

FOOD UPTAKE IN THE HARMFUL ALGA *PRYMNESIUM PARVUM* MEDIATED BY EXCRETED TOXINS

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ABSTRACT

We investigated the physiological role of toxin production and excretion in the mixotrophic flagellate *Prymnesium parvum* (Haptophyceae = Prymnesiophyceae). Below we present evidence that toxin excretion in *P. parvum* constitutes a mechanism to immobilize and seize motile prey. Cell-free filtrate of *P. parvum* batch cultures exerted a toxic effect on the motile dinoflagellate *Heterocapsa rotundata*, and feeding activity of *P. parvum* preying on *H. rotundata* was positively correlated with the measure of this toxic effect. When feeding on *H. rotundata*, feeding activity of *P. parvum* was low in dilute *P. parvum* cultures, but it was possible to induce an increase in feeding activity by adding filtrate from a denser *P. parvum* culture with a stronger toxic effect. When preying on a non-motile prey, the diatom *Thalassiosira pseudonana*, feeding activity of *P. parvum* was high even in a dilute *P. parvum* culture with low toxic effect.

INTRODUCTION

The question of why some algae produce toxins has been a point of much speculation, but few facts exist on the possible biological role of algal toxin production [17]. It has been suggested that toxins may function as grazing deterrents, affecting metazoan or protistan grazers [6, 16]. Allelopathy is another possibility, whereby the toxin-producing alga may inhibit and out-compete phytoplankton competitors [5, 9].

Here we present data on the possible role of toxin excretion in feeding by a toxic alga, the brackish water flagellate *Prymnesium parvum*. *Prymnesium parvum* is mixotrophic, i.e. it is photosynthetic, but it is also able to feed phagotrophically on other microorganisms as bacteria [7, 10] and even other protist [15]. *Prymnesium parvum* has a long history of producing harmful blooms in brackish and coastal waters. These blooms have been reported to occur worldwide [1], but particularly in European coastal waters and fishponds have these blooms had much damage to aquaculture industry as outcome [1].

The ichthyotoxic capability of *Prymnesium parvum* was reported already in the early 20th century [11], and since then cases of fish kills due to *P. parvum* blooms have occurred continuously until present time [1, 8, 12]. *Prymnesium parvum* is known to produce at least two related toxins, prymnesin-1 and prymnesin-2, and these toxic substances affect fish gills through damaging the wall of epithelia cells [13]. In addition, the haemolytic properties of *Prymnesium* toxins lead to cell lysis of many aquatic protist species [14, 15].

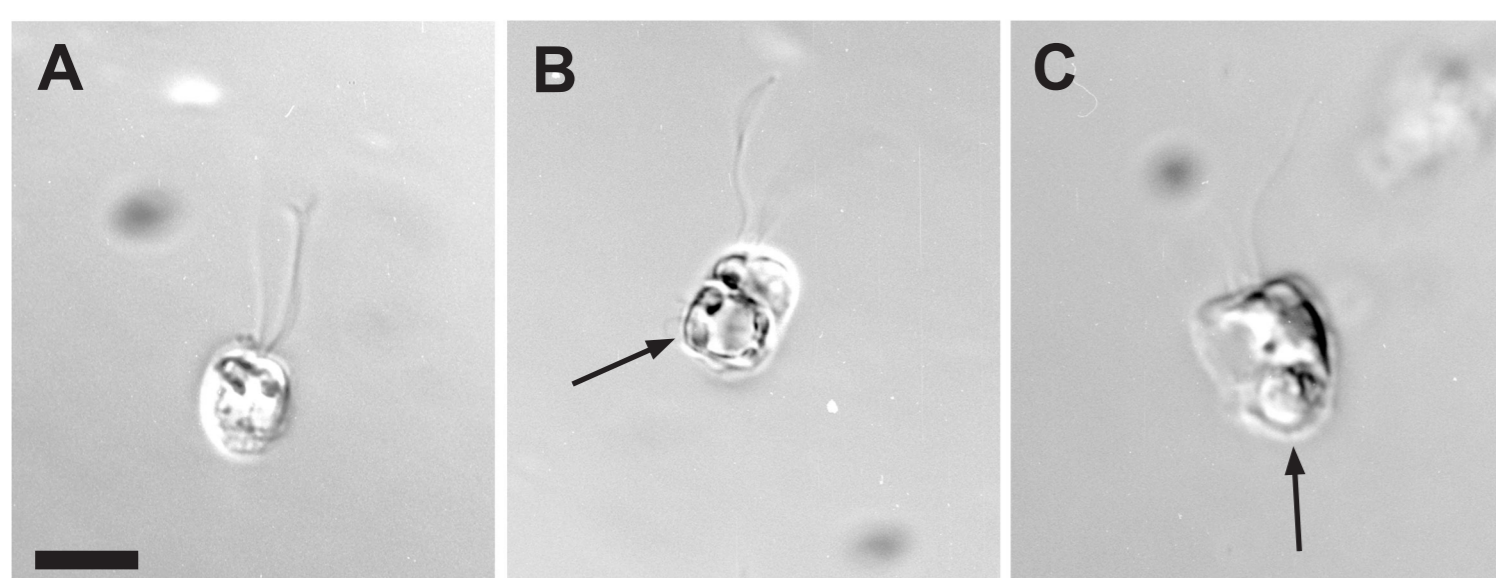


Fig. 1. The haptophyte *Prymnesium parvum*. A, cell without ingested food. B, cell with ingested *Thalassiosira pseudonana* (arrow). C, cell with food vacuole originating from ingested *Heterocapsa rotundata* (arrow). Scale bar ~10 µm.

METHODS

The algae were kept in batch cultures in f/10-medium [3] (salinity: 30‰, temperature: 15°C, irradiance: 90 µmol photons m⁻² s⁻¹). Triplicate cultures of *Prymnesium parvum* were set up and the development in cell densities were monitored for 22 days by microscopical counts of samples withdrawn every second day.

Toxicity of GF/C-filtered *Prymnesium parvum* cultures was quantified as the filtrate's capacity to provoke cell lysis or immobilization in *Heterocapsa rotundata* cells, according to the method previously described [5]. 500 *H. rotundata* cells were added to 0.3 ml cell-free, GF/C-filtered *P. parvum* culture. After 2 h, fractions of immobile or disintegrated *H. rotundata* cells were determined microscopically.

Feeding was quantified by determining the percentages of *Prymnesium parvum* cells that were phagotrophically active after 2 h of incubation with food. Using an inverted microscope, total numbers of *P. parvum* cells were counted (Fig. 1A) as well as the numbers of cells that either contained food vacuoles (Fig. 1B, C) or were fixed in the process of engulfing a prey cell. Prey concentration was constant in all feeding experiments, 5 × 10⁴ prey cells ml⁻¹.

As the *Prymnesium parvum* batch cultures grew denser, toxicity and feeding activity was measured simultaneously every second day using *Heterocapsa rotundata* as prey/target cell. At the end of the 22-days growth period, the *P. parvum* cultures were diluted to 5, 12.5, 25, 50, 75 and 90% of original density, respectively, by diluting with f/10-medium. Additional feeding experiments were then performed for each dilution using both *H. rotundata* and *Thalassiosira pseudonana* as prey cells.

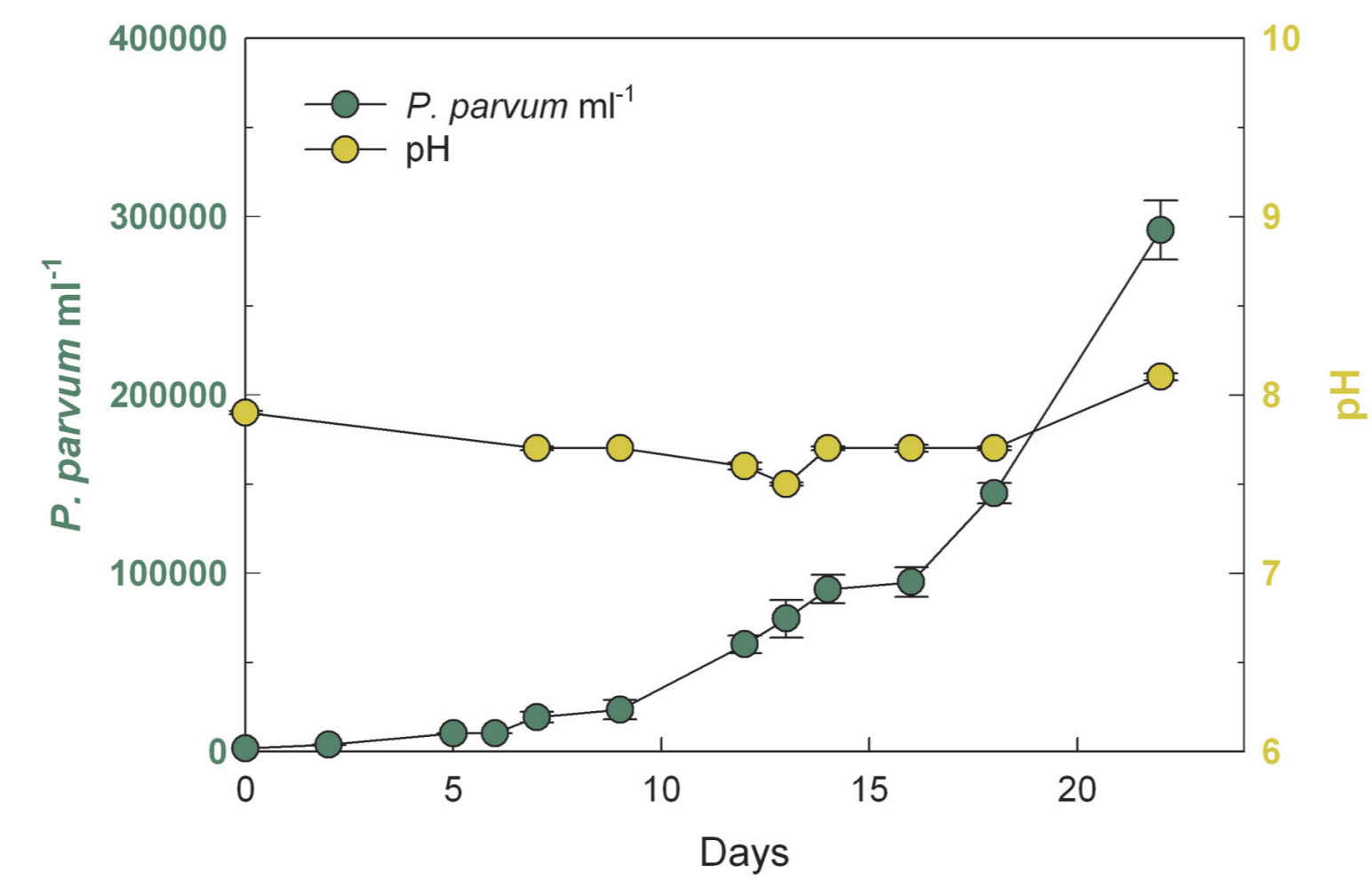


Fig. 2. Development of cell density and pH in *Prymnesium parvum* batch cultures. Symbols represent means of 3 replicates ± SE.

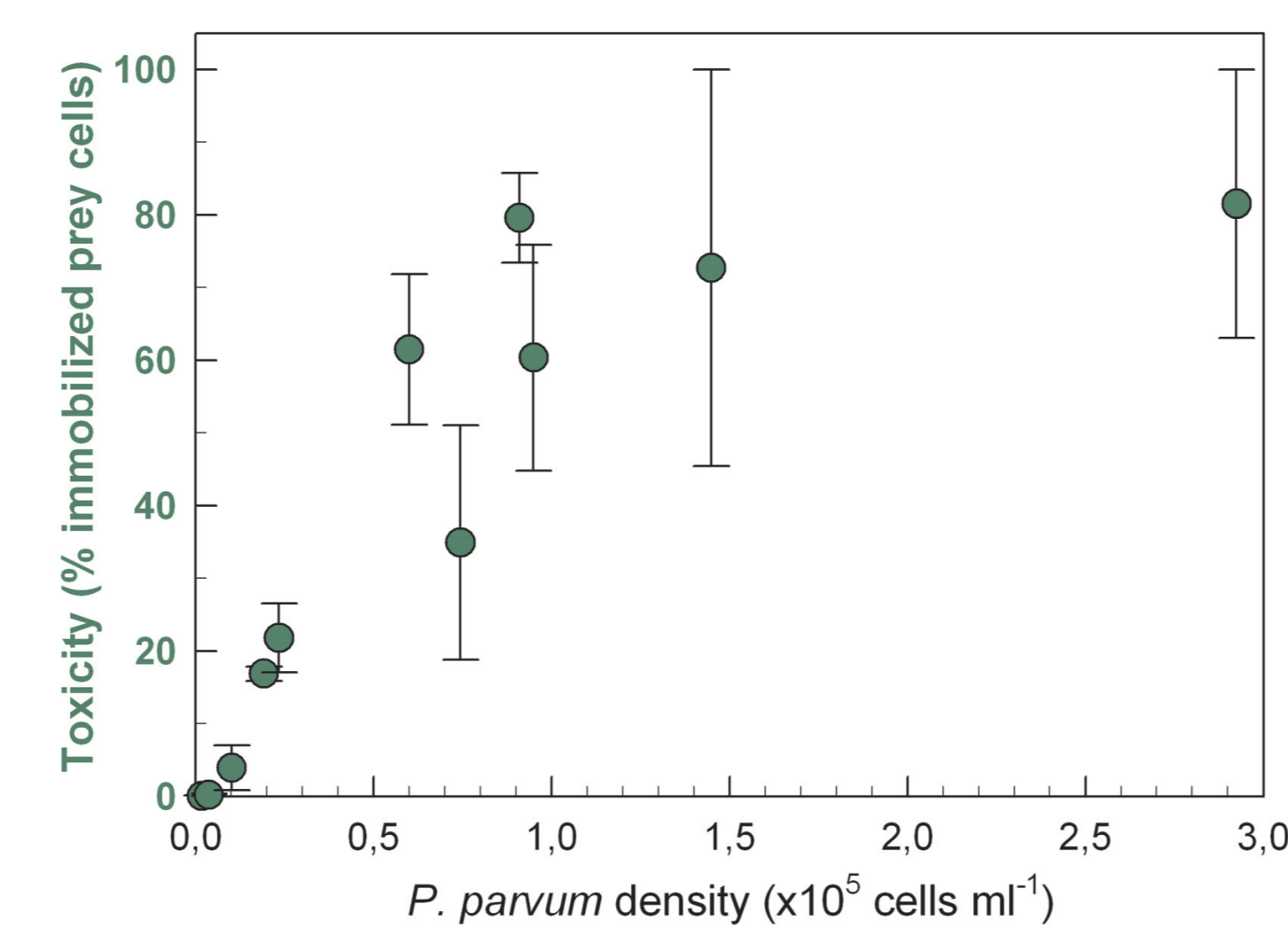


Fig. 3. Toxic effect of *Prymnesium parvum* filtrate on *Heterocapsa rotundata* as function of *P. parvum* cell density in batch cultures. *Prymnesium parvum* cell density varied with age of batch cultures. Toxicity was determined as the efficiency of *P. parvum* culture filtrate to immobilize prey, *H. rotundata*. Symbols represent means of 3 replicates ± SE.

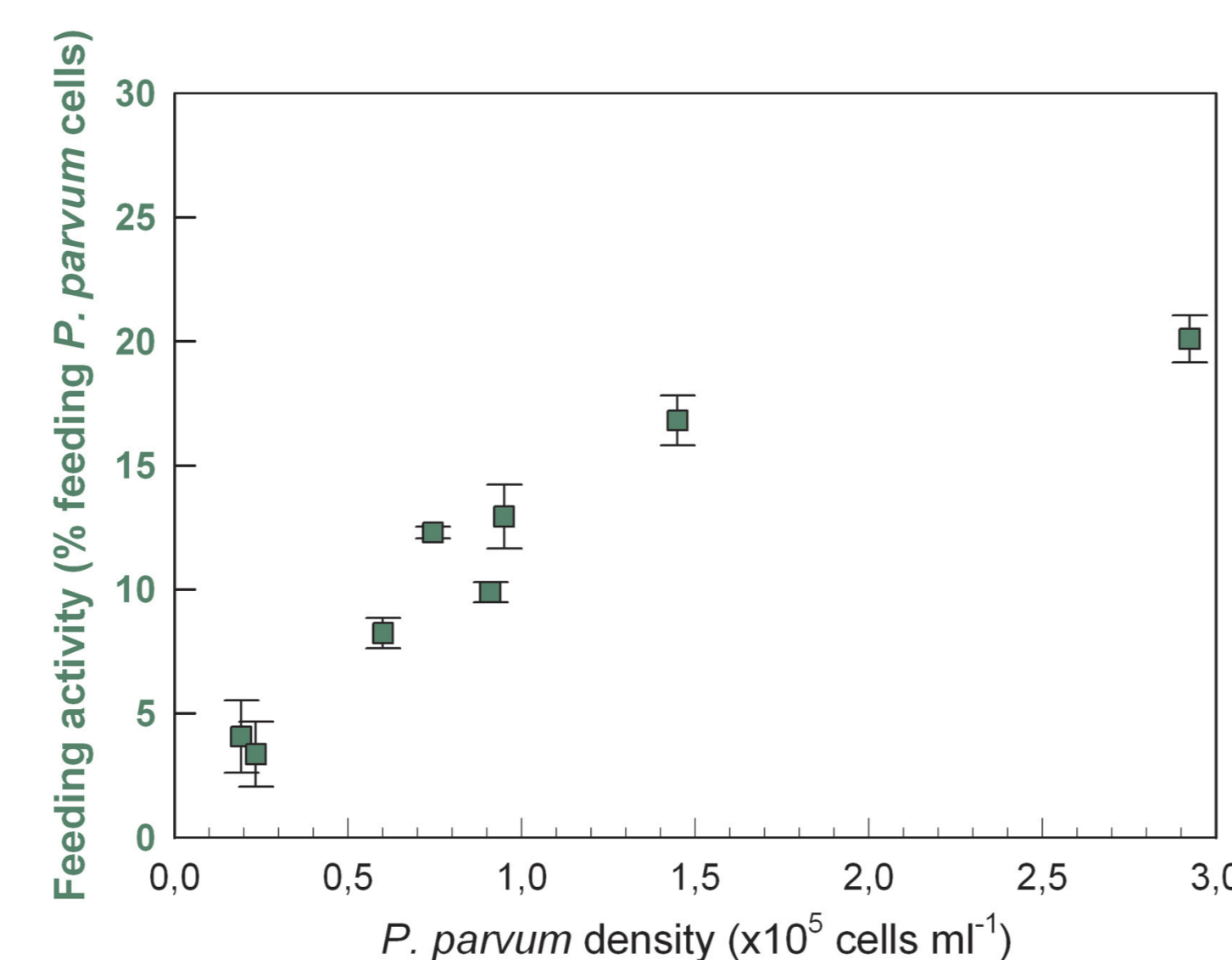


Fig. 4. Feeding activity of *Prymnesium parvum* feeding on *Heterocapsa rotundata* as function of *P. parvum* cell density in batch cultures. *Prymnesium parvum* cell density varied with age of batch cultures. Feeding activity was defined as the percentage of *P. parvum* cells feeding or containing food vacuoles after 2 h of incubation with prey. Prey concentrations were similar at all predator cell densities. Symbols represent means of 3 replicates ± SE.

RESULTS

When cell densities of *Prymnesium parvum* in batch cultures increased exponentially during the 22-day growth period (Fig. 2), toxic effect of the growth medium also increased (Fig. 3). Toxic effects on the target organism, *Heterocapsa rotundata* appeared at 0.1 × 10⁵ *P. parvum* cells ml⁻¹, at which point a minor fraction of the *H. rotundata* cells became immobile due to exposure to the filtrate (Fig. 3). When *P. parvum* cell densities had exceeded 1.0 × 10⁵ cells ml⁻¹, most *H. rotundata* cells (60-80%) were affected within 2 h after exposure to the filtrate (Fig. 3). It can be excluded that pH in the culture medium affected viability of *H. rotundata*, since pH never exceeded 8.1 (Fig. 2).

Feeding activity, expressed as the percentage of *Prymnesium parvum* cells exhibiting phagotrophic activity, also increased when the batch cultures grew denser (Fig. 4). At 1.6 × 10⁵ *P. parvum* cells ml⁻¹, only 0.1% of *P. parvum* cells were feeding 2 h after the addition of prey, but when the predator density had grown to 2.9 × 10⁵ *P. parvum* cells ml⁻¹, feeding activity increased to 20.1% (Fig. 4) even though the prey concentration was constant in all feeding experiments performed (5 × 10⁴ *Heterocapsa rotundata* ml⁻¹). Feeding activity was thus related to the cell density of the predator itself, and there was a positive correlation between toxicity of the culture medium and feeding activity in *P. parvum* (Fig. 5). Dilution of the dense *P. parvum* batch culture with f/10-medium yielded similar results: higher density of the predator itself led to higher feeding activity (Fig. 6).

The dinoflagellate prey *Heterocapsa rotundata* is a motile species. When *Prymnesium parvum* was fed a non-motile prey organism, the diatom *Thalassiosira pseudonana*, feeding activity was not related to predator density (Fig. 6), on the contrary, feeding activity was in this case always between 25 and 33%.

In a culture with low density of *Prymnesium parvum* (2 × 10⁴ cells ml⁻¹) and subsequent low toxic effect on *Heterocapsa rotundata* and low feeding activity, it was possible to induce a high feeding activity (30.5%) by adding cell-free filtrate from a dense (2 × 10⁵ cells ml⁻¹) *P. parvum* culture (Fig. 7). Following this addition of toxic filtrate, feeding activity was thus comparable to the feeding activity when the prey was the non-motile prey *Thalassiosira pseudonana* (Fig. 7).

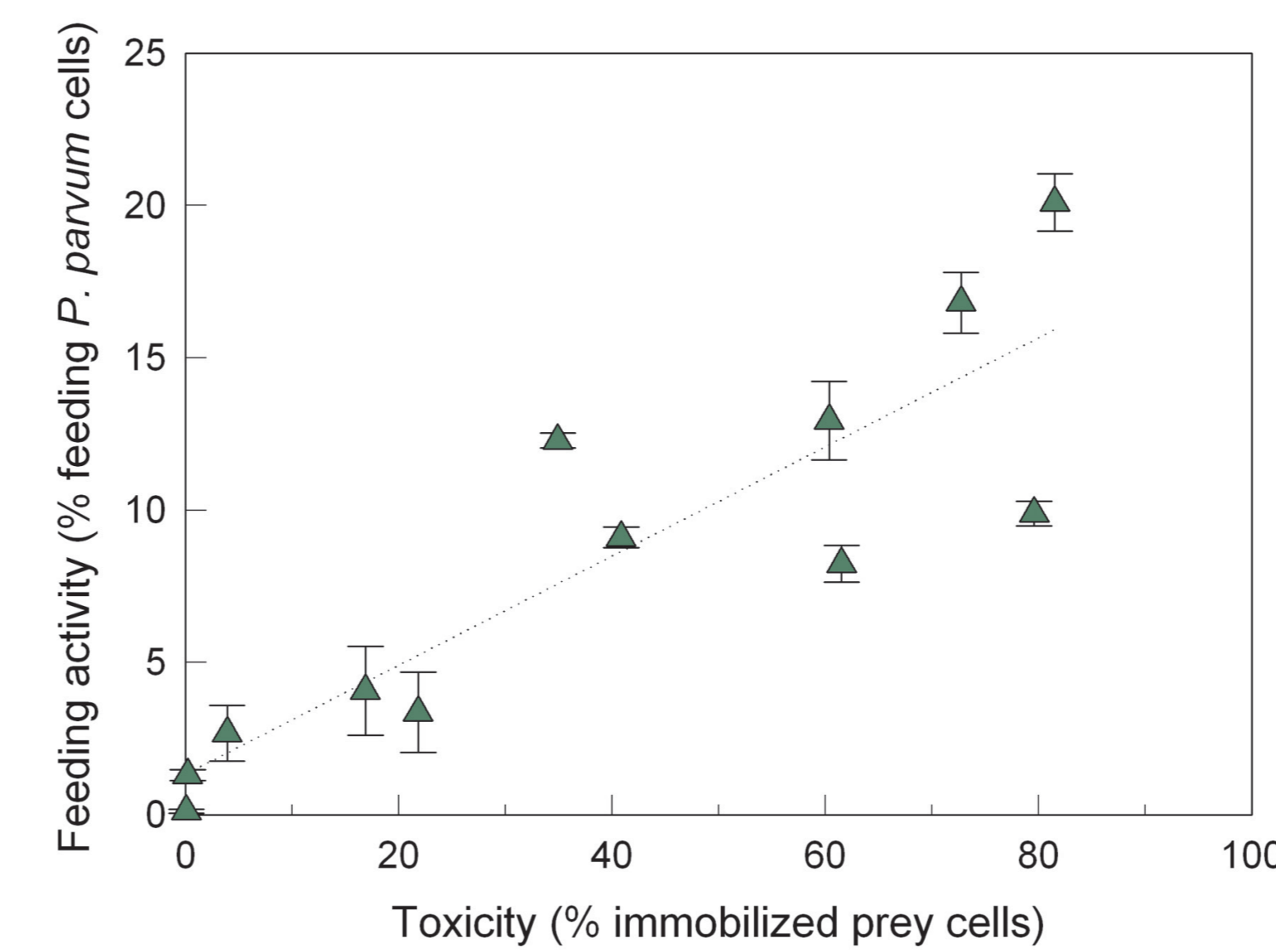


Fig. 5. Relationship between feeding activity and toxicity in *Prymnesium parvum* in batch cultures. Toxicity and feeding activity were determined using *Heterocapsa rotundata* as target/prey organism. Prey concentration was constant throughout the experiments; toxicity increased as result of increasing *P. parvum* cell densities. Symbols represent means of 3 replicates ± SE. Dotted line represents linear regression ($r^2 = 0.77$).

CONCLUSION

Feeding by *Prymnesium parvum* on motile prey occurred only when toxicity of the growth medium was sufficient to immobilize prey (Figs. 3-6), but when offered non-motile prey, feeding occurred even in medium with low toxin effect (Fig. 6). We conclude that one role of toxin production and excretion in *P. parvum* is to make potential prey exploitable for predation through immobilization of prey cells.

Prymnesium parvum does not possess any mechanism or morphological structure for capturing motile prey cells as it is the case for many other phagotrophic flagellates [2, 4]. When excretion of toxin by *P. parvum* serves as a mechanism to seize prey organisms, it thereby makes up for the lack of prey capturing mechanism that *P. parvum* is otherwise subject to.

The feeding strategy of *Prymnesium parvum* may explain why this species is so successful in producing blooms [1]: by excreting toxin, *P. parvum* is able not only to severely affect and even kill photosynthetic competitors, it may also eliminate potential grazers and, finally, by feeding on the affected organisms, *P. parvum* may be able to stimulate its own growth by gaining organic carbon or nutrients from the ingested prey.

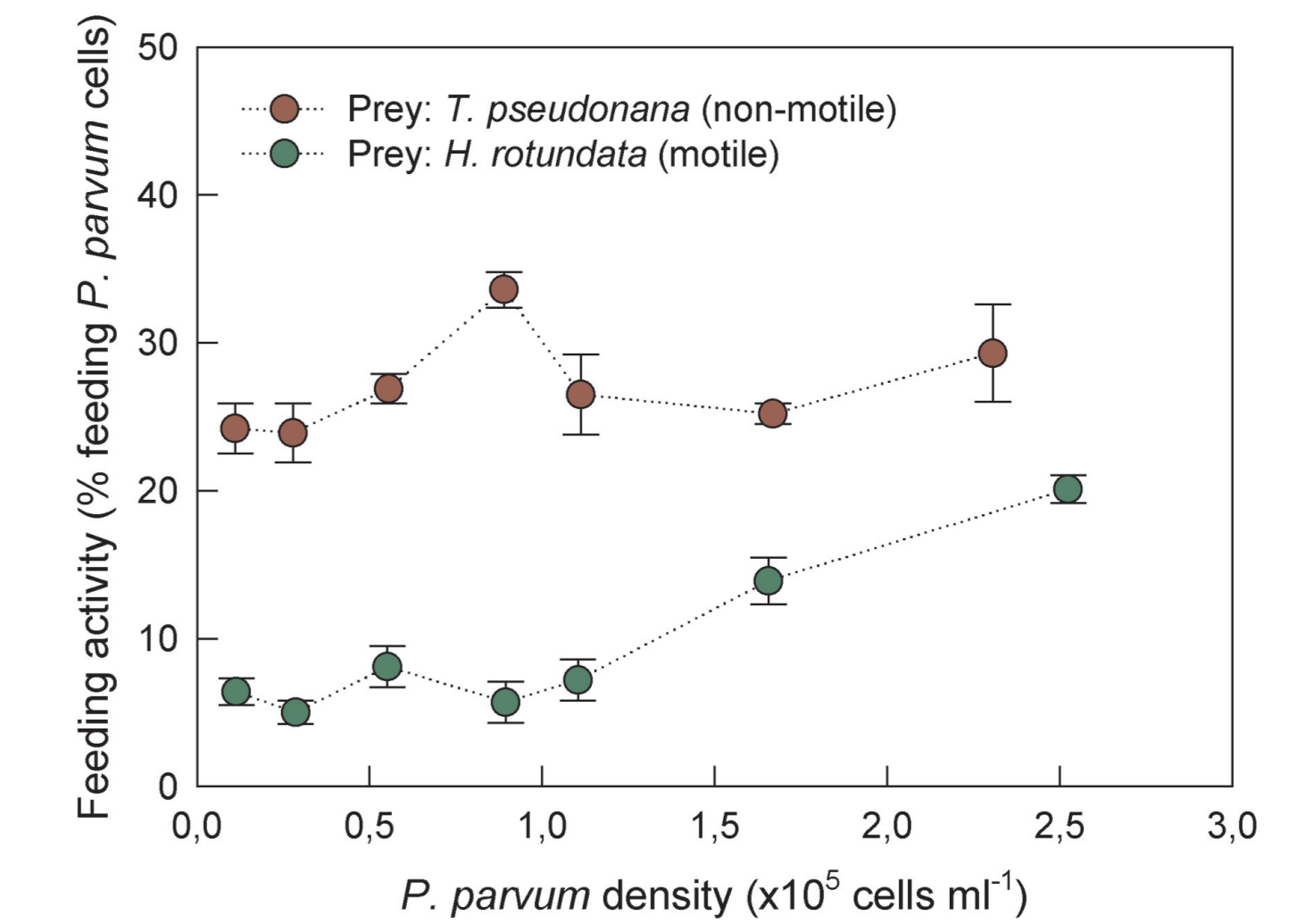


Fig. 6. Feeding activity of *Prymnesium parvum* feeding on *Heterocapsa rotundata* and on *Thalassiosira pseudonana* as functions of *P. parvum* cell density. Predator density was altered by diluting with f/10-medium. Prey concentration was similar at all predator cell densities. Symbols represent means of 3 replicates ± SE.

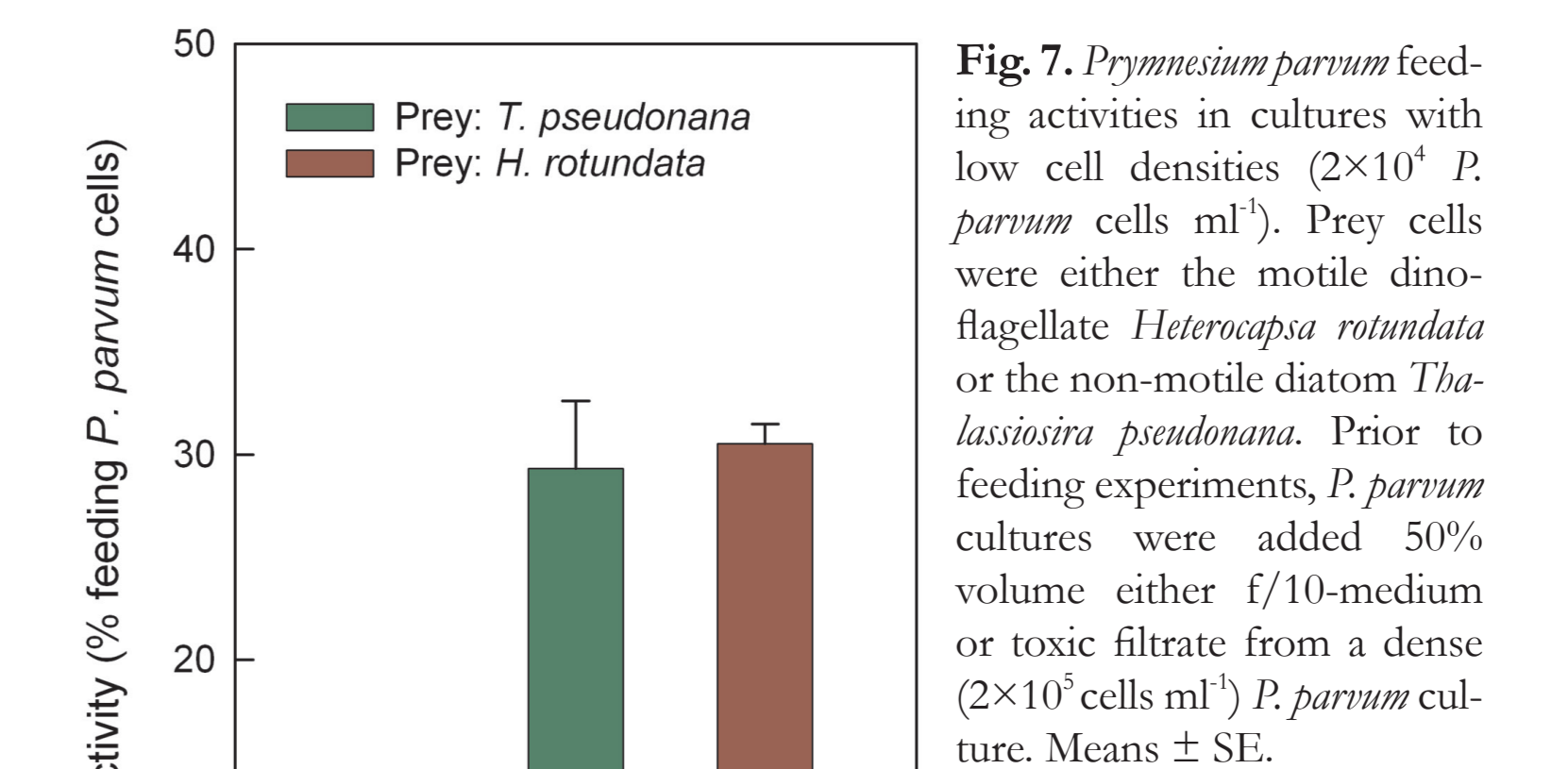


Fig. 7. *Prymnesium parvum* feeding activities in cultures with low cell densities (2 × 10⁴ *P. parvum* cells ml⁻¹). Prey cells were either the motile dinoflagellate *Heterocapsa rotundata* or the non-motile diatom *Thalassiosira pseudonana*. Prior to feeding experiments, *P. parvum* cultures were added 50% volume either f/10-medium or toxic filtrate from a dense (2 × 10⁵ cells ml⁻¹) *P. parvum* culture. Means ± SE.

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REFERENCES

- Edwards, B. and E. Ptasche. 1998. Bloom dynamics and physiology of *Prymnesium* and *Chrysochromulina*, p. 193-208. In D. M. Anderson, A. D. Cembella, and G. M. Hallegraeff [eds.], *Physiological Ecology of Harmful Algal Blooms*. Vol. G 41 NATO ASI Series. Springer.
- Gaines, G. and M. Elberichter. 1987. Heterotrophic nutrition, p. 224-268. In F. J. R. Taylor [ed.] *The biology of dinoflagellates*. Blackwell.
- Guillard, R. R. L., and J. H. Ryther. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Husted, and *Dinella confertosa* (Cleve) Gran. *Can. J. Microbiol.* 8: 229-239.
- Hansen, P. J. 1998. Phagotrophic mechanisms and prey selection in mixotrophic phytoflagellates, p. 525-537. In D. M. Anderson, A. D. Cembella, and G. M. Hallegraeff [eds.], *Physiological Ecology of Harmful Algal Blooms*. Vol. G 41 NATO ASI Series. Springer.
- Hansen, P. J., and L. E. Schmidt. 2001. Allelopathy in the prymnesiophyte *Chrysochromulina polytypica*: effect of cell concentration, growth phase and pH. *Mar. Ecol. Prog. Ser.* 216: 67-81.
- 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. *Mar. Ecol. Prog. Ser.* 28: 105-120.
- Legrand, C., N. Johansson, K. Y. Borsheim, and E. Granéli. 2001. Phagotrophy and toxicity variation in the mixotrophic *Prymnesium patelliferum* (Haptophyceae). *Limnol. Oceanogr.* 46: 1208-1214.
- Lindholm, T., Ohman, P., Kurki-Helasma, K., Kincaid, B., and Merilähti, J. 1999. Toxic algae and fish mortality in a brackish-water lake in Åland, SW Finland. *Hydrobiologia*. 397: 109-120.
- Maestrini, S.Y., and D. J. Bonin. 1981. Allelopathic relationships between phytoplankton species. *Can. Bull. Fish. Aquat. Sci.* 210: 323-338.
- Nygaard, K., and A. Tobiesen. 1993. Bacterivory in algae: A survival strategy during nutrient limitation. *Limnol. Oceanogr.* 38: 273-279.
- Otterstrom, C. V., and E. Steemann Nielsen. 1940. Two cases of extensive mortality in fishes caused by the flagellate *Prymnesium parvum*. *Carter. Rep. Danish Biol. Sta.* 44: 4-24.
- Sabour, B., Loudiki, M., Oudra, B., Oubrahim, S., Fawzi, B., Fadloui, S., Chlaidi, M., and Vasconcelos, V. 2000. Blooms of *Prymnesium parvum* associated with fish mortalities in a hypereutrophic brackish lake in Morocco. *Harmful Algae News*, p. 8-9.
- Shilo, M. 1971. Toxins of Chrysophyceae, p. 67-103. In S. Kadis, A. Giegler, and S. J. Ajl [eds.], *Microbial Toxins*. Academic.
- Shilo, M. and Rosenberger, R. F. 1960. Studies on the toxic principles formed by the chrysoomonad *Prymnesium parvum*. *Carter. Ann. N. Y. Acad. Sci.* 90: 866-876.
- Tillmann, U. 1998. Phagotrophy by a plastidic haptophyte, *Prymnesium patelliferum*. *Aquat. Microb. Ecol.* 14: 155-160.
- Verity, P.G., and D. K. Stoecker. 1982. Effects of *Olisthodiscus luteus* on growth and abundance of tintinnids. *Mar. Biol.* 72: 79-87.
- Wright, J. L. C., and A. D. Cembella. 1998. Ecophysiology and biosynthesis of polyether marine biotoxins, p. 427-451. In D. M. Anderson, A. D. Cembella, and G. M. Hallegraeff [eds.], *Physiological Ecology of Harmful Algal Blooms*. Vol. G 41 NATO ASI Series. Springer.