-defended organisms, benefiting the harmful taxa, and thus potentially contributing to the formation of HABs. Clarifying the complex dynamics underlying these predator–prey interactions is crucial for understanding the mechanistic drivers of HAB formation and their broader impacts on marine ecosystems.

Author Contributions

Paula Gonzalo-Valmala: Conceptualization; data curation; formal analysis; investigation; methodology; validation; visualization; writing – original draft preparation; writing – review and editing. Milad Pourdanandeh: Data curation; formal analysis; methodology; validation; visualization; writing – original draft preparation; writing – review and editing; supervision. Sandra Lage: Formal analysis; funding acquisition; investigation; writing – review and editing. Erik Selander: Conceptualization; formal analysis; funding acquisition; methodology; validation; project administration; resources; validation; writing – original draft preparation; writing – review and editing; supervision.

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Conflicts of Interest

None declared.

Data Availability Statement

The data supporting this study is available in Zenodo at https://doi.org/10.5281/zenodo.14883074.

References

Abdulhussain, A. H., K. B. Cook, A. D. Turner, A. M. Lewis, T. S. Bibby, and D. J. Mayor. 2021. "The Influence of the Toxin-Producing Dinoflagellate, *Alexandrium catenella* (1119/27), on the Survival and Reproduction of the Marine Copepod, *Acartia tonsa*, During Prolonged Exposure." *Frontiers in Marine Science* 8. https://doi.org/10.3389/fmars.2021.652225.

Abdulhussain, A. H., K. B. Cook, A. D. Turner, A. M. Lewis, M. A. Elsafi, and D. J. Mayor. 2020. "The Influence of the Toxin Producing Dinoflagellate, *Alexandrium catenella* (1119/27), on the Feeding and Survival of the Marine Copepod, Acartia tonsa." *Harmful Algae* 98: 101890. https://doi.org/10.1016/j.hal.2020.101890.

Álvarez, G., E. Uribe, R. Díaz, M. Braun, C. Mariño, and J. Blanco. 2011. "Bloom of the Yessotoxin-Producing Dinoflagellate *Protoceratium reticulatum* (Dinophyceae) in Northern

Chile." *Journal of Sea Research* 65, no. 4: 427–434. https://doi.org/10.1016/j.seares.2011.03.008.

Ambler, J. W., F. D. Ferrari, and J. A. Fornshell. 1991. "Population Structure and Swarm Formation of the Cyclopoid Copepod *Dioithona oculata* Near Mangrove Cays." *Journal of Plankton Research* 13, no. 6: 1257–1272. https://doi.org/10.1093/plankt/13.6.1257.

Anderson, D. M., E. Fachon, R. S. Pickart, et al. 2021. "Evidence for Massive and Recurrent Toxic Blooms of *Alexandrium catenella* in the Alaskan Arctic." *Proceedings of the National Academy of Sciences of the United States of America* 118, no. 41: e2107387118. https://doi.org/10.1073/pnas.2107387118.

Anderson, D. M., D. M. Kulis, J. J. Sullivan, S. Hall, and C. Lee. 1990. "Dynamics and Physiology of Saxitoxin Production by the Dinoflagellates *Alexandrium* spp." *Marine Biology* 104, no. 3: 511–524. https://doi.org/10.1007/BF01314358.

Arnoldt, S., M. Pourdanandeh, I. Spikkeland, M. X. Andersson, and E. Selander. 2024. "Mass Spectroscopy Reveals Compositional Differences in Copepodamides From Limnic and Marine Copepods." *Scientific Reports* 14, no. 1: 3147. https://doi.org/10.1038/s41598-024-53247-1.

Bagoien, E., A. Miranda, B. Reguera, and J. M. Franco. 1996. "Effects of Two Paralytic Shellfish Toxin Producing Dinoflagellates on the Pelagic Harpacticoid Copepod *Euterpina acutifrons*." *Marine Biology* 126, no. 3: 361–369. https://doi.org/10.1007/BF00354618.

Banse, K. 1982. "Cell Volumes, Maximal Growth Rates of Unicellular Algae and Ciliates, and the Role of Ciliates in the Marine Pelagial." *Limnology and Oceanography* 27, no. 6: 1059–1071. https://doi.org/10.4319/lo.1982.27.6.1059.

Bergquist, A. M., S. R. Carpenter, and J. C. Latino. 1985. "Shifts in Phytoplankton Size Structure and Community Composition During Grazing by Contrasting Zooplankton Assemblages." *Limnology and Oceanography* 30, no. 5: 1037–1045. https://doi.org/10.4319/lo.1985.30.5.1037.

Biggley, W. H., E. Swift, R. J. Buchanan, and H. H. Seliger. 1969. "Stimulable and Spontaneous Bioluminescence in the Marine Dinoflagellates, *Pyrodinium bahamense*, *Gonyaulax polyedra*, and *Pyrocystis lunula*." *Journal of General Physiology* 54, no. 1: 96–122. https://doi.org/10.1085/jgp.54.1.96.

Bravo, I., M. Vila, M. Masó, R. I. Figueroa, and I. Ramilo. 2008. "*Alexandrium catenella* and *Alexandrium minutum* Blooms in the Mediterranean Sea: Toward the Identification of Ecological Niches." *Harmful Algae* 7, no. 4: 515–522. https://doi.org/10.1016/j.hal.2007.11.005.

Bristow, L. A., W. Mohr, S. Ahmerkamp, and M. M. M. Kuypers. 2017. "Nutrients That Limit Growth in the Ocean." *Current Biology* 27, no. 11: R474–R478. https://doi.org/10.1016/j.cub.2017.03.030.

Burkenroad, M. D. 1943. "A Possible Function of Bioluminescence." *Journal of Marine Research* 5: 161–164.

Burson, A., M. Stomp, E. Greenwell, J. Grosse, and J. Huisman. 2018. "Competition for Nutrients and Light: Testing Advances in Resource Competition With a Natural Phytoplankton Community." *Ecology* 99, no. 5: 1108–1118. https://doi.org/10.1002/ecy.2187.

Colepicolo, P., T. Roenneberg, D. Morse, W. R. Taylor, and J. W. Hastings. 1993. "Circadian Regulation of Bioluminescence in the Dinoflagellate *Pyrocystis lunula*." *Journal of Phycology* 29, no. 2: 173–179. https://doi.org/10.1111/j.0022-3646.1993.00173.x.

Condie, S. A., E. C. J. Oliver, and G. M. Hallegraeff. 2019. "Environmental Drivers of Unprecedented *Alexandrium catenella* Dinoflagellate Blooms Off Eastern Tasmania, 2012–2018." *Harmful Algae* 87: 101628. https://doi.org/10.1016/j.hal.2019.101628.

Costa, P., A. Robertson, and M. Quilliam. 2015. "Toxin Profile of *Gymnodinium catenatum* (Dinophyceae) From the Portuguese Coast, as Determined by Liquid Chromatography Tandem Mass Spectrometry." *Marine Drugs* 13, no. 4: 2046–2062. https://doi.org/10.3390/md13042046.

Cusick, K. D., and E. A. Widder. 2020. "Bioluminescence and Toxicity as Driving Factors in Harmful Algal Blooms: Ecological Functions and Genetic Variability." *Harmful Algae* 98: 101850. https://doi.org/10.1016/j.hal.2020.101850.

Dunlap, J. C., and J. W. Hastings. 1981. "The Biological Clock in *Gonyaulax* Controls Luciferase Activity by Regulating Turnover." *Journal of Biological Chemistry* 256, no. 20: 10509–10518. https://doi.org/10.1016/S0021-9258(19)68651-5.

Esaias, W. E., and H. C. Curl. 1972. "Effect of Dinoflagellate Bioluminescence on Copepod Ingestion Rates." *Limnology and Oceanography* 17, no. 6: 901–906. https://doi.org/10.4319/lo.1972.17.6.0901.

Field, C. B., M. J. Behrenfeld, J. T. Randerson, and P. Falkowski. 1998. "Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components." *Science* 281, no. 5374: 237–240. https://doi.org/10.1126/science.281.5374.237.

Fox, J., and S. Weisberg. 2019. An R Companion to Applied Regression. 3rd ed. Sage. https://www.john-fox.ca/Companion/.

Frangópulos, M., C. Guisande, I. Maneiro, I. Riveiro, and J. Franco. 2000. "Short-Term and Long-Term Effects of the Toxic Dinoflagellate *Alexandrium minutum* on the Copepod *Acartia clausi*." *Marine Ecology Progress Series* 203: 161–169. https://doi.org/10.3354/meps203161.

Frederiksen, M., M. Edwards, A. J. Richardson, N. C. Halliday, and S. Wanless. 2006. "From Plankton to Top Predators: Bottom-Up Control of a Marine Food Web Across Four Trophic Levels." *Journal of Animal Ecology* 75, no. 6: 1259–1268. https://doi.org/10.1111/j.1365-2656.2006.01148.x.

Froneman, P. W. 2001. "Seasonal Changes in Zooplankton Biomass and Grazing in a Temperate Estuary, South Africa." *Estuarine, Coastal and Shelf Science* 52, no. 5: 543–553. https://doi.org/10.1006/ecss.2001.0776.

Gelman, A., and J. Hill. 2006. "Data Analysis Using Regression and Multilevel/Hierarchical Models." In Analytical Methods for Social Research. Cambridge University Press. https://doi.org/10.1017/CBO9780511790942.

Glass, G. V., P. D. Peckham, and J. R. Sanders. 1972. "Consequences of Failure to Meet Assumptions Underlying the Fixed Effects Analyses of Variance and Covariance." *Review of Educational Research* 42, no. 3: 237–288. https://doi.org/10.3102/00346543042003237.

Grebner, W., E. C. Berglund, F. Berggren, et al. 2019. "Induction of Defensive Traits in Marine Plankton—New Copepodamide Structures." *Limnology and Oceanography* 64, no. 2: 820–831. https://doi.org/10.1002/lno.11077.

Griffin, J. E., G. Park, and H. G. Dam. 2019. "Relative Importance of Nitrogen Sources, Algal Alarm Cues and Grazer Exposure to Toxin Production of the Marine Dinoflagellate *Alexandrium catenella*." *Harmful Algae* 84: 181–187. https://doi.org/10.1016/j.hal.2019.04.006.

Grønning, J., and T. Kiørboe. 2020. "Diatom Defence: Grazer Induction and Cost of Shell-Thickening." *Functional Ecology* 34, no. 9: 1790–1801. https://doi.org/10.1111/1365-2435.13635.

Guillard, R. R. L., and P. E. Hargraves. 1993. "*Stichochrysis immobilis* Is a Diatom, Not a Chrysophyte." *Phycologia* 32, no. 3: 234–236. https://doi.org/10.2216/i0031-8884-32-3-234.1.

Guisande, C., M. Frangópulos, I. Maneiro, A. Vergara, and I. Riveiro. 2002. "Ecological Advantages of Toxin Production by the Dinoflagellate *Alexandrium minutum* Under Phosphorus Limitation." *Marine Ecology Progress Series* 225: 169–176. https://doi.org/10.3354/meps225169.

Hamner, W. M., and J. H. Carleton. 1979. "Copepod Swarms: Attributes and Role in Coral Reef Ecosystems." *Limnology and Oceanography* 24, no. 1: 1–14. https://doi.org/10.4319/lo.1979.24.1.0001.

Han, J., J. S. Park, Y. Park, J. Lee, H. H. Shin, and K.-W. Lee. 2021. "Effects of Paralytic Shellfish Poisoning Toxin-Producing Dinoflagellate *Gymnodinium catenatum* on the Marine Copepod *Tigriopus japonicus*." *Marine Pollution Bulletin* 163: 111937. https://doi.org/10.1016/j.marpolbul.2020.111937.

Hanley, K. A., and E. A. Widder. 2017. "Bioluminescence in Dinoflagellates: Evidence That the Adaptive Value of Bioluminescence in Dinoflagellates is Concentration Dependent." *Photochemistry and Photobiology* 93, no. 2: 519–530. https://doi.org/10.1111/php.12713.

Harwell, M. R., E. N. Rubinstein, W. S. Hayes, and C. C. Olds. 1992. "Summarizing Monte Carlo Results in Methodological Research: The One- and Two-Factor Fixed Effects ANOVA Cases." *Journal of Educational Statistics* 17, no. 4: 315–339. https://doi.org/10.2307/1165127.

Hastings, J. 2013. "Circadian Rhythms in Dinoflagellates: What is the Purpose of Synthesis and Destruction of Proteins?" *Microorganisms* 1, no. 1: 26–32. https://doi.org/10.3390/microorganisms1010026.

Hedges, L. V., J. Gurevitch, and P. S. Curtis. 1999. "The Meta-Analysis of Response Ratios in Experimental Ecology." *Ecology* 80, no. 4: 1150–1156. https://doi.org/10.1890/0012-9658(1999)080[1150:TMAORR]2.0.CO;2.

Heneghan, R. F., J. D. Everett, J. L. Blanchard, and A. J. Richardson. 2016. "Zooplankton Are Not Fish: Improving Zooplankton Realism in Size-Spectrum Models Mediates Energy Transfer in Food Webs." *Frontiers in Marine Science* 3: 201. https://doi.org/10.3389/fmars.2016.00201.

Hope, R. M. 2022. "*Rmisc: Ryan Miscellaneous*." https://CRAN.R-project.org/package=Rmisc.

Huntley, M., P. Sykes, S. Rohan, and V. Marin. 1986. "Chemically-Mediated Rejection of Dinoflagellate Prey by the Copepods *Calanus pacificus* and *Paracalanus parvus*: Mechanism, Occurrence and Significance." *Marine Ecology Progress Series* 28: 105–120. https://doi.org/10.3354/meps028105.

John, U., R. W. Litaker, M. Montresor, S. Murray, M. L. Brosnahan, and D. M. Anderson. 2014. "Formal Revision of the *Alexandrium tamarense* Species Complex (Dinophyceae) Taxonomy: The Introduction of Five Species With Emphasis on Molecular-Based (rDNA) Classification." *Protist* 165, no. 6: 779–804. https://doi.org/10.1016/j.protis.2014.10.001.

Kang, H.-M., J. Lee, Y.-J. Lee, et al. 2022. "Transcriptional and Toxic Responses to Saxitoxin Exposure in the Marine Copepod *Tigriopus japonicus*." *Chemosphere* 309: 136464. https://doi.org/10.1016/j.chemosphere.2022.136464.

Kassambara, A. 2023. "*ggpubr*: '*ggplot2*' *Based Publication Ready Plots*." https://CRAN.R-project.org/package=ggpubr.

Kenitz, K. M., A. W. Visser, P. Mariani, and K. H. Andersen. 2017. "Seasonal Succession in Zooplankton Feeding Traits Reveals Trophic Trait Coupling." *Limnology and Oceanography* 62, no. 3: 1184–1197. https://doi.org/10.1002/lno.10494.

Koike, K., Y. Horie, T. Suzuki, et al. 2006. "*Protoceratium reticulatum* in Northern Japan: Environmental Factors Associated With Seasonal Occurrence and Related Contamination of Yessotoxin in Scallops." *Journal of Plankton Research* 28, no. 1: 103–112. https://doi.org/10.1093/plankt/fbi103.

Kunimitsu, K., and N.-U. Keiko. 1986. "Distribution of Taurolipid in *Tetrahymena* Cells." *Biochimica et Biophysica Acta (BBA)—Lipids and Lipid Metabolism* 878, no. 2: 281–283. https://doi.org/10.1016/0005-2760(86)90157-8.

Ladds, M., J. Smith, V. Strohm, and C. Gobler. 2024. "*Acartia tonsa* Grazing on the Harmful Dinoflagellate *Dinophysis acuminata* Reduces Copepod Survival and Increases Extracellular Toxin Concentrations." *Marine Ecology Progress Series* 745: 25–40. https://doi.org/10.3354/meps14666.

Lage, S., P. R. Costa, A. V. M. Canário, and J. P. Da Silva. 2022. "LC–HRMS Profiling of Paralytic Shellfish Toxins in *Mytilus galloprovincialis* after a *Gymnodinium catenatum* Bloom." *Marine Drugs* 20, no. 11: 680. https://doi.org/10.3390/md20110680.

Lindström, J., W. Grebner, K. Rigby, and E. Selander. 2017. "Effects of Predator Lipids on Dinoflagellate Defence

Mechanisms—Increased Bioluminescence Capacity." *Scientific Reports* 7, no. 1: 13104. https://doi.org/10.1038/s41598-017-13293-4.

Litchman, E., C. A. Klausmeier, O. M. Schofield, and P. G. Falkowski. 2007. "The Role of Functional Traits and Trade-Offs in Structuring Phytoplankton Communities: Scaling From Cellular to Ecosystem Level." *Ecology Letters* 10, no. 12: 1170–1181. https://doi.org/10.1111/j.1461-0248.2007.01117.x.

Lix, L. M., J. C. Keselman, and H. J. Keselman. 1996. "Consequences of Assumption Violations Revisited: A Quantitative Review of Alternatives to the One-Way Analysis of Variance 'F' Test." *Review of Educational Research* 66, no. 4: 579–619. https://doi.org/10.2307/1170654.

Llewellyn, L., A. Negri, and M. Quilliam. 2004. "High Affinity for the Rat Brain Sodium Channel of Newly Discovered Hydroxybenzoate Saxitoxin Analogues From the Dinoflagellate *Gymnodinium catenatum*." *Toxicon* 43, no. 1: 101–104. https://doi.org/10.1016/j.toxicon.2003.10.016.

Long, J. D., G. W. Smalley, T. Barsby, J. T. Anderson, and M. E. Hay. 2007. "Chemical Cues Induce Consumer-Specific Defenses in a Bloom-Forming Marine Phytoplankton." *Proceedings of the National Academy of Sciences* 104, no. 25: 10512–10517. https://doi.org/10.1073/pnas.0611600104.

Lundholm, N., B. Krock, U. John, et al. 2018. "Induction of Domoic Acid Production in Diatoms—Types of Grazers and Diatoms Are Important." *Harmful Algae* 79: 64–73. https://doi.org/10.1016/j.hal.2018.06.005.

Lürling, M., and E. Van Donk. 2000. "Grazer-Induced Colony Formation in *Scenedesmus*: Are There Costs to Being Colonial?" *Oikos* 88, no. 1: 111–118. https://doi.org/10.1034/j.1600-0706.2000.880113.x.

Manzi Marinho, M., and V. L. de Moraes Huszar. 2002. "Nutrient Availability and Physical Conditions as Controlling Factors of Phytoplankton Composition and Biomass in a Tropical Reservoir (Southeastern Brazil)." *Fundamental and Applied Limnology* 153, no. 3: 443–468. https://doi.org/10.1127/archiv-hydrobiol/153/2002/443.

McCauley, E., and F. Briand. 1979. "Zooplankton Grazing and Phytoplankton Species Richness: Field Tests of the Predation Hypothesis." *Limnology and Oceanography* 24, no. 2: 243–252. https://doi.org/10.4319/lo.1979.24.2.0243.

Meunier, C. L., M. Boersma, K. H. Wiltshire, and A. M. Malzahn. 2016. "Zooplankton Eat What They Need: Copepod Selective Feeding and Potential Consequences for Marine Systems." *Oikos* 125, no. 1: 50–58. https://doi.org/10.1111/oik.02072.

Moschonas, G., R. J. Gowen, R. F. Paterson, et al. 2017. "Nitrogen Dynamics and Phytoplankton Community Structure: The Tole of Organic Nutrients." *Biogeochemistry* 134, no. 1–2: 125–145. https://doi.org/10.1007/s10533-017-0351-8.

Negri, A., D. Stirling, M. Quilliam, et al. 2003. "Three Novel Hydroxybenzoate Saxitoxin Analogues Isolated From the Dinoflagellate *Gymnodinium catenatum*." *Chemical Research in Toxicology* 16, no. 8: 1029–1033. https://doi.org/10.1021/tx034037j.

Negri, A. P., C. J. S. Bolch, S. Geier, D. H. Green, T.-G. Park, and S. I. Blackburn. 2007. "Widespread Presence of Hydrophobic Paralytic Shellfish Toxins in *Gymnodinium catenatum*." *Harmful Algae* 6, no. 6: 774–780. https://doi.org/10.1016/j.hal.2007.04.001.

Olesen, A. J., F. Ryderheim, B. Krock, N. Lundholm, and T. Kiørboe. 2022. "Costs and Benefits of Predator-Induced Defence in a Toxic Diatom." *Proceedings of the Royal Society B: Biological Sciences* 289, no. 1972: 20212735. https://doi.org/10.1098/rspb.2021.2735.

Pančić, M., and T. Kiørboe. 2018. "Phytoplankton Defence Mechanisms: Traits and Trade-Offs." *Biological Reviews* 93, no. 2: 1269–1303. https://doi.org/10.1111/brv.12395.

Pane, L., M. Feletti, B. Fancomacaro, and G. L. Mariottini. 2004. "Summer Coastal Zooplankton Biomass and Copepod Community Structure Near the Italian Terra Nova Base (Terra Nova Bay, Ross Sea, Antarctica)." *Journal of Plankton Research* 26, no. 12: 1479–1488. https://doi.org/10.1093/plankt/fbh135.

Park, G., L. Norton, D. Avery, and H. G. Dam. 2023. "Grazers Modify the Dinoflagellate Relationship Between Toxin Production and Cell Growth." *Harmful Algae* 126: 102439. https://doi.org/10.1016/j.hal.2023.102439.

Park, J. Y., D. H. Choi, N. Y. Kim, et al. 2024. "Effects of Zooplankton Extracts on the Production of Paralytic Shellfish Toxins by *Gymnodinium catenatum* and *Alexandrium pacificum*." *Ocean Science Journal* 59: 50. https://doi.org/10.1007/s12601-024-00178-7.

Pedersen, T. L. 2024. "*patchwork: The Composer of Plots*." https://patchwork.data-imaginist.com.

Pershing, A. J., K. E. Mills, N. R. Record, et al. 2015. "Evaluating Trophic Cascades as Drivers of Regime Shifts in Different Ocean Ecosystems." *Philosophical Transactions of the Royal Society, B: Biological Sciences* 370, no. 1659: 20130265. https://doi.org/10.1098/rstb.2013.0265.

Pourdanandeh, M., V. Séchet, L. Carpentier, et al. 2025. "Effects of Copepod Chemical Cues on Intra- and Extracellular Toxins in Two Species of *Dinophysis*." *Harmful Algae* 142: 102793. https://doi.org/10.1016/j.hal.2024.102793.

Prevett, A., J. Lindström, J. Xu, B. Karlson, and E. Selander. 2019. "Grazer-Induced Bioluminescence Gives Dinoflagellates a Competitive Edge." *Current Biology* 29, no. 12: R564–R565. https://doi.org/10.1016/j.cub.2019.05.019.

Quinn, G. P., and M. J. Keough. 2023*a*. "Simple Linear Models With One Predictor." In Experimental Design and Data Analysis for Biologists, edited by G. P. Quinn and M. J. Keough, 2nd ed., 76–114. Cambridge University Press. https://doi.org/10.1017/9781139568173.007.

Quinn, G. P., and M. J. Keough. 2023*b*. "Introduction to Linear Models." In Experimental Design and Data Analysis for Biologists, edited by G. P. Quinn and M. J. Keough, 2nd

ed., 45–61. Cambridge University Press. https://doi.org/10.1017/9781139568173.005.

R Core Team. 2024. "*R: A Language and Environment for Statistical Computing*." https://doi.org/10.4103/idoj.idoj_1037_24. https://www.R-project.org/.

Ram, K., and H. Wickham. 2023. "*wesanderson: A Wes Anderson Palette Generator*." https://CRAN.R-project.org/package=wesanderson.

Ribeiro, S., A. Amorim, T. J. Andersen, F. Abrantes, and M. Ellegaard. 2012. "Reconstructing the History of an Invasion: The Toxic Phytoplankton Species *Gymnodinium catenatum* in the Northeast Atlantic." *Biological Invasions* 14, no. 5: 969–985. https://doi.org/10.1007/s10530-011-0132-6.

Riegman, R., M. de Boer, and L. d. S. Domis. 1996. "Growth of Harmful Marine Algae in Multispecies Cultures." *Journal of Plankton Research* 18, no. 10: 1851–1866. https://doi.org/10.1093/plankt/18.10.1851.

Rigby, K., E. Berdalet, C. Berglund, et al. 2024. "Direct and Indirect Effects of Copepod Grazers on Community Structure." *Journal of Plankton Research* 46, no. 5: 515–524. https://doi.org/10.1093/plankt/fbae047.

Rigby, K., A. Kinnby, J. Grønning, et al. 2022. "Species-Specific Responses to Grazer Cues and Acidification in Phytoplankton: Winners and Losers in a Changing World." *Frontiers in Marine Science* 9: 875858. https://doi.org/10.3389/fmars.2022.875858.

Rigby, K., and E. Selander. 2021. "Predatory Cues Drive Colony Size Reduction in Marine Diatoms." *Ecology and Evolution* 11, no. 16: 11020–11027. https://doi.org/10.1002/ece3.7890.

Ritz, C., F. Baty, J. C. Streibig, and D. Gerhard. 2015. "Dose–Response Analysis Using R." *PLoS One* 10, no. 12: e0146021. https://doi.org/10.1371/journal.pone.0146021.

Robinson, D., A. Hayes, and S. Couch. 2024. "*broom: Convert Statistical Objects Into Tidy Tibbles*." https://broom.tidymodels.org/.

Ruxton, G. D., and G. Beauchamp. 2008. "Time for Some a Priori Thinking About Post Hoc Testing." *Behavioral Ecology* 19, no. 3: 690–693. https://doi.org/10.1093/beheco/arn020.

Ryderheim, F., E. Selander, and T. Kiørboe. 2021. "Predator-Induced Defence in a Dinoflagellate Generates Benefits Without Direct Costs." *ISME Journal* 15, no. 7: 2107–2116. https://doi.org/10.1038/s41396-021-00908-y.

Sailley, S. F., L. Polimene, A. Mitra, A. Atkinson, and J. I. Allen. 2015. "Impact of Zooplankton Food Selectivity on Plankton Dynamics and Nutrient Cycling." *Journal of Plankton Research* 37, no. 3: 519–529. https://doi.org/10.1093/plankt/fbv020.

Schindelin, J., I. Arganda-Carreras, E. Frise, et al. 2012. "Fiji: An Open-Source Platform for Biological-Image Analysis." *Nature Methods* 9, no. 7: 676–682. https://doi.org/10.1038/nmeth.2019.

Schultz, M., and T. Kiørboe. 2009. "Active Prey Selection in Two Pelagic Copepods Feeding on Potentially Toxic and Non-Toxic Dinoflagellates." *Journal of Plankton Research* 31, no. 5: 553–561. https://doi.org/10.1093/plankt/fbp010.

Selander, E., E. C. Berglund, P. Engström, et al. 2019. "Copepods Drive Large-Scale Trait-Mediated Effects in Marine Plankton." *Science Advances* 5, no. 2: eaat5096. https://doi.org/10.1126/sciadv.aat5096.

Selander, E., T. Fagerberg, S. Wohlrab, and H. Pavia. 2012. "Fight and Flight in Dinoflagellates? Kinetics of Simultaneous Grazer-Induced Responses in *Alexandrium tamarense*." *Limnology and Oceanography* 57, no. 1: 58–64. https://doi.org/10.4319/lo.2012.57.1.0058.

Selander, E., J. Heuschele, G. M. Nylund, et al. 2016. "Solid Phase Extraction and Metabolic Profiling of Exudates From Living Copepods." *PeerJ* 4: e1529. https://doi.org/10.7717/peerj.1529.

Selander, E., H. H. Jakobsen, F. Lombard, and T. Kiørboe. 2011. "Grazer Cues Induce Stealth Behavior in Marine Dinoflagellates." *Proceedings of the National Academy of Sciences* 108, no. 10: 4030–4034. https://doi.org/10.1073/pnas.1011870108.

Selander, E., J. Kubanek, M. Hamberg, M. X. Andersson, G. Cervin, and H. Pavia. 2015. "Predator Lipids Induce Paralytic Shellfish Toxins in Bloom-Forming Algae." *Proceedings of the National Academy of Sciences* 112, no. 20: 6395–6400. https://doi.org/10.1073/pnas.1420154112.

Selander, E., P. Thor, G. Toth, and H. Pavia. 2006. "Copepods Induce Paralytic Shellfish Toxin Production in Marine Dinoflagellates." *Proceedings of the Royal Society B: Biological Sciences* 273, no. 1594: 1673–1680. https://doi.org/10.1098/rspb.2006.3502.

Signorell, A. 2025. "*DescTools: Tools for Descriptive Statistics*." https://github.com/andrisignorell/desctools.

Singmann, H., B. Bolker, J. Westfall, F. Aust, and M. S. Ben-Shachar. 2024. "*afex: Analysis of Factorial Experiments*." https://CRAN.R-project.org/package=afex.

Smayda, T. J. 1997. "Harmful Algal Blooms: Their Ecophysiology and General Relevance to Phytoplankton Blooms in the Sea." *Limnology and Oceanography* 42, no. 5part2: 1137–1153. https://doi.org/10.4319/lo.1997.42.5_part_2.1137.

Smayda, T. J. 2002. "Adaptive Ecology, Growth Strategies and the Global Bloom Expansion of Dinoflagellates." *Journal of Oceanography* 58, no. 2: 281–294. https://doi.org/10.1023/A:1015861725470.

Smayda, T. J., and C. S. Reynolds. 2003. "Strategies of Marine Dinoflagellate Survival and Some Rules of Assembly." *Journal of Sea Research* 49, no. 2: 95–106. https://doi.org/10.1016/S1385-1101(02)00219-8.

Strauss, S. Y., J. A. Rudgers, J. A. Lau, and R. E. Irwin. 2002. "Direct and Ecological Costs of Resistance to Herbivory." *Trends in Ecology & Evolution* 17, no. 6: 278–285. https://doi.org/10.1016/S0169-5347(02)02483-7.

Teegarden, G. 1999. "Copepod Grazing Selection and Particle Discrimination on the Basis of PSP Toxin Content." *Marine Ecology Progress Series* 181: 163–176. https://doi.org/10.3354/meps181163.

Turner, J. 2004. "The Importance of Small Planktonic Copepods and Their Roles in Pelagic Marine Food Webs." *Zoological Studies* 42, no. 2: 255–266.

Turner, J. T. 2006. "Harmful Algae Interactions With Marine Planktonic Grazers." In Ecology of Harmful Algae, 259–270. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-540-32210-8_20.

Valiadi, M., and D. Iglesias-Rodriguez. 2013. "Understanding Bioluminescence in Dinoflagellates—How Far Have We Come?" *Microorganisms* 1, no. 1: 3–25. https://doi.org/10.3390/microorganisms1010003.

White, H. H. 1979. "Effects of Dinoflagellate Bioluminescence on the Ingestion Rates of Herbivorous Zooplankton." *Journal of Experimental Marine Biology and Ecology* 36, no. 3: 217–224. https://doi.org/10.1016/0022-0981(79)90117-5.

Wickham, H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York https://ggplot2.tidyverse.org.

Wickham, H., M. Averick, J. Bryan, et al. 2019. "Welcome to the Tidyverse." *Journal of Open Source Software* 4, no. 43: 1686. https://doi.org/10.21105/joss.01686.

Wickham, H., and J. Bryan. 2023. "*readxl: Read Excel Files*." https://readxl.tidyverse.org; https://github.com/tidyverse/readxl.

Wilke, C. O. 2024. "*cowplot: Streamlined Plot Theme and Plot Annotations for 'ggplot2'*." https://wilkelab.org/cowplot/.

Wilke, C. O., and B. M. Wiernik. 2022. "*ggtext: Improved Text Rendering Support for 'ggplot2'*." https://CRAN.R-project.org/package=ggtext.

Xu, J., and T. Kiørboe. 2018. "Toxic Dinoflagellates Produce True Grazer Deterrents." *Ecology* 99, no. 10: 2240–2249. https://doi.org/10.1002/ecy.2479.

Yamamoto, T., and K. Tarutani. 1999. "Growth and Phosphate Uptake Kinetics of the Toxic Dinoflagellate *Alexandrium tamarense* From Hiroshima Bay in the Seto Inland Sea, Japan." *Phycological Research* 47, no. 1: 27–32. https://doi.org/10.1046/j.1440-1835.1999.00149.x.

Yu, G. 2023. "*ggimage: Use Image in 'ggplot2'*." https://CRAN.R-project.org/package=ggimage.

Zhu, H. 2024. "*kableExtra: Construct Complex Table With 'kable' and Pipe Syntax*." https://CRAN.R-project.org/package=kableExtra.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

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