1 **ORIGINAL PAPER**

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3 **ACryptic Marine Ciliate Feeds on Progametes of *Noctiluca* spp.**

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The dinoflagellate *Noctiluca scintillans* (*Noctiluca*) has the ability to reproduce sexually, which may help to increase or restore its population size during periods of blooms or environmental stress. Here, we documented for the first time a marine ciliate, *Strombidium* sp. that feeds on *Noctiluca*'s progametes undergoing stages 5 to 9 of nuclear division. This ciliate frequently swam on or around gametogenic and some vegetative *Noctiluca* cells. The ciliates associated with gametogenic cells had significantly lower swimming speed and changed direction more frequently than those associated with vegetative cells, which overall increased their time spent around the food patches (progametes). This trophic interaction constitutes an upside-down predator-prey link, in which ciliates within the typical size range of *Noctiluca* prey, become the predators. Based on the phylogenetic tree (maximum-likelihood), there are 14 environmental clones similar to *Strombidium* sp. found in other coastal waters, where *Noctiluca* presence or blooms have been reported. This novel predator-prey relationship could therefore be common in other *Noctiluca* habitats. An additional study is needed to assess the magnitude of its impacts on *Noctiluca* population dynamics and plankton bloom succession.

Keywords: *Strombidium* sp.; feeding behavior; swimming pattern; *Noctiluca*; progametes.

Introduction

Noctiluca scintillans (hereafter *Noctiluca*) is a large dinoflagellate that can cause enormous blooms in many coastal regions (Elbrächter and Qi 1998; Harrison et al. 2011). The red *Noctiluca* studied here lacks the symbiotic prasinophyte *Protoeuglenanoctilucae* and is an obligate heterotroph with high dietary flexibility; its prey include bacteria, detritus, phytoplankton, protozoa, copepods and eggs of copepods and fish (Kirchner et al. 1996; Quevedo et al. 1999; Umani et al. 2004). To date, most field and laboratory studies took a bottom-up approach and focused on the role of the availability and quality of food items in shaping *Noctiluca* abundance (Kiørboe and Titelman 1998; Nakamura 1998a,b; Zhang et al. 2015). A few other studies, however, have explored potential top-down regulation of *Noctiluca* populations, i.e., predation by copepods, fish larvae and hydromedusae (Daan 1989; Fock and Greve 2002; Lehto et al. 1998). Still, the role of *Noctiluca*'s reproductive strategies, especially sexual reproduction, on controlling its population dynamics is less studied.

Noctiluca is diploid in the vegetative stage, and it can reproduce asexually by binary fission and sexually by gametogenesis (Fukuda and Endoh 2006; Ishikawa 1894; Zingmark 1970). Vegetative cells are programmed to become gametogenic after a defined number of binary fissions (Sato et al. 1998), or under unfavorable food condition (Dewey 1976, Sriwong et al. 2008). Fukuda and Endoh (2006) showed that some vegetative cells could transform into gametogenic cells by undergoing two successive nuclear divisions without cell division. These nuclei, each accompanied by a small amount of cytoplasm, protrude through the cell surface membrane and further divide up to 10 times, forming up to 1024 "progametes" over 24 hours. When matured, the progametes are released as zoospores which later fuse to form a trophont (Fukuda and Endoh 2006; Zingmark 1970). This distinctive reproductive strategy significantly enhances the reproductive capability of individual *Noctiluca* cells, and in turn, contributes towards the dramatic outbursts of *Noctiluca* in the field (Miyaguchi et al. 2006, 2008; Sato et al. 1998).

Despite the potential significance of sexual reproduction in *Noctiluca* bloom formation and succession (Miyaguchi et al. 2006, 2008; Sato et al. 1998), zoospore

formation has not been definitively linked to its population variations (Miyaguchi et al. 2008; Nakamura 1998a; Uhlig et al. 1995). This lack of information is, in part, due to the rarity of gametogenic cells in field samples (e.g., <5% of total population in Seto Island Sea, Japan; Nakamura, 1998a) and the difficulty of distinguishing zoospores from other dinoflagellates (Elbrächter and Qi 1998; Fukuda and Endoh 2006; Miyaguchi et al. 2008). Therefore, the contribution and importance of sexual reproduction for formation of *Noctiluca* blooms remain unclear.

Ciliates often play important ecological roles in the microzooplankton communities of coastal waters as major consumers of picoplankton and nanoplankton (Bernard and Rassoulzadegan 1990; Stoecker and Evans 1985) and as large and nutritious food particles for mesozooplankton (Löder et al. 2011; Nielsen and Kiørboe 1994). Consequently, they represent a significant link in energy transfer from microbial components in the food web to higher trophic levels (Azam et al. 1983; Calbet and Saiz 2005; Sherr et al. 1986). In the present study, we found that a cryptic marine ciliate *Strombidium* sp. was able to feed on the progametes of *Noctiluca* that had undergone 5 to 9 nuclear divisions (i.e., corresponding to 64 to 1,024-progamete cells). However, *Noctiluca* was not observed to feed on this ciliate. This novel trophic interaction constitutes an unusual upside-down trophic link in marine food web that may reduce the effectiveness of sexual reproduction as a survival or bloom strategy for *Noctiluca*. If our observations are representative of other systems, this novel interaction could potentially account for the scarcity of gametogenic cells in the field and have implications for food web structure and *Noctiluca* bloom succession.

Results

Morphological Characteristics and Occurrence of *Strombidium* sp.

The ciliate repeatedly observed on the surfaces of gametogenic cells and some vegetative cells of *Noctiluca* (Fig. 1A, Supplementary Material Video S1) had cell dimensions of 20–37 μm in length and 18–34 μm in width. Its general morphology was similar to that of other *Strombidium* species (Agatha 2004): an open adoral zone of membranelles with a gap on the ventral, endoral membranelles on the inner wall of

buccal lip and somatic ciliature reduced to kinety (Fig. 1B, C). Based on both morphological and molecular analysis, this ciliate was a new species belonging to the genus *Strombidium* (Shen et al. unpubl. observ.). It is distinguished from other *Strombidium* species by its deep and prominent buccal cavity (10–12 μm wide; 10–15 μm long) and a short but strong adoral membranelles that terminate about half way down the cell and are used as walking or crawling appendages (Fig. 1C). In addition, this *Strombidium* sp. showed no auto-fluorescence under blue, green or UV light.

During our sampling period, the seawater temperature ranged from 17 to 24 °C and salinity was 31.6 to 32.6 psu (Fig. 2). Oligotrichia, including *Pelagostrobilidium*, *Pseudotontonia*, *Cyrtostrombidium*, tintinnid and other *Strombidium* species, accounted for the majority of the ciliates observed on all sampling days, averaging 65% of total ciliate abundance. *Myrionecta* sp. in the subclass Haptoria were also abundant in the samples, contributing 35% on average. The abundance of our target ciliate (i.e., *Strombidium* sp.) varied dramatically throughout the sampling period, ranging from 50 to 25,700 cells L^{-1} (Fig. 3A). The high abundance co-occurred with *Noctiluca* blooms on 30 November, 15 December and 24 December 2014 (Fig. 3A), accounting for 26%, 40% and 19% of Oligotrichia, respectively, on those sample dates (Fig. 3B).

Comparison of the 18S rRNA gene sequences of *Strombidium* sp. in the present study with environmental clones from the NCBI nucleotide database (Johnson et al. 2008) revealed high similarity ($\geq 96\%$ similarity in nucleotide) between *Strombidium* sp. and 14 environmental clones from different coastal waters (Fig. 4).

Feeding Behaviors and Swimming Patterns of *Strombidium* sp.

Strombidium sp. used its adoral zone of membranelles for swimming and crawling, with the ventral side facing down (Supplementary Material Videos S2 and S3). Video recordings clearly showed active feeding on *Noctiluca*’ progametes that were in the 5 to 9 nuclear division stages. The ciliates usually aggregated around clusters of progametes and rapidly scrabbled the clusters with their membranelles (Supplementary Material Videos S2 and S3). *Strombidium* sp. then grabbed the posterior ends of individual progametes firmly with its buccal region tightened and rotated clockwise or counterclockwise with its cilia beating vigorously. Feeding was accompanied by violent writhing and twisting of the *Strombidium* sp. cell (Supplementary Material Video S2). After progamete ingestion, *Strombidium* sp. remained around the clusters

and attacked other progametes in the vicinity. To accommodate larger and multiple prey, *Strombidium* sp. would also enlarge its body volume during feeding, retaining up to 10 progametes at a time (Supplementary Material Video S3).

Once all progametes of agametogenic *Noctiluca* cell were consumed, *Strombidium* sp. left the now cleared 'ghost cell' and swam to another *Noctiluca*, either vegetative or gametogenic (Supplementary Material Videos S2 and S3). The surfaces of the ghost cell remained intact after attack, but the cells were not observed to divide further. When vegetative *Noctiluca* cell cannibalized a ghost gametogenic cell that had just been cleared of progametes by *Strombidium* sp. (Supplementary Material Video S4), the cannibalistic *Noctiluca* kept clear of and did not consume the ciliates that remained crawling around on the ghost cell.

A rough estimation of the ingestion potential of *Strombidium* sp. on *Noctiluca* progametes showed that, *Strombidium* sp. ingested at a rate of 0.5 progametes ciliate⁻¹ h⁻¹ on a gametogenic cell at the 64 to 256-progamete stage, and 13 progametes ciliate⁻¹ h⁻¹ on an agametogenic cell at the >256-progamete stage (progametes of smaller size than those of earlier stages).

Analysis of the swimming behaviors of *Strombidium* sp. revealed that the ciliates associated with gametogenic cells swam in circular paths with lower gross and net speeds compared to those associated with vegetative cells, which swam in more linearly directed or helical paths that often traversed the entire *Noctiluca* cell (Figs 5, 6). *Strombidium* sp. that were swimming on gametogenic cells changed direction more frequently than those on vegetative cells, as indicated by the shorter time between turns (1.16±0.51 vs 2.01±0.7 s, Fig. 6C), which reflected their overall slower swimming and longer residence times in the vicinity of prey items.

Discussion

We report for the first time an undescribed marine ciliate, *Strombidium* sp. feeding on the progametes of *Noctiluca*. *Noctiluca* appeared unable to feed on or trap *Strombidium* sp. on the mucus clump attached to the tips of their tentacles. Ingestion of progametes by *Strombidium* sp. thus constitutes an unusual upside-down trophic linkage that

contrasts with the general pattern of energy transfer from smaller to successively larger organisms in pelagic food webs.

The size range of prey ingested by ciliates is determined by the oral diameter and cytostome (Jonsson 1986), and for free-swimming tintinnid ciliates, the maximum length of prey cells is ~50% of the ciliate's oral diameter (Dolan et al. 2013). In our observations, the buccal cavity of *Strombidium* sp. only ranged from 10 to 12 μ m in width and from 10 to 15 μ m in length, however, the ellipsoid progametes had cell dimensions of 11–23 μ m x 6–23 μ m. These dimensions represent 2–4 fold higher prey: predator size ratios than expected for suspension-feeding ciliates (Hansen et al. 1994). While surface-associated *S. oculatum* and *S. stylifer*, which also feed on gametes of ulvaceous green macroalgae, are suspension feeders and only consume the gametes after they are shed from the thallus (McManus et al. 2004). The effective handling and engulfing of relatively large food items by this *Strombidium* sp. may be novel and was facilitated by feeding on the solid surfaces of the *Noctiluca* cell.

Unlike many planktonic ciliates, which display red fluorescence under an epifluorescence microscope due to feeding upon or incorporating the functional plastids of algal prey (Stoecker et al. 1987, 1989), the *Strombidium* sp. studied did not contain any pigment, suggesting a heterotrophic life style. In addition, we observed that *Strombidium* sp. disappeared within 1–2 days in natural seawater without *Noctiluca*, even when the water was enriched with rice grains to stimulate heterotrophic bacteria (Fenchel 1968), or with added monoalgal strains of *Rhodomonas* sp. or *Dunaliella* sp. (Jakobsen and Hansen 1997; Kirby 1950; Park et al. 2007; Verity 1988). On the contrary, *Strombidium* sp. remained abundant in the isolations where *Noctiluca* was the only food present, until the *Noctiluca* cells died out due to starvation a week later, suggesting feeding preference. Additional field observations are needed to study the dietary breadth of this ciliate.

Strombidium sp. associated with vegetative and gametogenic cells swam differently and the more circular paths around food path highlighted chemotaxis. Many planktonic ciliates are known for their chemosensory abilities, which allow them to exploit concentrated prey patches by active chemotaxis (Fenchel and Blackburn 1999; Montagnes and Lessard 1999; Verity 1988). For example, Fenchel and Blackburn (1999) found that the bacterivorous ciliates were able to aggregate within minutes around point sources of food placed at distances of a few centimeters.

Verity (1988) also showed that the ciliates *Tintinnopsis dadayi* and *Strombidium* sp. were able to locate and identify their preferred prey.

The ciliate *Strombidium* sp. was also found swimming around or travelling on some of the vegetative cells (Figs 1, 5). However, the localization of ciliates on gametogenic cells and only certain vegetative cells, while absent on most of the neighboring cells suggests that the aggregations were not incidental or transient (Supplementary Material Video S1). Fukuda and Endoh (2006) have shown that the motile zoospores of *Noctiluca* often creep over the surfaces of vegetative cells. It is thus possible that the ciliates on vegetative cells were searching for zoospores (Fukuda and Endoh 2006). Alternatively, *Strombidium* sp. might be aggregating on certain vegetative cells waiting for gametes to emerge, we observed that the ciliates concentrate on gametogenic cells in the four nuclei stage, a very early stage of gametogenesis (Supplementary Material Video S5). We hypothesize that the ciliates could be attracted by chemical stimuli released from gametogenic or vegetative cells preparing to undergo gametogenesis.

A very rough estimate of the ingestion potential of *Strombidium* sp. on *Noctiluca* progametes was made based on two cells and should be interpreted with caution. Sample size was limited because only a small part of the *Noctiluca* population (<1%) was gametogenic, not all gametogenic cells could be clearly observed for loss of progametes, and the transient nature of the gametogenesis (~2 hours; Fukuda and Endoh 2006). If these crude estimates are representative, given the mean ciliate biovolume of ~13,000 μm^3 (20 to 37 μm in length and 18 to 34 μm in width), the observed ingestion rates represent 45% of ciliate body volume h^{-1} for the smaller progametes, and 6% of ciliate body volume h^{-1} for the larger progametes. Assuming comparable nutritional density of the ciliates and progametes, and a typical ciliate gross growth efficiency of 30% (Calbet and Landry 2004; Straile 1997), such ingestion rates of the ciliates could support instantaneous growth rates of 1.8 d^{-1} . This would be comparable to typical maximum rates for suspension-feeding ciliates at high concentrations of phytoplankton (Hansen et al. 1997; Jonsson 1986). In addition, *Strombidium* sp. reached a maximum concentration of 25,700 cells L^{-1} when the abundance of *Noctiluca* was 26,100 cells L^{-1} during the field study (Fig. 3A). If these limited laboratory based ingestion estimates can be extrapolated, the seeding

stock of *Strombidium* sp. could potentially remove all progametes in as short as 4 hours, assuming 5% of the existing *Noctiluca* cells were at the 1,024-progamete stage.

Our intriguing and novel observation of this upside-down trophic link could have further implications for community dynamics. Progametes contain oil droplets and are likely to be nutritious food items for grazers (Fukuda and Endoh 2006). Once consumed and converted to ciliate biomass by *Strombidium* sp., this may benefit other pelagic consumers, e.g. copepods or fish larvae (Broglia et al. 2003; Stoecker and Capuzzo 1990), which are otherwise deprived of adequate planktonic prey in *Noctiluca* blooms but unable to feed directly on large *Noctiluca* cells (usually >200 µm) (Elbrächter and Qi 1998; Harrison et al. 2011; Huang and Qi 1997; Schaumann et al. 1988).

Most of the places that have environmental clones similar to *Strombidium* sp. are also locations where *Noctiluca* presence or blooms have been reported (Fig. 4; Edvardsen et al. 2003; Harrison et al. 2011; Okolodkov and Dodge 1996). The trophic interaction we observed between *Strombidium* sp. and *Noctiluca* could be occurring elsewhere, providing a possible explanation for the scarcity of gametogenic cells in field populations (Nakamura 1998a; Uhlig and Sahling 1990; Uhlig et al. 1995). Many planktonic dinoflagellates undergo sexual reproduction during their life cycle or under environmental stress (Brawley and Johnson 1992; Persson et al. 2008; Pfiester 1987), if gamete consumption by ciliates is common in the marine food web, the effectiveness of sexual reproduction as an alternate reproduction and/or survival strategy of *Noctiluca* or other dinoflagellates may need to be re-assessed.

Conclusions

We documented a novel predator-prey interaction in which a marine ciliate, *Strombidium* sp., in the prey size range of *Noctiluca* feeds voraciously instead on *Noctiluca* progametes. We suspect that the ciliate that swims on or around gametogenic and certain vegetative *Noctiluca* cells is attracted by chemical stimuli associated with gametogenesis. This newly discovered ciliate species and predatory behavior illustrates that there is still much to learn about microbial trophic interactions in marine food webs.

Methods

Water sampling and processing: We first observed a marine ciliate swimming on or around *Noctiluca* cells in a water sample collected at the pier of Port Shelter, Hong Kong (22° 20.453' N, 114° 17.703' E) on 30 November 2014. Samplings were subsequently conducted on 10 occasions during the *Noctiluca* blooms in December 2014. Water temperature and salinity were measured in situ with a multi-probe sensor YSI 556 Multiprobe.

Subsamples for enumeration of *Noctiluca* and ciliate abundances were preserved in acidic Lugol's solution (final concentration 2%). To determine *Noctiluca* abundances, water subsamples of 5–50 mL were settled in Petri dishes and counted at $\times 10$ magnification under a dissecting microscope (Leica MZ6). If the abundance was low, 2 L seawater samples were concentrated with a 102- μ m mesh before counting. For determining ciliate abundances, subsamples were settled in a sedimentation chamber and counted at $\times 200$ and/or $\times 400$ magnification (Olympus BX51 and Olympus IX 51 microscope). We counted at least 50 individuals of the target ciliate (the ciliate associated with *Noctiluca*), or for samples with low overall ciliate counts, the target species was identified in the first two hundred ciliates enumerated. Duplicate counts were performed for each sample.

Morphology and phylogeny of the ciliate associated with *Noctiluca*: Live ciliates associated with *Noctiluca* were observed under an inverted microscope (Olympus IX51) and checked with blue, green and UV light for auto-fluorescence. Infraciliature of this ciliate was revealed by protargol impregnation as described by Foissner (2014) and observed under an upright microscope (Nikon 80i).

In order to estimate the global distribution of the target ciliate, its 18S ribosomal RNA (18S rRNA) sequence (**GenBank accession no. KT730064**) was used to search the nucleotide sequences database on NCBI via nucleotide BLAST (BLASTn) webpage (Johnson et al. 2008). Environmental clone sequences with ≥ 96 % similarity with the 18S rRNA sequence of our target ciliate were selected for analysis. The 18S rRNA sequence of our target ciliate, the selected environmental clones, 4 other species of the *Strombidium* genus and a out group *Strombidinopsis acuminata* were

then aligned and used to construct a Maximum-likelihood tree with MEGA 6.0 (Tamura et al. 2013).

Feeding behavior and swimming patterns of the ciliate associated with *Noctiluca*

Ciliates associated with *Noctiluca* cells were photographed in bright field under an inverted microscope (Olympus CK30), and the sizes of individual ciliates and *Noctiluca* were measured with an ocular micrometer. Videos clips were captured at 30 frames sec⁻¹ (fps) on the day of collection with an Olympus Tough TG-3 video camera. Videos were divided into 10-sec clips with iMove (Apple Inc.) and uncompressed to AVI format with the open source software ffmpeg. These clips were processed with the customized software Avidemux 2.4 to remove background noise, set thresholds for size and brightness, and collect pixel coordinates of individual ciliates in each frame (Chan and Grünbaum 2010). Cell dimensions of each *Noctiluca* observed were used to calibrate the video clip in which it was recorded, converting the pixel coordinates into physical coordinates. These coordinates collected were then used to reconstruct trajectories of individual ciliates with the Matlab program Tracker3D (Chan and Grünbaum 2010; Chan et al. 2015). Only paths that were followed for over 30 frames were included in statistical analyses. A total of 298 ciliate paths were tracked in association with 6 different sets of gametogenic *Noctiluca* cells, and a total of 156 ciliate paths were tracked in association with 4 different sets of vegetative *Noctiluca* cells.

By applying smoothing splines on the reconstructed trajectories to remove frame rate noise, the average gross speed of each path was computed as the first derivative with respect to time of the smoothing spline. Net speeds for each path were computed by dividing the distance between the start and end points by the path duration. Turning points of each path were also identified by resolving smoothed curves into line segments with the Ramer-Douglas-Peucker algorithm, and the time between turn (sec) was computed as the path duration divided by the number of turns. Students' *t* tests (2-tailed) were used to compare swimming speeds and turning rates of ciliates associated with gametogenic and vegetative cells of *Noctiluca*.

Estimation of ingestion rates on *Noctiluca*'s progametes

A very preliminary estimate of the ciliate's ingestion rate could be made from the digitized time-series images taken of two gametogenic cells. These rates were

determined by measuring the disappearance of progametes relative to the number of ciliates present on gametogenic cell surfaces (Supplementary Fig. S1). At the beginning of the recordings, one gametogenic *Noctiluca* cell (627 μm) contained ~211 progametes ($\text{ESD} = 9.4 \pm 1.0 \mu\text{m}$, $n = 20$), the other gametogenic cell (685 μm) contained ~34 progametes ($\text{ESD} = 14.5 \pm 1.5 \mu\text{m}$, $n = 10$). From the size, shape and remaining number of progametes, the former gametogenic cell was likely in stage 8 or 9 of nuclear division, while the latter was in stage 5 to 7 based on the descriptions of life cycle of *Noctiluca* by Fukuda and Endoh (2006).

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References

- Agatha S**(2004). A cladistic approach for the classification of Oligotrichid ciliates (Ciliophora: Spirotricha). *ActaProtozool***43**: 201-217
- Azam F, Fenchel T, Field J, Gray J, Meyer-Reil L, Thingstad F** (1983) The ecological role of water-column microbes in the sea. *Mar Ecol Prog Ser***10**: 257-263
- Bernard C, Rassoulzadegan F** (1990) Bacteria or microflagellates as a major food source for marine ciliates: Possible implications for the microzooplankton. *Mar Ecol Prog Ser***64**: 147-155
- Brawley SH, Johnson LE** (1992) Gametogenesis, gametes and zygotes: an ecological perspective on sexual reproduction in the algae. *Br Phycol J* **27**: 233-252
- Broglia E, Jonasdottir S, Calbet A, Jakobsen HH, Saiz E** (2003)Effect of heterotrophic versus autotrophic food on feeding and reproduction of the calanoid copepod *Acartia tonsa*: relationship with prey fatty acid composition. *AquatMicrobEcol***31**: 267-278
- Calbet A, Landry MR** (2004) Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. *Limnol Oceanogr* **49**: 51-57
- Calbet A, Saiz E** (2005) The ciliate-copepod link in marine ecosystems. *AquatMicrobEcol***38**: 157-167
- Chan K, Grünbaum D** (2010) Temperature and diet modified swimming behaviors of larval sand dollar. *Mar Ecol Prog Ser***415**: 49-59
- Chan KYK, García E, Dupont S** (2015) Acidification reduced growth rate but not swimming speed of larval sea urchins. *Sci Rep* **5**: 9764
- Daan R** (1989) Factors controlling the summer development of copepod populations in the Southern Bight of the North Sea. *Neth J Sea Res* **23**: 305-322
- Dewey JM** (1976) Rates of feeding, respiration, and growth of the rotifer *Branchionus plicatilis* and the dinoflagellate *Noctiluca miliaris* in the laboratory. Ph. D. thesis, University of Washington
- Dolan JR, Landry MR, Ritchie ME** (2013) The species-rich assemblages of tintinnids (marine planktonic protists) are structured by mouth size. *The ISME J***7**: 1237-1243

385 **Edvardsen B, Shalchian-Tabrizi K, Jakobsen KS, Medlin LK, Dahl E, Brubak S,**
386 **Paasche E** (2003) Genetic variability and molecular phylogeny of Dinophysis
387 species (Dinophyceae) from Norwegian waters inferred from single cell analyses
388 of rDNA. J Phycol **39**: 395-408

389 **Elbrächter M, Qi Y** (1998) Aspect of *Noctiluca* (Dinophyceae) population
390 dynamics. In: Anderson MD (ed) Ecology of harmful algal blooms. Springer,
391 London, pp 315-335

392 **Fenchel T** (1968) The ecology of marine microbenthos II. The food of marine benthic
393 ciliates. Ophelia **5**: 73-121

394 **Fenchel T, Blackburn N** (1999) Motile chemosensory behaviour of phagotrophic
395 protists: mechanisms for and efficiency in congregating at food patches. Protist
396 **150**: 325-336

397 **Fock H, Greve W** (2002) Analysis and interpretation of recurrent spatio-temporal
398 patterns in zooplankton dynamics: a case study on *Noctiluca scintillans*
399 (Dinophyceae) in the German Bight (North Sea). Mar Biol **140**: 59-73

400 **Foissner W** (2014) An update of ‘basic light and scanning electron microscopic
401 methods for taxonomic studies of ciliated protozoa’. Int J Syst Evol Microbiol **64**:
402 271-292

403 **Fukuda Y, Endoh H** (2006) New details from the complete life cycle of the red-tide
404 dinoflagellate *Noctiluca scintillans* (Ehrenberg) McCartney. Eur J Protistol **42**:
405 209-219

406 **Hansen B, Bjørnsen PK, Hansen PJ** (1994) The size ratio between planktonic
407 predators and their prey. Limnol Oceanogr **39**: 395-403

408 **Hansen PJ, Bjørnsen PK, Hansen BW** (1997) Zooplankton grazing and growth:
409 Scaling within the 2-2,000 µm body size range. Limnol Oceanogr **42**: 687-704

410 **Harrison P, Furuya K, Glibert P, Xu J, Liu H, Yin K, Lee JHW, Anderson D,**
411 **Gowen R, Al-Azri A** (2011) Geographical distribution of red and green
412 *Noctiluca scintillans*. Chin J Oceanol Limnol **29**: 807-831

413 **Huang C, Qi Y** (1997) The abundance cycle and influence factors on red tide
414 phenomena of *Noctiluca scintillans* (Dinophyceae) in Dapeng Bay, the South
415 China Sea. J Plankton Res **19**: 303-318

416 **Ishikawa C** (1894) Studies of reproductive elements. 2. *Noctiluca miliaris*, Sur.; its
417 division and spore-formation. J Cell Sci Imp Univ Tokyo **6**: 297-344

418 **Jakobsen HH, Hansen PJ** (1997) Prey size selection, grazing and growth response
 419 of the small heterotrophic dinoflagellate *Gymnodinium* sp. and the ciliate
 420 *Balanion comatum*-a comparative study. Mar EcolProg Ser**158**: 75-86

421 **Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S, Madden TL**
 422 (2008) NCBI BLAST: a better web interface. Nucleic Acids Res **36**: W5-W9

423 **Jonsson PR** (1986) Particle size selection, feeding rates and growth dynamics of
 424 marine planktonic oligotrichous ciliates (Ciliophora: Oligotrichina). Mar Ecol
 425 Prog Ser **33**: 265-277

426 **Kjørboe T, Titelman J** (1998) Feeding, prey selection and prey encounter
 427 mechanisms in the heterotrophic dinoflagellate *Noctiluca scintillans*. J Plankton
 428 Res**20**: 1615-1636

429 **Kirby H** (1950) Materials and Methods in the Study of Protozoa. Univ of California
 430 Press, Berkeley

431 **Kirchner M, Sahling G, Uhlig G, Gunkel W, Klings KW** (1996) Does the red tide-
 432 forming dinoflagellate *Noctiluca scintillans* feed on bacteria? Sarsia **81**: 45-55

433 **Lehto J, Sulkin S, Strom S, Johnson D** (1998) Protists and detrital particles as prey
 434 for the first larval stage of the brachyuran crab, *Hemigrapsus oregonensis*. J Exp
 435 Mar BiolEcol**230**: 213-224

436 **Löder MGJ, Kraberg AC, Aberle N, Peters S, Wiltshire KH** (2011)
 437 Dinoflagellates and ciliates at Helgoland Roads, North Sea. Helgol Mar Res **66**:
 438 11-23

439 **McManus GB, Zhang H, Lin S** (2004) Marine planktonic ciliates that prey on
 440 macroalgae and enslave their chloroplasts. Limnol Oceanogr **49**: 308-313

441 **Miyaguchi H, Fujiki T, Kikuchi T, Kuwahara VS, Toda T** (2006) Relationship
 442 between the bloom of *Noctiluca scintillans* and environmental factors in the
 443 coastal waters of Sagami Bay, Japan. J Plankton Res **28**: 313-324

444 **Miyaguchi H, Kurosawa N, Toda T** (2008) Real-time polymerase chain reaction
 445 assays for rapid detection and quantification of *Noctiluca scintillans* zoospore.
 446 Mar Biotechnol **10**: 133-140

447 **Montagnes DJ, Lessard EJ** (1999) Population dynamics of the marine planktonic
 448 ciliate *Strombidinopsis multiauris*: its potential to control phytoplankton blooms.
 449 AquatMicrobEcol**20**: 167-181

450 **Nakamura Y** (1998a) Biomass, feeding and production of *Noctiluca scintillans* in the
 451 Seto Inland Sea, Japan. J Plankton Res **20**: 2213-2222

452 **Nakamura Y** (1998b) Growth and grazing of a large heterotrophic dinoflagellate,
453 *Noctiluca scintillans*, in laboratory cultures. J Plankton Res **20**: 1711-1720

454 **Nielsen TG, Kiørboe T** (1994) Regulation of zooplankton biomass and production in
455 a temperate, coastal ecosystem. 2. Ciliates. Limnol Oceanogr **39**: 508-519

456 **Okolodkov YB, Dodge JD** (1996) Biodiversity and biogeography of planktonic
457 dinoflagellates in the Arctic Ocean. J Exp Mar Biol Ecol **202**: 19-27

458 **Park JS, Myung G, Kim HS, Cho BC, Yih W** (2007) Growth responses of the
459 marine photosynthetic ciliate *Myrionecta rubra* to different cryptomonad strains.
460 Aquat Microb Ecol **48**: 83-90

461 **Persson A, Smith BC, Wikfors GH, Alix JH** (2008) Dinoflagellate gamete
462 formation and environmental cues: Observations, theory, and synthesis. Harmful
463 Algae **7**: 798-801

464 **Pfiester L** (1987) Dinoflagellate reproduction. In: Taylor FJR (ed) The biology of
465 dinoflagellates. Blackwell, Boston, pp 611-648

466 **Quevedo M, Gonzalez-Quiros R, Anadon R** (1999) Evidence of heavy predation by
467 *Noctiluca scintillans* on *Acartia clausi* (Copepoda) eggs off the central
468 Cantabrian coast (NW Spain). Oceanol Acta **22**: 127-131

469 **Sato MS, Suzuki M, Hayashi H** (1998) The density of a homogeneous population of
470 cells controls resetting of the program for swarmer formation in the unicellular
471 marine microorganism *Noctiluca scintillans*. Exp Cell Res **245**: 290-293

472 **Schaumann K, Gerdes D, Hesse K** (1988) Hydrographic and biological
473 characteristics of a *Noctiluca scintillans* red tide in the German Bight, 1984.
474 Meeresforschung-Rept **32**: 77-91

475 **Sherr E, Sherr BF, Fallon RD, Newell SY** (1986) Small, aloricate ciliates as a major
476 component of the marine heterotrophic nanoplankton. Limnol Oceanogr **31**: 177-
477 183

478 **Sriwoon R, Pholpunthin P, Lirdwitayaprasit T, Kishino M, Furuya K** (2008)
479 Population dynamics of green *Noctiluca Scintillans* (Dinophyceae) associated
480 with the monsoon cycle in the upper Gulf of Thailand. J Phycol **44**: 605-615

481 **Stoecker DK, Capuzzo JM** (1990) Predation on protozoa: its importance to
482 zooplankton. J Plankton Res **12**: 891-908

483 **Stoecker DK, Evans GT** (1985) Effects of protozoan herbivory and carnivory in a
484 microplankton food web. Mar Ecol Prog Ser **25**: 159-167

485 **Stoecker DK, Michaels AE, Davis LH** (1987) Large proportion of marine planktonic
486 ciliates found to contain functional chloroplasts. *Nature* **326**: 790-792

487 **Stoecker DK, Taniguchi A, Michaels AE** (1989) Abundance of autotrophic,
488 mixotrophic and heterotrophic planktonic ciliates in shelf and slope waters.
489 *MarEcol Prog Ser* **50**: 241-254

490 **Straile D** (1997) Gross growth efficiencies of protozoan and metazoan zooplankton
491 and their dependence on food concentration, predator-prey weight ratio, and
492 taxonomic group. *Limnol Oceanogr* **42**: 1375-1385

493 **Sulkin S, Lehto J, Strom S, Hutchinson D** (1998) Nutritional role of protists in the
494 diet of first stage larvae of the Dungeness crab *Cancer magister*. *Mar Ecol Prog*
495 *Ser***169**: 237-242

496 **Tamura K, Stecher G, Peterson D, Filipski A, Kumar S** (2013) MEGA6:
497 molecular evolutionary genetics analysis version 6.0. *Mol Bio Evol***30**: 2725-
498 2729

499 **Uhlig G, Sahling G** (1990) Long-term studies on *Noctiluca scintillans* in the German
500 Bight population dynamics and red tide phenomena 1968-1988. *Neth J Sea Res*
501 **25**: 101-112

502 **Uhlig G, Sahling G, Hanslik M** (1995) *Noctiluca scintillans*: zeitliche Verteilung bei
503 Helgoland und räumliche Verbreitung in der Deutschen Bucht (Langzeitreihen
504 1970-1993). *BerBiolAnstHelgoland***9**: 1-127

505 **Umani SF, Beran A, Parlato S, Virgilio D, Zollet T, De Olazabal A, Lazzarini B,**
506 **Cabrini M** (2004) *Noctiluca scintillans* MACARTNEY in the Northern Adriatic
507 Sea: long-term dynamics, relationships with temperature and eutrophication, and
508 role in the food web. *J Plankton Res* **26**: 545-561

509 **Verity PG** (1988) Chemosensory behavior in marine planktonic ciliates. *Bull Mar Sci*
510 **43**: 772-782

511 **Zhang S, Liu H, Chen B, Wu C-J** (2015) Effects of diet nutritional quality on the
512 growth and grazing of *Noctiluca scintillans*. *Mar Ecol Prog Ser***527**: 73-85

513 **Zingmark RG** (1970) 1970. Sexual reproduction in the dinoflagellate *Noctiluca*
514 *miliaris* Suriray 1. *J Phycol***6**: 122-126

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Figure and Video Legends

Fig. 1 (A) Frame grabbed micrographs of *Strombidium* sp. swarming on or around gametogenic (black arrow) and vegetative (white arrows) *Noctiluca* cells; (B) Micrographs of *Strombidium* sp. fixed in Lugol's solution; (C) Micrograph of ventral view of the oral area of *Strombidium* sp. after protargol impregnation under microscope. AM, anterior membranelles; VM, ventral membranelles. The longer bar indicates the cell size, the shorter bar indicates the length of oral area. Scale bars are in μm .

Fig. 2 Temperature ($^{\circ}\text{C}$) and salinity (psu) in Port Shelter from 30 November to 29 December 2014.

Fig. 3 (A) Abundances (cells L^{-1}) of *Noctiluca* and *Strombidium* sp.; (B) Percentage of *Strombidium* sp. in Oligotrichia (%) and the abundance of Oligotrichia (cells L^{-1}) in Port Shelter from 30 November to 29 December 2014 (averaged based on duplicate counts). Note the different y-axis scales.

Fig. 4 Phylogenetic tree (maximum-likelihood) showing the relationship of the 18S rRNA sequences of *Strombidium* sp. (black arrow) and the highly similar environmental clones recovered in different marine environments. Sequences of the environmental clones are labeled with their original places. 4 other species from the *Strombidium* genus are included for comparison. Bootstrap resampling was performed 1,000 times and the values higher than 50 % were displayed on the branches.

Fig. 5 (A) Frame grabbed micrographs of *Strombidium* sp. associated with gametogenic *Noctiluca* cell and examples of the swimming trajectories of 3 individual ciliates, cells inscribed by the blue circles are *Noctiluca*'s progametes; (B) Frame grabbed micrographs *Strombidium* sp. associated with vegetative *Noctiluca* and examples of the swimming trajectories of 3 individual ciliates. Units on scale bars and axes are μm .

Fig. 6 Net (A) and gross (B) swimming speed ($\mu\text{m s}^{-1}$), and the time between turns (sec; C) of *Strombidium* sp. associated with gametogenic and vegetative *Noctiluca* cells (mean $\pm$ s.d.). *** indicates statistically significant difference at $P < 0.001$ level (Student's *t* test, 2-tailed, $n = 298$).

546 Video 1. *Strombidium* sp. swimming on gametogenic (black arrow) and vegetative
547 (white arrows) *Noctiluca* cells.

548 Video 2. Snapshot of the feeding behavior of *Strombidium* sp. on
549 *Noctiluca*'s progamete. The arrow indicates *Strombidium* sp. feeding upon a progamete,
550 as indicated by the scrapping and spinning motion.

551 Video 3. Clearance of the progametes of a gametogenic *Noctiluca* cell by
552 *Strombidium* sp.. Duration of the whole process was 1h.

553 Video 4. A vegetative *Noctiluca* cell cannibalizing a ghost gametogenic cell that has
554 just been cleared of progametes by *Strombidium* sp..

555 Video 5. *Strombidium* sp. swimming on the gametogenic *Noctiluca* cell at four nuclei
556 division stage.

Figure

A

B

C

Figure

Figure

Figure

0.01

Figure
A

B

Figure