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Paralytic shellfish toxin concentration and cell density changes in *Pyrodinium bahamense – Noctiluca scintillans* feeding experiments

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ABSTRACT

For the first time the potential of *Noctiluca scintillans*, a non-toxic mixotrophic dinoflagellate, in bioconverting and/or excreting saxitoxin has been illustrated, thus contributing to the limited knowledge on the aspects of toxin pathways in the food chain/web and predator-prey preferences. *Noctiluca* growth rate increased with higher *Pyrodinium* concentration but the ratio of *Noctiluca* to *Pyrodinium* should at least be 1:250 cells per mL. *Noctiluca* fed with *Pyrodinium* alone was found to decrease in number suggesting that the nutrients from this prey were insufficient. This was confirmed by the improved cell density of *Noctiluca* upon addition of 0.01% casitone to the *Pyrodinium*-fed *Noctiluca*. The alternative prey (*Gymnodinium sanguineum*) slowed down the grazing impact of *Noctiluca* on *Pyrodinium*. *Noctiluca* depleted *Gymnodinium* earlier than *Pyrodinium* showing preference over a prey with less saxitoxin. After the feeding experiments, total saxitoxin levels decreased to 72% in the *Noctiluca–Pyrodinium* setup whereas no saxitoxin was detected in the *Noctiluca* culture fed with *Pyrodinium* and *G. sanguineum*. It is possible that *Gymnodinium* can provide some nutrients needed to make *Noctiluca* more efficient in bioconverting saxitoxin.

1. Introduction

Pyrodinium bahamense var. *compressum* has caused major public health and economic problems in the Asia-Pacific region by producing toxic algal blooms and causing paralytic shellfish poisoning (PSP). In the Philippines, *Pyrodinium* blooms usually starts at the beginning of southwest monsoon or end of summer (April, May or June) (Azanza and Taylor, 2001). An almost yearly occurrence of *Pyrodinium* blooms from 1988 to 1998 was experienced in Manila Bay. During the period 1997 to 2001 it was observed however that the significant decline in *Pyrodinium* population was coupled to an increase in cell counts of *Noctiluca scintillans*, a green alga known to feed on various smaller phytoplanktons including *Pyrodinium* (Hansen et al., 2004). This observation of plankton succession led to the suggestion that *Noctiluca* could be a potential natural biological control against *Pyrodinium*.

Harmful algal bloom (HAB) management options include preventive, control and mitigating schemes. Whereas preventive measures try to avert algal blooms from actually happening, control and mitigating options try to stop or at least alleviate. HAB's when they occur by employing biological or chemical means. For example, the use of grazers that can feed on the HAB-causing organism is a biological means of controlling algal blooms. This top-down control is an important type of interspecies interactions in aquatic ecosystems.

Phytoplankton grazers are believed to contribute to the top-down control of phytoplankton especially the harmful ones (Ostromouv, 2002). In a study conducted by Uye (1986), copepods selectively grazed on *Chattonella antiqua*

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during the outbreak of a bloom. Ingestion rates were found to increase linearly with increasing cell concentrations until a maximum level was reached, beyond which the rates remained constant. More recently in the Philippines, Hansen et al. (2004) studied the growth and grazing responses of the heterotrophic dinoflagellate *Noctiluca* fed with the autotrophic dinoflagellate *Pyrodinium* as a function of irradiance and food concentration in laboratory cultures. It was found that the growth rate of *Noctiluca* increased as prey concentration increases. *Noctiluca* preyed actively on *Pyrodinium* and no signs of reduced ingestion or satiation by the toxic cells were observed.

Some studies illustrate prey preference among planktonic grazers, effect of light intensity, and yearly variation in prey-predator relationship. Food uptake by *Fragilidium subglobosum* stimulated photosynthesis at low prey concentrations. When *Ceratium tripos* cells were added as food in excess of *F. subglobosum*, growth and ingestion rates increased more with light intensity within the studied range (9–45 μmol photons $m^{-2}s^{-1}$) than with *Ceratium furca* and *Ceratium fusus* (Hansen and Nielsen, 1997). In a study done by Smalley, et al. (1999), it was observed that *C. furca* as predator preferred ciliates of the genus *Strobilidium*.

Although planktonic copepods are major suspension feeders in the sea, the impact of their grazing pressure upon red-tide flagellates has not been fully investigated. The available information on the diversity of grazer-prey relationship is limited and the role of grazing in the control of toxic algal populations is poorly understood. Predator-prey relationship between dinoflagellates and their grazers should be explored since it is important in understanding the mechanisms of outbreak and maintenance of algal blooms.

In this study, the ability of *Noctiluca* to feed on *Pyrodinium* as affected by other nutrient sources, i.e. organic materials and other dinoflagellate species, was analyzed for the first time. Different concentrations of the prey and predator that could possibly alter the grazing activity of this organism were examined. Moreover, the effect of grazing on saxitoxin level was studied.

2. Materials and methods

2.1. Maintenance of culture and cell growth analysis

Samples of *N. scintillans* were collected in Limay, Bataan using plankton net with 20-μm mesh and transported to the Marine Science Institute laboratory in an ice chest. These were washed with sterile filtered seawater and acclimatized in the laboratory for 24 h. They were cultured under the same conditions used for *P. bahamense* var *compressum* and *Gymnodinium sanguineum*.

The *Pyrodinium* cells utilized in the experiments were taken from existing cultures in the laboratory maintained at 24 + 2 °C, 150 $\mu Em^{-2}s^{-1}$ under a 12:12 h light:dark (L:D) cycle as described by Azanza-Corrales and Hall (1993).

Growth of both organisms was monitored using cell counts under a Carl Zeiss Axioskop II microscope at a magnification of 100X with the aid of a Sedgewick Rafter Chamber (Andersen and Throndsen, 2003). The growth and grazing responses of *Noctiluca* were determined by noting the changes on prey and predator concentrations by cell counting every two days following the procedures of Hansen et al. (2004). Sub-samples were taken every two days by withdrawing 50 mL (10% of the total volume) from the culture flasks then replacing it with the same amount of fresh F/2 medium (Guillard and Ryther, 1962). Prior to the withdrawal of sub-samples, the culture flasks were gently shaken to disaggregate the cells to ensure even distribution. *Noctiluca* cells were counted under a DP11 Olympus stereoscope while *Pyrodinium* and *Gymnodinium* cells were counted using Carl Zeiss Axioskop II microscope. Ratio of cell counts (C_1/C_2, where C_T = cell count at time t and C_o = initial count) where calculated at 0 day to day 10.

2.2. Feeding experiments

2.2.1. Noctiluca *feeding on* Pyrodinium

Following the techniques used by Hansen et al. (2004), *Noctiluca* cells with a concentration of 1, 3 and 5 cells mL^{-1} were added in the culture flasks with *Pyrodinium* concentrations of 500, 750 and 1000 cells mL^{-1}. *Noctiluca* and *Pyrodinium* setups grown alone were prepared for all the test concentrations as controls. All experiments were carried out in 1-L culture flasks with two replicates each.

Cultures were incubated for 10 days under optimum laboratory conditions using F/2 medium. Sub-samples were drawn from each culture flask every two days for cell counting. Paralytic shellfish toxin (PST) levels were determined upon termination of the experiment.

2.2.2. Addition of organic nutrient in the media

To determine whether *Noctiluca* would graze effectively on *Pyrodinium* even in the presence of an alternative food source, 0.01% of casitone was added in the media as source of organic nutrient. The same concentrations of *Noctiluca* and *Pyrodinium* were tested for feeding following the same procedure, laboratory conditions and sampling schemes as above.

2.2.3. Addition of other phytoplankton (G. sanguineum) as Prey

To assess whether *Noctiluca* has food preference when given two prey species simultaneously, same concentrations of *Gymnodinium* and *Pyrodinium* were added to the media with known concentrations of *Noctiluca*. The G:P:N cell count ratio used were 250:250:1, 375:375:1, 250:250:3, 375:375:3. Procedures as described above were followed.

2.3. Toxin analysis

To verify the ability of *Pyrodinium* cells to produce paralytic shellfish toxins (PST's), three isolates of *P. bahamense* var. *compressum* (M93, M94 and M95) isolated from Masinloc Bay, Zambales at different years (1993, 1994 and 1995 respectively) were grown in F/2 medium at an initial cell density of 500 cells/mL. Cell counts and levels of PST's were monitored for 28 days.

Monitoring of the relative toxicities of the three *Pyrodinium* isolates was done using ECOS-HPLC as described below.

2.3.1. Sampling method

Extraction of PST's was done using a modification of the acetic acid extraction method of Oshima (1995). Samples