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Interactions between *Noctiluca scintillans* and Three Co-Occurring Microalgae in Response to Varying Nutrient Levels

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Abstract: The dinoflagellate *Noctiluca scintillans* is a globally distributed bloom-forming species. Previous studies have shown that the primary reason for the frequent occurrence of *N. scintillans* blooms may be the proliferation of microalgae due to eutrophication, which provides a sufficient source of food. Meanwhile, *N. scintillans* may release nutrients into the environment, thus affecting the population dynamics of microalgae. Thus, to investigate the interaction between *N. scintillans* and co-occurring microalgae, this study examined the population dynamics of *N. scintillans* and their interaction with three representative microalgae species in response to varying nutrient levels. The findings indicate that the growth of *N. scintillans* is slow when co-cultured with diatom *Skeletonema costatum*. Moreover, a high density and rapid growth rate of *S. costatum* may have an inhibitory effect on the growth of *N. scintillans*. Conversely, the population abundance of *N. scintillans* increased with the rise in the population density and nutritional level of *Heterocapsa steinii* (dinoflagellate) and *Heterosigma akashiwo* (raphidophyceae). Notably, *N. scintillans* can discharge specific nutrients into the aquatic environment, which can subsequently be assimilated and exploited by *H. steinii*. Thus, the interaction between the species and population dynamics of plankton, as well as changes in nutrient levels within the ecosystem, played a significant role in influencing the growth and population dynamics of *N. scintillans*. The mutualistic association between *N. scintillans* and microalgae may establish a transient closed loop, thereby fostering the sustained proliferation and subsequent expansion of *N. scintillans*.

Keywords: dinoflagellate; *Noctiluca scintillans*; algal bloom; trophic interactions

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1. Introduction

Harmful algal blooms (HABs) are the sudden proliferation or accumulation of marine microalgae and other plankton in aquatic environments, which cause significant damage to the marine ecosystem, human health, aquaculture, and tourism [1–3]. The dinoflagellate *Noctiluca scintillans* (Macartney) Kofoid and Swezy, a globally distributed bloom-forming species in numerous coastal waters worldwide, poses a potentially harmful threat to fishery resources and marine ecosystems [4,5]. Although *N. scintillans* does not directly produce any toxins, its extensive proliferation results in the secretion of copious amounts of mucus and ammonia, which leads to the creation of an anoxic environ-

ment. This phenomenon has detrimental impacts on aquaculture and aquatic environments [6–8]. Furthermore, the food vacuoles of *N. scintillans* contain toxigenic microalgae, indicating the potential role of *N. scintillans* as a carrier of phycotoxins to higher trophic levels [9–12].

Based on the nutritional requirements and bloom color of *N. scintillans*, they can be categorized into two types: red and green [13]. The red *N. scintillans* is a heterotrophic plankton that relies solely on feeding for its growth and reproduction, with carotenoids contributing to its distinctive orange-red color. In contrast, the green *N. scintillans* is a mixoplankton that harbors the photosynthetic symbiont *Pedinomonas noctilucae* (Subrahmanyan) Sweeney, and the proliferation of this species is frequently linked to the shoaling of hypoxic water [14,15]. Notably, food intake also plays a crucial role in sustaining its state [16,17]. This study focused on red *N. scintillans*, as there are mainly red *N. scintillans* in Chinese coastal waters [13].

The proliferation of *N. scintillans* typically occurs in nearshore coastal regions with substantial nutrient inputs, such as areas that are influenced by upwelling phenomena [13,18]. These environments facilitate the growth of microalgae, which serve as the sole energy source for the development of red *N. scintillans*. Hence, *N. scintillans* assumes a predatory role within marine food webs, exerting control over plankton populations through its feeding activities, which facilitates its proliferation, and it often becomes the dominant species [19–21]. However, the growth and population dynamics of *N. scintillans* are influenced by the dynamics of planktonic species, population fluctuations, and nutrient variations. For instance, a study by Zhang et al. [22] in the Pingtan Sea revealed substantial variations in the population growth of *N. scintillans* when feeding on distinct microalgae, even when subjected to equivalent biomass levels. Moreover, Ara et al. [23] revealed a significant positive association between the abundance of *N. scintillans* populations and chlorophyll a in Sangmo Bay, Japan, suggesting that there is a bottom-up effect between *N. scintillans* and microalgae.

In addition to its role in the regulation of carbon transfer within marine food webs, *N. scintillans* also serves as a crucial factor for nutrient regeneration [13,24]. This organism can accumulate and regenerate substantial quantities of dissolved nutrients (e.g., NH_4^+ and PO_4^{3-}) and organic substances [23,25,26]. The discharge of these nutrients into the surrounding water fosters the proliferation of co-occurring microalgae species within the ecosystem. For instance, Santiwat [27] suggested that the NH_4-N in *N. scintillans* contributed to the NH_4-N levels in the water column of the Seto Inland Sea, with a range of 0 to 119% during the spring season. In situ studies conducted by Montani et al. [6] demonstrated that *N. scintillans* can induce short-term eutrophication through the discharge of regenerated nitrogen and phosphorus into the surrounding marine area, leading to the occurrence of red tides that are caused by other microalgae.

Previous research has primarily focused on in situ monitoring, with limited laboratory studies, particularly regarding the interaction between *N. scintillans* and microalgae. In this study, the population dynamics of *N. scintillans* were investigated when co-cultured with three typical microalgae species under varying nutrient conditions. Additionally, the research examines the interplay between *N. scintillans* and microalgae through the observation of nutrient release by *N. scintillans* and the subsequent response of microalgae to these nutrients. The objective of this study was to explore the response of *N. scintillans* to fluctuations in nutrient availability and the presence of microalgae, as well as to establish the relationship between *N. scintillans* and microalgae.

2. Materials and Methods

2.1. Noctiluca scintillans Cultures

The red *N. scintillans* strain was initially isolated from the coastal waters of Lianyungang, China, in October 2020. A single cell of *N. scintillans* was separated with a large-

diameter pipette based on its morphology and cultured in sterilized seawater with a salinity of 30‰. This was prepared by filtration through a 0.22 μm filter and subsequent autoclaving of in situ seawater.

The culture was incubated at 20 °C, following a light–dark cycle of 14:10 h, and it had a photon flux of 100 μmol m^{-2} s^{-1}. Additionally, Tetraselmis subcordiformis (Wille) Butcher was used as the prey [28,29]. To minimize the influence of the prey residues in vivo and externally on the experiments, *N. scintillans* were starved for 48 h and then washed 2~3 times through a 100 μm mesh using sterilized seawater before the formal experiments.

2.2. Population Dynamics of Noctiluca scintillans and Their Typical Prey Species under Different Nutrient Concentrations

The population dynamics of *N. scintillans* and its typical prey species, including mixoplankton [30] (dinoflagellate (*Heterocapsa steinii* Tillmann, Gottschling, Hoppenrath, Kusber and Elbrächter), raphidophyte (*Heterosigma akashiwo* (Y.Hada) Hada ex Hara and Chihara)) and phytoplankton (diatom (*Skeletonema costatum* (Greville) Cleve)), were examined under different nutrient concentrations. The *S. costatum* used in the study were obtained from the Center for Collection of Marine Algae at Xiamen University, and the remaining algae were isolated from Lianyungang, China. The *S. costatum* were cultured in f/2 medium, while the other algae were cultured in f/2-Si medium [31]. The algal cultures were incubated as described above. For the experiments, cells in the exponential growth phase were specifically chosen.

The *N. scintillans* were co-cultured with three prey species. For the co-culture experiments, three different nutrient concentration co-culture systems were prepared, which were f/2, f/8 (one-fourth the concentration of f/2 medium), and f/32 (one-sixteenth the concentration of f/2 medium) medium, respectively, and an equal volume of sterilized seawater was used as the control group. The culture vessels were 100 mL flasks with 50 mL of culture, and they were incubated as described above. The initial densities of *N. scintillans*, *H. steinii*, *H. akashiwo*, and *S. costatum* were 10, 1.0×10^4, 1.0×10^4, and 4.2×10^5 cells/mL, respectively. Three replicates were set up in each experimental group. The algal cells were counted every 2 days. Moreover, the carbon yield for *N. scintillans* and three prey species in the culture system was estimated based on cell carbon content and cell concentration. The carbon content values for *N. scintillans* (35.34 ng C $cell^{-1}$), *H. steinii* (0.15 ng C $cell^{-1}$), *H. akashiwo* (0.11 ng C $cell^{-1}$), and *S. costatum* (0.026 ng C $cell^{-1}$) were obtained from published literature [32–34].

The cell density of *N. scintillans* was counted using macroscopic observation due to the cells' considerable size range of approximately 200~2000 μm in diameter, making them visible to the unaided eye [35]. To obtain a representative sample, the algal culture was gently agitated, and 1 mL of the culture was collected for cell counting. Importantly, the cultures were obtained using a large-bore pipette tip. The algal cell density of the other cultures was assessed using a Sedgwick Rafter chamber under a microscope (Nikon E100, Nikon, Tokyo, Japan). The counting process was conducted in triplicate, with each count being performed three times to ensure accuracy.

2.3. Release of Nutrients from Noctiluca scintillans

Based on the above experiments, *N. scintillans* had the most food vacuoles when they were co-cultured with *H. steinii*. Therefore, the two were co-incubated for 48 h to ensure that *N. scintillans* completed feeding. The satiated *N. scintillans* were subsequently subjected to the aforementioned methods of washing and filtration to eliminate surplus prey, and they were then carefully transferred to sterilized seawater. The culture vessels were 100 mL flasks containing 50 mL of culture, and they were incubated according to the previously mentioned protocol. The initial density of *N. scintillans* was 50 cells/mL. The digestion of *N. scintillans* was judged by observing its content and the volume of food vacuoles every day. After complete digestion, the *N. scintillans* suspension was collected and

filtered with a 0.22 μm membrane to obtain the filtrates. In addition, the filtrated suspensions on day 0 of the experiment were collected as control. The filtered water was stored at −20 °C until required for nutrient analysis.

The quantification of dissolved inorganic phosphorus (DIP) was performed using the molybdenum blue method [36], while the concentrations of nitrate (NO_3-N) and nitrite (NO_2-N) were determined through the copper–cadmium reduction method [37] and the diazo–azo colorimetric method [38], respectively. Ammonium (NH_4-N) levels were measured using the sodium bromate oxidation method [39]. Soluble total nitrogen was assessed following persulfate oxidation [40], and the concentration of dissolved organic nitrogen was calculated by subtracting soluble inorganic nitrogen from soluble total nitrogen.

2.4. Response of Heterocapsa steinii to the Nutrients That Are Released by Noctiluca scintillans

The filtrates that were collected above were used for *H. steinii* growth culture to explore the utilization of the nutrients that were released by *N. scintillans*. Six culture conditions were set up for the experiment, which were: sterilized seawater, filtrate + f/2-Si-P, filtrate + f/2-Si-N, filtrate + f/2-Si-N-P, filtrate, and sterilized seawater + f/2-Si (the control group). Briefly, the stock culture of *H. steinii* (0.5 mL; cell concentrations: 1.0×10^5 cells/mL) was inoculated into fresh batch of their corresponding medium (4.5 mL). *H. steinii* were cultured in 6-well plates at a concentration of 1.0×10^4 cells/mL in a volume of 5 mL, and they were incubated as described above. Three replicates were set up in each experiment group, and the algal cells were counted using a Sedgwick Rafter chamber under a microscope on the 5th day.

2.5. Data Statistics and Analysis

The experiments were conducted in triplicate, and the data are reported as the mean ± standard deviation. The results were plotted using Origin 2023, and the error bars indicate the standard deviation (n = 3). The statistical analyses were carried out using SPSS 16.0 software, and the significant differences among the treatments in the study were assessed using a one-way analysis of variance ($p < 0.05$).

3. Results

3.1. Population Dynamics of Noctiluca scintillans and Typical Prey Species under Different Nutrient Conditions

To investigate the response of the population dynamics of *N. scintillans* and three typical prey species (*H. steinii*, *H. akashiwo*, and *S. costatum*) to changes in nutrients, the prey species were co-cultured with *N. scintillans* under different nutrient concentrations. As shown in Figures 1–3, an increase in the nutrient concentration led to a corresponding increase in the growth rate of the three prey species. On the sixth day of the experiment, the cell densities of *H. steinii*, *H. akashiwo*, and *S. costatum* in the treatment group with a nutrient concentration of f/2 exhibited a significant increase from the initial values of 1.0×10^4, 1.0×10^4, and 4.2×10^4 cells/mL to the final values of $(1.5 \pm 0.10) \times 10^5$, $(3.6 \pm 0.1) \times 10^5$, and $(5.4 \pm 0.1) \times 10^5$ cells/mL, respectively. Among the three experimental groups, *N. scintillans* had the highest growth rate when co-cultured with *H. steinii* (Figure 1A,C). The population density of *N. scintillans* in the f/2-Si treatment group increased to (109.0 ± 3.9) cells/mL on experimental day 6, which was significantly higher than that in the other culture systems (Figure 1). Furthermore, its population density increased with the increase in the population abundance of *H. steinii*, and abundant and well-filled food vacuoles were observed in the *N. scintillans* cells (Figure 4A). Similarly, in the co-culture treatment with *H. akashiwo*, the population density of *N. scintillans* exhibited a positive correlation with the population density of *H. akashiwo* (Figure 2A,C), and there were also abundant and well-filled food vacuoles (Figure 4C). Moreover, on the sixth day of the experiment, the population density of *N. scintillans* in the f/2-Si treatment group increased to (38.6 ± 3.2)

cells/mL (Figure 2A). In contrast, when co-cultured with *S. costatum*, *N. scintillans* had sluggish growth across all the treatment groups (Figure 3A,C). The population density of *N. scintillans* in the f/2 treatment group exhibited an initial increase followed by a subsequent decline, with the highest density observed on day 4 (Figure 3A). Furthermore, minimal presence of food vacuoles was noted in *N. scintillans* when co-cultured with *S. costatum* (Figure 4B).

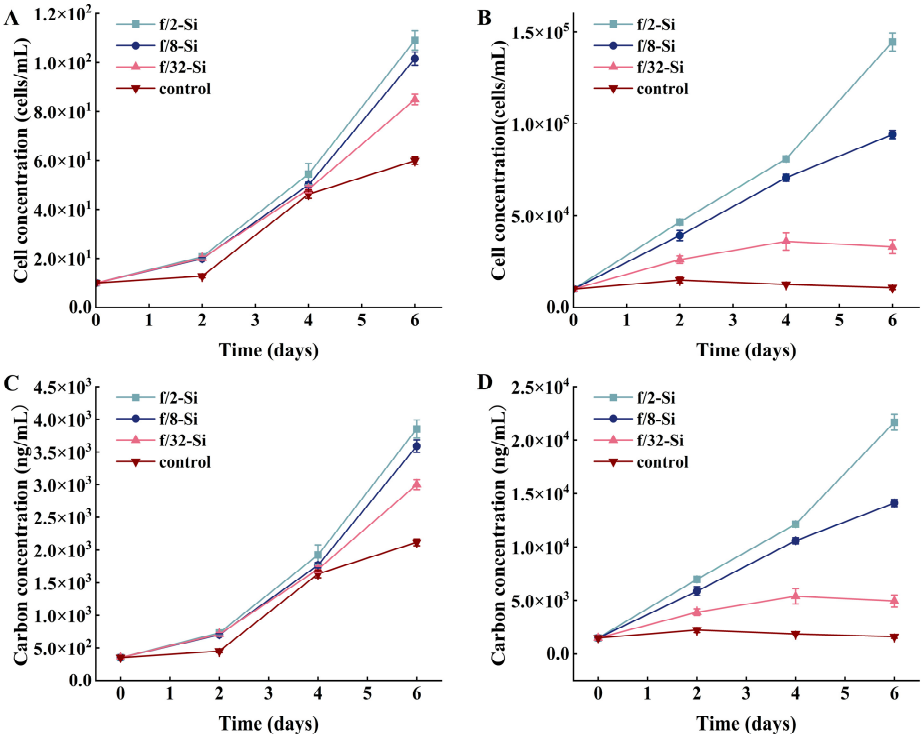

Figure 1. (**A**): Growth of *N. scintillans* when co-cultured with *H. steinii* under different nutrient conditions. (**B**): Growth of *H. steinii* when co-cultured with *N. scintillans* under different nutrient conditions. (**C**): The carbon yield of *N. scintillans* when co-cultured with *H. steinii* under different nutrient conditions. (**D**): The carbon yield of *H. steinii* when co-cultured with *N. scintillans* under different nutrient conditions. f/2-Si: f/2 medium without silicate; f/8-Si: one-fourth the concentration of f/2 medium (without silicate); f/32-Si: one-sixteenth the concentration of f/2 medium (without silicate). Control: sterilized seawater without f/2 medium.

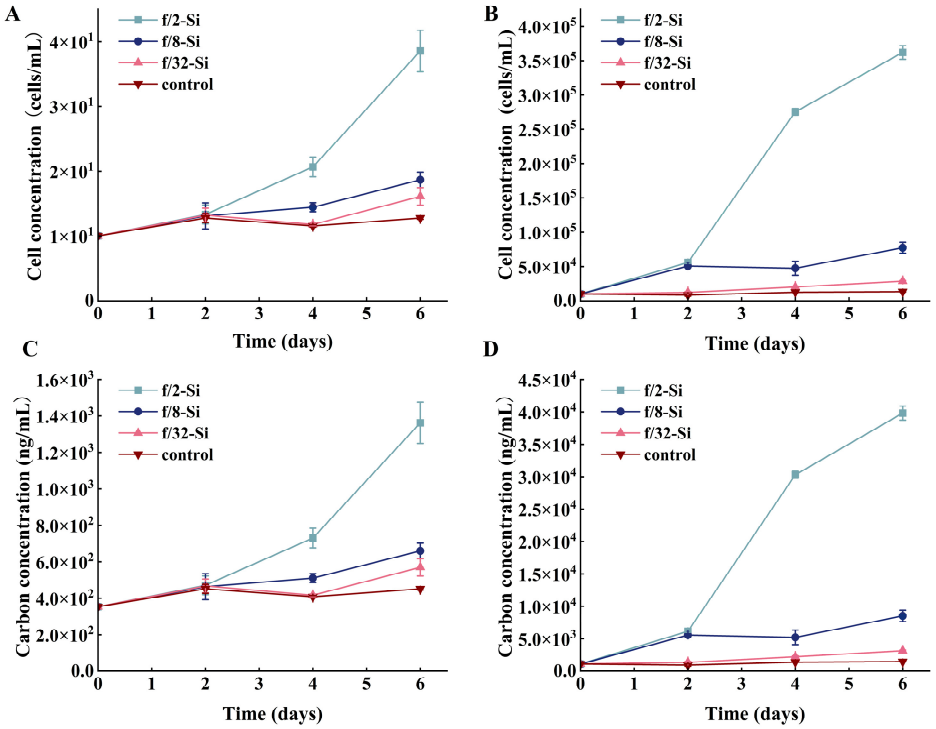

Figure 2. (**A**): Growth of *N. scintillans* when co-cultured with *H. akashiwo* under different nutrient conditions. (**B**): Growth of *H. akashiwo* when co-cultured with *N. scintillans* under different nutrient conditions. (**C**): The carbon yield of *N. scintillans* when co-cultured with *H. akashiwo* under different nutrient conditions. (**D**): The carbon yield of *H. akashiwo* when co-cultured with *N. scintillans* under different nutrient conditions. f/2-Si: f/2 medium without silicate; f/8-Si: one-fourth the concentration of f/2 medium (without silicate); f/32-Si: one-sixteenth the concentration of f/2 medium (without silicate). Control: sterilized seawater without f/2 medium.

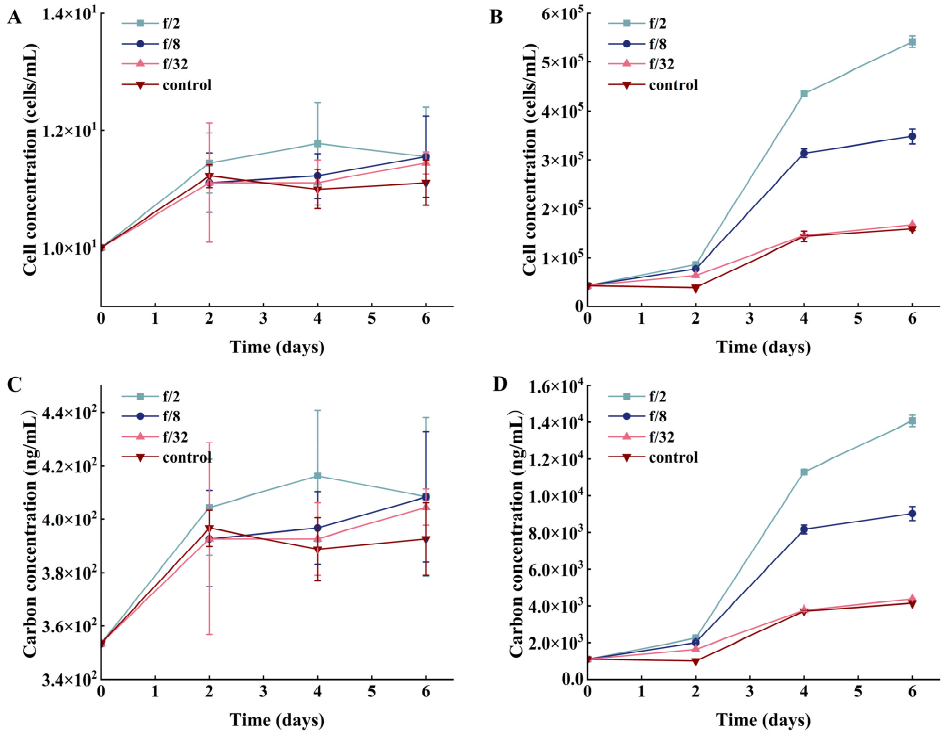

Figure 3. (**A**): Growth of *N. scintillans* when co-cultured with *S. costatum* under different nutrient conditions. (**B**): Growth of *S. costatum* when co-cultured with *N. scintillans* under different nutrient conditions. (**C**): The carbon yield of *N. scintillans* when co-cultured with *S. costatum* under different

nutrient conditions. (**D**): The carbon yield of *S. costatum* when co-cultured with *N. scintillans* under different nutrient conditions. f/2-Si: f/2 medium without silicate; f/8-Si: one-fourth the concentration of f/2 medium (without silicate); f/32-Si: one-sixteenth the concentration of f/2 medium (without silicate). Control: sterilized seawater without f/2 medium.

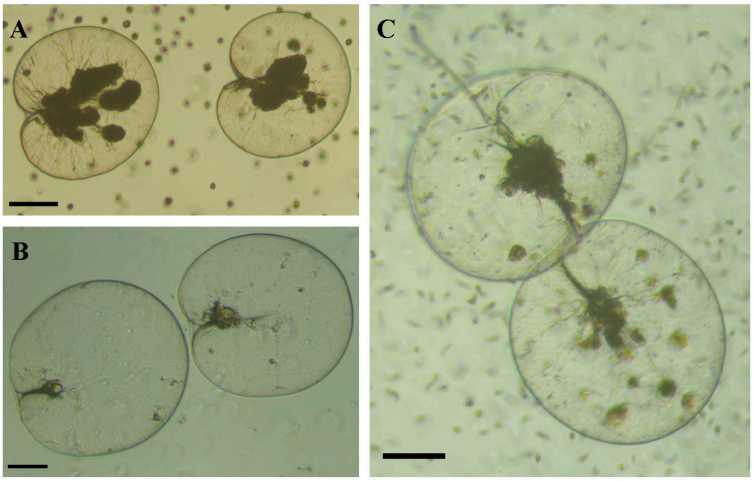

Figure 4. (**A**): Microscopic images of *N. scintillans* when co-incubated with *H. steinii*. (**B**): Microscope images of *N. scintillans* when co-incubated with *S. costatum*. (**C**): Microscope images of *N. scintillans* when co-incubated with *H. akashiwo*. The scales in Figures **A**–**C** are 100 μm.

3.2. Release of Nutrients from Noctiluca scintillans

Digestion was confirmed by microscopic observation, which revealed an obvious decline in both the quantity and volume of food vacuoles in *N. scintillans* on experimental day 5 (Figure 5). Consequently, the *N. scintillans* filtrate was collected on day 5 for the subsequent analysis of its nutrient composition and concentration. The findings indicated that *N. scintillans* released 57.36 μM of total soluble nitrogen within 5 d, and the dissolved organic nitrogen was at the highest proportion at 54.14 μM, which was followed by the nitrate nitrogen (NO_3-N) at 3 μM and the ammonia nitrogen (NH_4-N) (0.21 μM). Dissolved inorganic phosphate was not detected in this study (Table 1); however, it is possible that it may be present in some form of dissolved organic phosphate.

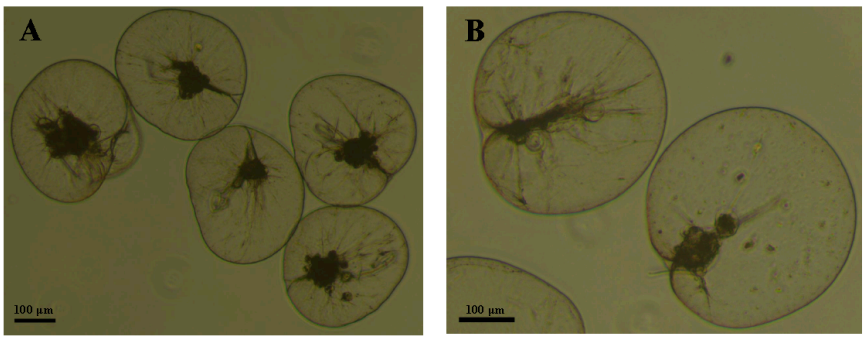

Figure 5. (**A**): *N. scintillans* in a satiated state; (**B**): *N. scintillans* digestion for 5 d. The scale in Figures **A** and **B** are 100 μm.

Table 1. Release of nutrients from *N. scintillans* after 5 d of prey digestion.

Nutrients	Control (μM)	Filtered Water (μM)	Release of Nutrients (μM)
Ammonia Nitrogen (NH_4-N)	0.93	1.14	0.21
Nitrate Nitrogen (NO_3-N)	7.86	10.86	3.00
Nitrite Nitrogen (NO_2-N)	0.00	0.00	0.00
Dissolved organic Nitrogen	8.29	62.43	54.14

Total Soluble Nitrogen	17.07	74.43	57.36
Dissolved inorganic phosphate (DIP)	0.00	0.00	0.00

3.3. Heterocapsa steinii Used the Nutrients That Were Regenerated by Noctiluca scintillans

The use of the nutrients in the *N. scintillans* filtrate by *H. steinii* is shown in Figure 6. The growth of *H. steinii* varied under different culture conditions. On the fifth day of the culture period, the *H. steinii* in the filtrate supplemented with f/2-Si-N demonstrated superior growth, resulting in a cell density increase to (8.3 ± 0.7) × 10^4 cells/mL, which was not significantly different from the control group (sterilized seawater + f/2-Si). However, the cell densities of *H. steinii* when it was cultured in the filtrate, filtrate supplemented with f/2-Si-P, filtrate supplemented with f/2-P-N, and natural seawater were (4.1 ± 0.3) × 10^4, (4.1 ± 0.3) × 10^4, (3.8 ± 0.1) × 10^4, and (3.6 ± 0.7) × 10^4 cells/mL, respectively. These values were significantly lower than those that were observed in the control group and the filtrate supplemented with f/2-Si-N treatment group. These results indicated that *H. steinii* was capable of absorbing and using the nitrogenous nutrients in the filtrate of *N. scintillans* for its growth and reproduction.

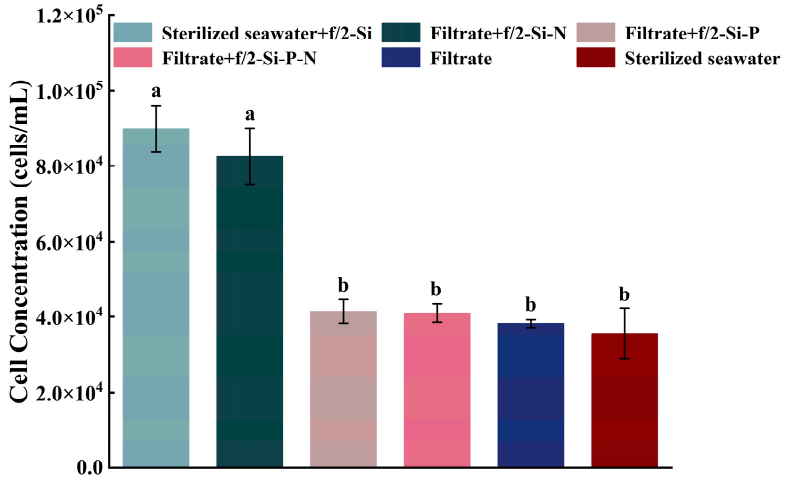

Figure 6. The cell density of *H. steinii* on the fifth day under different culture conditions. The error bars represent the standard deviation (n = 3). Different lowercase letters represent significant differences between the groups ($p < 0.05$).

4. Discussion

Red *N. scintillans* is a typically heterotrophic organism, and numerous studies have indicated that the primary factor that contributes to the frequent occurrence of *N. scintillans* red tide is the proliferation of microalgae resulting from eutrophication, which provides ample food for *N. scintillans* [41–43]. The growth and population dynamics of *N. scintillans* are significantly influenced by the species and dynamics of the plankton and the fluctuations in nutrient availability. For instance, Hallegraeff et al. [44] found that there was a significant difference in the average growth rate of *N. scintillans* when feeding on different algae. Additionally, Zhang et al. [45] found that the growth rate of *N. scintillans* was likely to increase in tandem with an increased prey density, but certain marginal effects are also present, such as a decline in population abundance and mortality when exposed to high-density *Platymonas subcordiformis* (Wille) Hazen.

The findings of this study indicated that *N. scintillans* had a sluggish growth rate within a co-culture system with *S. costatum*, and there was a minimal presence of feeding vacuoles in the cells (Figures 3A and 4B). This phenomenon can be attributed to the sedimentation of *S. costatum* under experimental conditions, which diminishes the likelihood of interacting with *N. scintillans* and subsequently reduces its feeding rate. In addition, the population density of *N. scintillans* exhibited a gradual decline in the f/2 treatment group

(Figure 3A), suggesting that the high density and rapid growth rate of *S. costatum* may have impeded the growth of *N. scintillans* through allelopathy [10,46]. In accordance with previous research by Garrido et al. [10], it was determined that the interactions between *N. scintillans* and its toxic dinoflagellate prey, *Dinophysis acuminate* and *Alexandrium minutum*, were predominantly influenced by negative allelopathic effects. This finding likely contributes to a plausible explanation for the succession of red tides caused by *S. costatum* and *N. scintillans*. In situ monitoring has shown that the occurrence of *N. scintillans* red tide typically follows that of the diatom blooms [8,21,47]. It is possible that the rapid proliferation of diatoms during the initial phase of the blooms hinders the growth of *N. scintillans*. However, as the diatom proliferation progresses into its later stage, the growth of *N. scintillans* is no longer inhibited. Concurrently, the heterotrophic attributes of *N. scintillans* expedite the termination of the diatom blooms.

In contrast to *S. costatum*, the high density and rapid growth rate of *H. steinii* and *H. akashiwo* did not hinder the growth of *N. scintillans*. The population abundance of *N. scintillans* increased as the population density and nutrient concentration of the two prey species increased, which was accompanied by the presence of abundant and fully formed food vacuoles within the cells. This suggested that *N. scintillans* can effectively feed on *H. steinii* and *H. akashiwo* and use their nutrients for growth and reproduction, which could potentially lead to an outbreak of bilateral red tides involving *N. scintillans*, *H. steinii*, and *H. akashiwo*. For instance, an in situ survey in Dinghai Bay, Fujian, revealed the occurrence of a bilateral red tide consisting of *N. scintillans* and *Prorocentrum donghaiense* Lu [48]. Similarly, in Port Shelter, Hong Kong, a bilateral red tide composed of *N. scintillans* and *Mesodinium rubrum* (Lohmann) was observed [49].

Like metazoan zooplankton species, *N. scintillans* plays a dual role, as it not only consumes microalgae for growth and reproduction but also releases inorganic and organic nutrients that facilitate the proliferation of other algae in its surroundings [6,23,25]. Under normal growth conditions, the metabolites that are produced by *N. scintillans* can serve as a significant nitrogen and phosphorus source, supplying approximately one-third and one-half, respectively, of the required nutrients for the growth of microalgae in the ecosystem [23]. However, during *N. scintillans* red tide, the nitrogen and phosphorus emissions from *N. scintillans* can potentially meet or even surpass the demands of the phytoplankton [23]. For example, Luo et al. [29] conducted an in situ investigation at Seeb Jetty in the Oman Sea, which revealed a significant positive correlation ($p < 0.001$) between the nutrient concentration in the water and the abundance of green *N. scintillans*, suggesting that the feeding process served as the primary mechanism for *N. scintillans* to accumulate substantial quantities of nutrients. The findings of this study indicated that *N. scintillans*, when in a fed state, released nitrogen into the water, while no release of phosphorus was observed (Table 1). This finding is supported by Zhang et al. [50], who proposed that actively feeding *N. scintillans* tend to retain phosphorus and selectively release nitrogen. Additionally, Mohanty et al. [51] observed a similar rise in nitrate levels during the *N. scintillans* bloom in the Bay of Bengal. This likely represents a stoichiometric strategy to maintain internal C:N:P ratios [50,52]. Evidently, the nutrient-release behavior of *N. scintillans*, including various types of nutrients, appears to be influenced by both the nutritional composition of its prey and its physiological state [8,50]. It is recognized that stoichiometric imbalances between predators and prey impact nutrition and ecosystems [53,54]. Moreover, the confinement of a single prey in laboratory settings might restrict the nutrient release by *N. scintillans*. In natural marine environmental conditions, *N. scintillans* encounter a diverse range of prey sources, which potentially results in a more extensive variety of released nutrients. Once released, these nutrients can be assimilated and used by other microalgae (Figure 6). Therefore, it is plausible that *N. scintillans* could potentially contribute to the proliferation of red tides formed by other organisms.

Biotic interactions, whether advantageous or detrimental, play a pivotal role in the initiation, persistence, and severity of HABs. These interactions are often undervalued due to the difficulties that are associated with their detection [10]. HABs encompass a diverse

array of organisms, each with distinct population dynamics and diverse patterns. Prior research, as well as our findings, have provided initial evidence of a reciprocal symbiotic relationship between *N. scintillans* and certain microalgae, suggesting the possibility of a beneficial feedback loop (Figure 7). The release of terrestrial nutrients or the upwelling of nutrient-rich deep-sea waters triggers eutrophication, which results in a substantial proliferation of microalgae within the surrounding ecosystem. Under suitable environmental conditions (e.g., temperature and salinity), an abundance of prey triggers a rapid proliferation of *N. scintillans*. During feeding, *N. scintillans* releases a substantial quantity of nutrients into the surrounding water, which are subsequently absorbed and used by other microalgae. These regenerated nutrient sources facilitate the repopulation of microalgae in the ecosystem, establishing a transient closed loop that fosters the persistence of outbreaks and the continued expansion of *N. scintillans* [29]. It also provides a theoretical basis for the prediction and formation mechanism of *N. scintillans* red tide. Notably, the interplay between the regeneration of the limiting nutrient and nutrient consumption by microalgae plays a crucial role in the formation of blooms [55]. Additionally, the nutritional composition of prey, such as the ratio of carbon, nitrogen, and phosphorus, may impact the positive feedback loop between *N. scintillans* and prey, warranting further study [53].

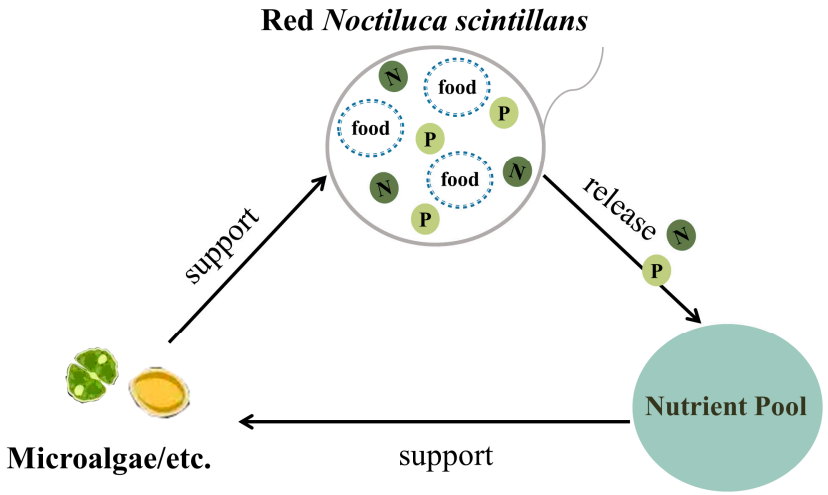

Figure 7. Schematic of the interaction between red *N. scintillans* and *H. steinii*.

In conclusion, different plankton have different effects on *N. scintillans*, influencing population dynamics or nutrient regeneration. Therefore, when investigating the prediction and occurrence mechanisms of *N. scintillans* red tide, it is crucial to comprehensively consider the presence of different plankton species and environmental factors. Future studies on the interaction between *N. scintillans* and microalgae are needed. Additionally, in the examination of the feeding habits of *N. scintillans*, particularly those individuals that secrete toxins, it is imperative to consider the potential transmission or alteration of these toxins throughout the feeding cycle. It is also necessary to explore the effects of various environmental conditions, organisms, and *N. scintillans* on nutrient regeneration under the conditions of satiety, starvation, or rupture to reveal the "switch" and influencing factors of nutrient release.

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