Oceanologia (2020) 62, 402—407

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.journals.elsevier.com/oceanologia

SHORT COMMUNICATION

Low-active high-density *Noctiluca scintillans* cells in surface seawater

Kuninao Tada[a,b,*], Toshimasa Asahi[a], Saho Kitatsuji[a,c], Mika Nomura[a], Hitomi Yamaguchi[a], Kazuhiko Ichimi[d]

[a] *Faculty of Agriculture, Kagawa University, Ikenobe, Japan*
[b] *Seto Inland Sea Regional Research Center, Kagawa University, Saiwai-cho, Takamatsu, Kagawa, Japan*
[c] *National Research Institute of Fisheries and Environment of Inland Sea, Japan Fisheries Research and Education Agency, Maruishi, Hatsukaichi, Hiroshima, Japan*
[d] *Aji Marine Station, Seto Inland Sea Regional Research Center, Kagawa University, Kamano, Aji, Takamatsu, Kagawa, Japan*

Received 10 November 2019; accepted 29 February 2020
Available online 13 March 2020

KEYWORDS
Noctiluca scintillans;
Heterotrophic dinoflagellate;
Cell density;
Coastal water;
ATP;
Activity

Summary *Noctiluca scintillans* is an important member of the mesozooplankton in terms of biomass and production in the Seto Inland Sea, Japan. The densities and adenosine triphosphate (ATP) contents of *N. scintillans* cells were measured. Vertical profiles of *N. scintillans* cellular activity in the coastal water were determined and the ATP contents were high at middle layers, with a maximum depth of 10 m. ATP contents were low in the surface and lower layers. These results suggest that active *N. scintillans* cells in subsurface layers with low density play an important role in the coastal ecosystem, and high-density cells in the surface water are not active.

Noctiluca scintillans is a large heterotrophic dinoflagellate that has a worldwide distribution (e.g. Elbrächter and Qi, 1998). *N. scintillas* is also one of the most common red-tide-forming dinoflagellates in temperate and tropical coastal regions, and is a frequent cause of water discoloration. Many field observations have been conducted and long- or short-term variations in *N. scintillans* and phytoplankton abundances have been reported (e.g. Huang and Qi, 1997; Tada et al., 2004; Uhling and Sahling, 1990). Although we showed that the biomass of *N. scintillans* cannot be ignored even when a red tide outbreak is not occurring (Tada et al., 2004), the ecological role of *N. scintillans* is

* Corresponding author at: Faculty of Agriculture, Kagawa University, 2393 Ikenobe, Miki, Kagawa 761—0795, Japan.
E-mail address: tada@ag.kagawa-u.ac.jp (K. Tada).
Peer review under the responsibility of the Institute of Oceanology of the Polish Academy of Sciences.

Production and hosting by Elsevier

https://doi.org/10.1016/j.oceano.2020.02.005

still not well understood. Specifically, it is not clear whether *N. scintillans* exists as a large biomass with or without predation among other phytoplankton organisms, mainly phytoplankton. However, some studies have discussed the role of *N. scintillans* in a nutrient environment and aquatic ecosystems: *N. scintillans* can play an important role as a nutrient regenerator and supplier owing to the significant amount of NH_4^+-N and PO_4^{3-}-P regeneration, the pools within the cells, and its supply to phytoplankton primary production (Ara et al., 2013; Montani et al., 1998; Pithakpol et al., 2000a, b). We believe that it is very important to determine the activity of *N. scintillans* cells in the field.

Adenosine triphosphate (ATP) is the energy material in all organisms, and the amount of ATP in particulate material is a valuable indication of biomass of live microorganisms. Several authors have determined the ATP contents of seawater and marine sediment samples using a luciferin-luciferase reaction (e.g. Holm-Hansen and Booth, 1966). ATP has been used both to estimate biomass (e.g. Holm-Hansen and Booth, 1966) and metabolic activity (e.g. Hobbie et al., 1972; Patterson et al., 1970). Holm-Hansen (1970) reported that during extreme nitrogen or phosphorous deficiency, the algal cellular levels of ATP decreased to 20%–50% of that found in exponentially-growing cells. Brezonik et al. (1975) showed that algal ATP responded rapidly to mercury addition and pH changes, indicating its usefulness as a measurement of toxicity. They also showed that the rapid response of ATP following nutrient additions to starved algal cultures suggests that ATP may be useful as a tool in nutrient bioassay studies. In addition, Skjoldal and Båmstedt (1975) reported that the ATP concentration in zooplankton showed marked seasonal change with increasing values during periods of reproduction, which is assumed to reflect an inherent seasonal rhythm in metabolism. Recently, Hyun et al. (2018) studied ballast water and reported that the ATP concentration of living phytoplankton cells obtained from a mono-culture experiment was much higher than living cells in the field. Moreover, they reported that the ATP concentration in plankton cells in ballast water was low because of a decline in their biological activity under the extended exposure to dark conditions. We applied this method to determine the ATP contents of *N. scintillans* cells to examine the vertical profiles of *N. scintillans* cellular activity in a coastal water column.

The oceanographic observation was conducted on 1 May 2018 at Harima-Nada, the eastern part of the Seto Inland Sea, Japan (using the r/v *Calanus III* (19 tons) of the Seto Inland Sea Regional Research Center, Kagawa University, Japan). On the observation day, we looked for areas where *N. scintillans* occurred at high densities. We collected seawater samples at two points: Stn. SS1 and Stn. SS2 (Fig. 1, Table 1). At Stn. SS2, we observed a minor water discoloration by *N. scintillans* in the surface seawater. At both stations, seawater samples were collected from seven depths (0, 2.5, 5, 7.5, 10, 20, and 30 m), using a clean bucket for the surface layer and 10-L Van Dorn bottles for the other layers. A total of 1 L of seawater from each depth was passed through a 150 μm mesh plankton net, and the cell numbers were counted using a magnifying glass, with or without appropriate dilution to account for high density.

For ATP determination, 0.1 to 2 L of collected seawater was immediately filtered on board ship through a 47 mm diameter 200 μm mesh screen (Tanaka Sanjiro Co., Ltd., Japan). The mesh screens were then transferred to glass test tubes which contained 3 mL of boiling Tris buffer. After 3 minutes in boiling water, the glass tubes were cooled in an ice bath and thereafter preserved in a deep freezer until analysis. When *N. scintillans* cells were counted, no large phytoplankton and no other zooplankton except *N. scintillans* were observed. The 200 μm mesh screen samples contained only *N. scintillans* cells, and measured ATP was only produced by *N. scintillans*. For chlorophyll *a* (Chl *a*) determination, 0.2 L of seawater from each depth was filtered through a Whatman GF/F filter (47 mm) and extracted in 90% acetone. Extracts were stored overnight in dark at 4°C until analysis. For particulate organic carbon (POC) determination, 1 L of seawater from each depth was filtered through a pre-combusted Whatman GF/F filter (450°C, 2 h), and the filters were rinsed with a small volume of 1N HCl and re-distilled water.

Table 1 Locations and depth of the sampling stations.

Sampling station	Location	Water depth
Stn.SS1	34°25′577″N, 134°19′875″E	48.3 m
Stn.SS2	34°25′876″N, 134°21′118″E	44.7 m

The ATP contents of *N. scintillans* cells were determined by the method of Holm-Hansen and Booth (1966) with some modifications suggested by Bulleid (1978) using a luminometer (Luminescencer PSN AB-2200, Atto, Japan), as described by Parsons et al. (1984).

Chl *a* concentrations of seawater were determined by the fluorescence method (Holm-Hansen et al., 1965) using a fluorometer (10-AU, Turner Designs, USA), described by Parsons et al. (1984). POC concentrations of seawater were determined using a CHN analyzer (Micro Corder JM10, J-Science Lab, Japan).

On the sampling day, cell densities of *N. scintillans* varied from 0 to 328 cells/L at Stn. SS1, and 0 to 21,500 cells/L at Stn. SS2 (Figs. 2 and 3). As described previously, we observed a minor coloration of surface water by *N. scintillans* at Stn. SS2. The cell densities decreased with depth, and no cells were found at the depth of 30 m at either station. ATP concentrations varied from 2.61 to 124 ng/L at Stn. SS1 and 0 to 15,600 ng/L at Stn. SS2. The ATP concentrations were high in the surface layer and were low in the lower layer, with a maximum concentration of 124 ng/L at the 2.5 m depth at Stn. SS1 and 15,600 ng/L at the 0 m depth at Stn. SS2. As previously mentioned, when *N. scintillans* cells were counted for the seawater samples collected by 150 μm mesh plankton nets, no large phytoplankton and no other zooplankton except *N. scintillans* were observed. Therefore, it was thought these ATP contents that accumulated on the 200 μm mesh screen were produced only by *N. scintillans* cells. The ATP contents of *N. scintillans* varied from 0 to 15.1 ng/cell at Stn. SS1 and 0 to 53.8 ng/cell at Stn. SS2. The ATP contents were high at the middle layer with the maximum at the 10 m depth, and low in surface and deeper layers for both stations. However, Chl

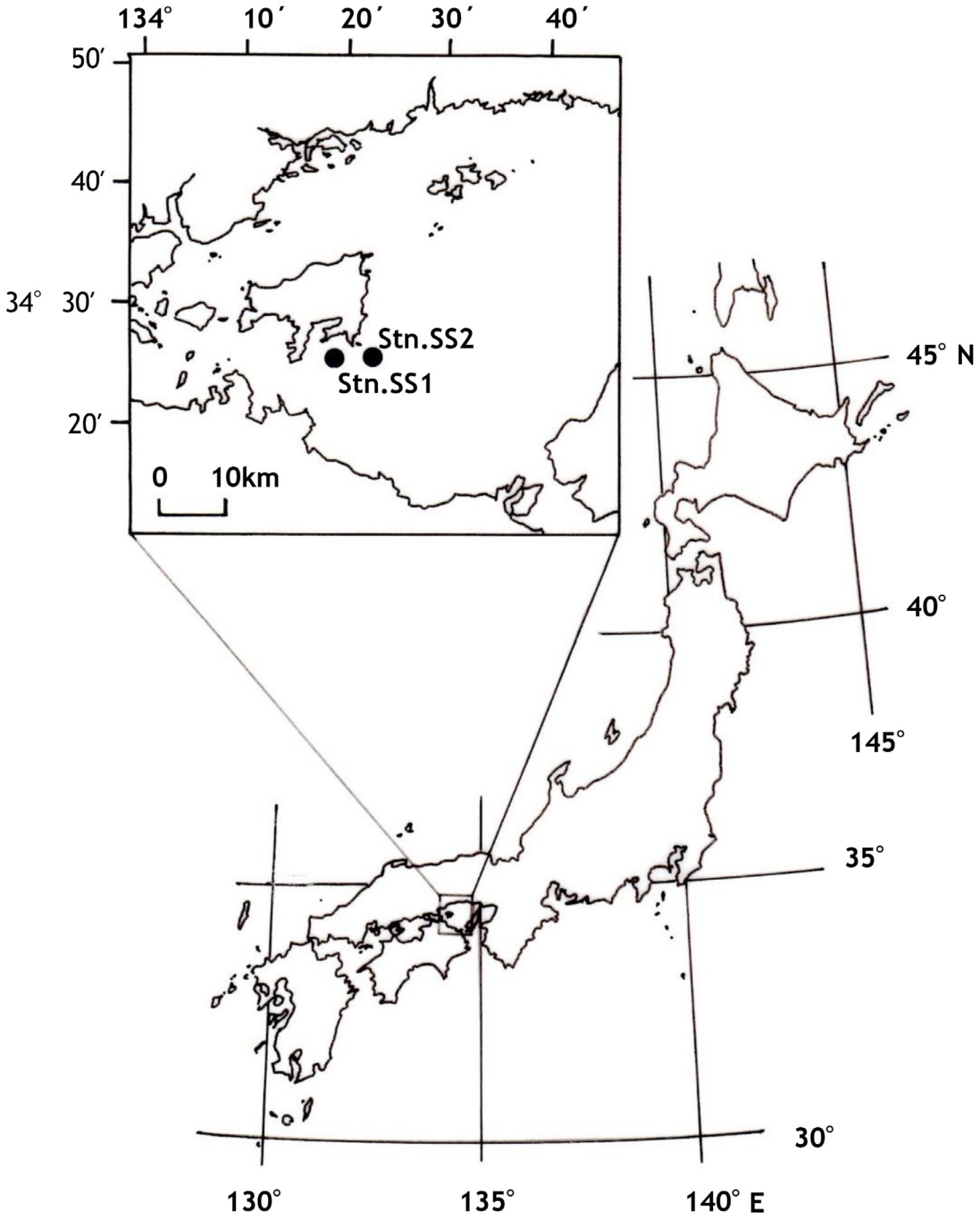

Figure 1 Sampling stations in Harima-Nada, Seto Inland Sea, Japan.

a concentrations varied from 1.88 to 5.02 μg/L and 1.50 to 2.61 μg/L for Stn. SS1 and Stn. SS2, respectively. For both stations, maximum Chl *a* concentrations were observed at the 10 m depth and were lower in surface or deeper layers.

POC concentrations varied from 220 to 351 μg/L at Stn. SS1 and 244 to 12,500 μg/L at Stn. SS2. POC concentrations at both stations tended to decrease with increasing depth.

In the water column at the two stations, POC concentrations varied from 220 to 12,500 μg/L, and decreased with increasing depth (Fig. 4). We estimated phytoplankton carbon from the Chl *a* concentration, using the C/Chl *a* ratio of 56.5 reported for this region (Tada and Morishita, 1997). The biomass of *N. scintillans* was also estimated from its cell density, using the average value for the cellular carbon content (0.353 μg/cell) of field samples that were obtained from this region (Tada et al., 2000). The *Noctiluca* carbon biomass varied from 0% to 62% of POC in the water column at both stations. *Noctiluca* carbon biomass was similar to or about 56 times higher than the phytoplankton biomass at 0 m at Stn. SS1 and Stn. SS2. Except for the 0 m level, the *Noctiluca* carbon varied from 0% to 64% of phytoplankton carbon (phytoplankton biomass) at the two stations. Interestingly, the maximum values of Chl *a* concentrations and ATP contents of *N. scintillans* cells were observed at the 10 m depth

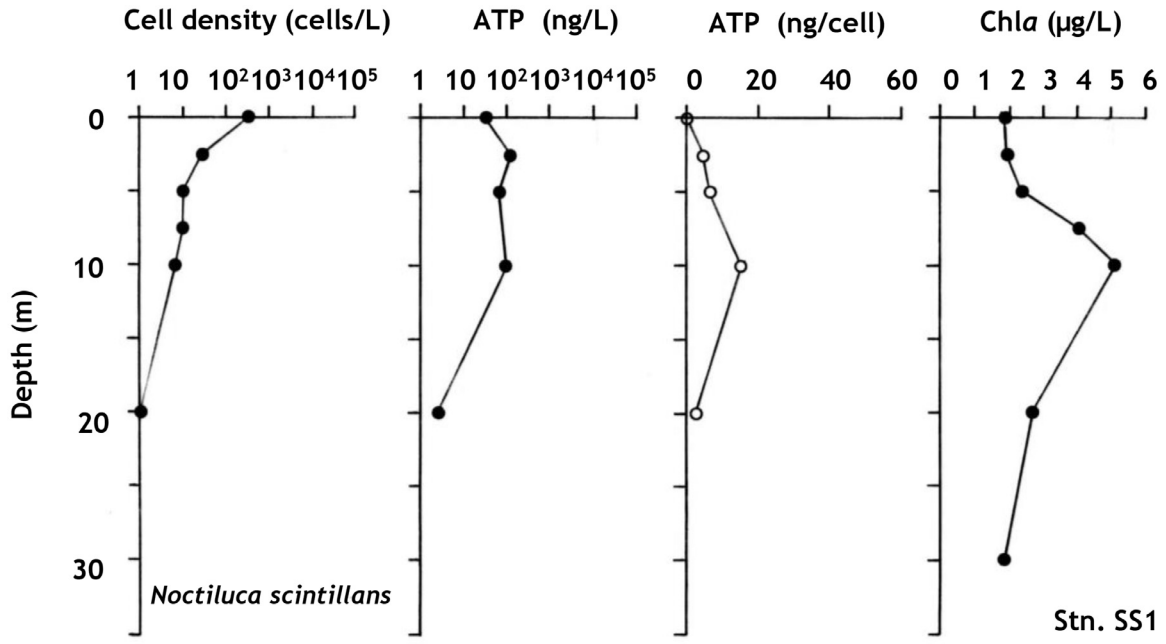

Figure 2 Vertical distributions of the cell density and ATP content of *Noctiluca scintillans* and Chl *a* concentrations in the seawater at Stn. SS1.

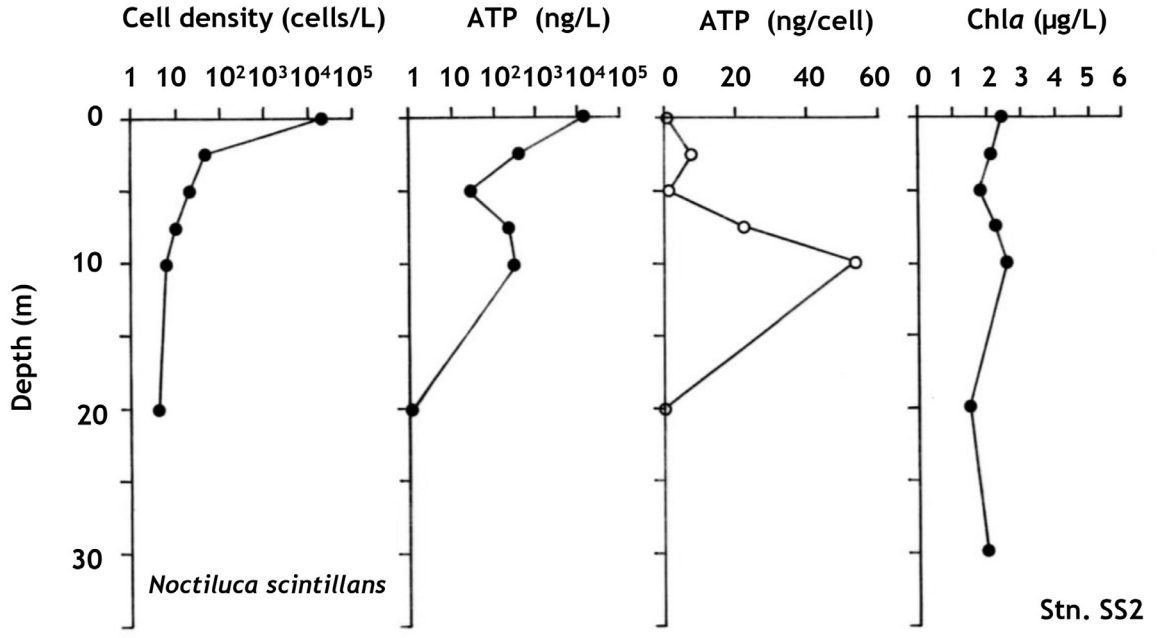

Figure 3 Vertical distributions of the cell density and ATP content of *Noctiluca scintillans* and Chl *a* concentrations in the seawater at Stn. SS2.

at both stations, although maximum cell densities of *N. scintillans* were observed at the 0 m depth. The biomass of *N. scintillans* at the 10 m depth with maximum Chl *a* concentrations at both stations, was about 1% of the phytoplankton biomass, and we believe *N. scintillans* had sufficient food.

Although cell densities decreased with increasing depth, with a maximum density in the surface layer, the ATP contents of *N. scintillans* cells in the surface layer were not high, and the maximum ATP contents were observed at the 10 m for both stations. Our results showed that high cell densities in the surface layer were not active, and *N. scintillans* cells fed actively at a depth of 10 m. From our results, we believe that *N. scintillans* cells with high intracellular ATP were feeding on phytoplankton actively, and they were associated with the maximum depth of Chl *a*. At both stations, *N. scintillans* abundances were very high at the surface (0 m depth), while Chl *a* concentrations were higher at the 10 m depth. Thus *N. scintillans* at the 10 m depth could feed on more phytoplankton than at the 0 m depth, and consequently intracellular ATP contents at the

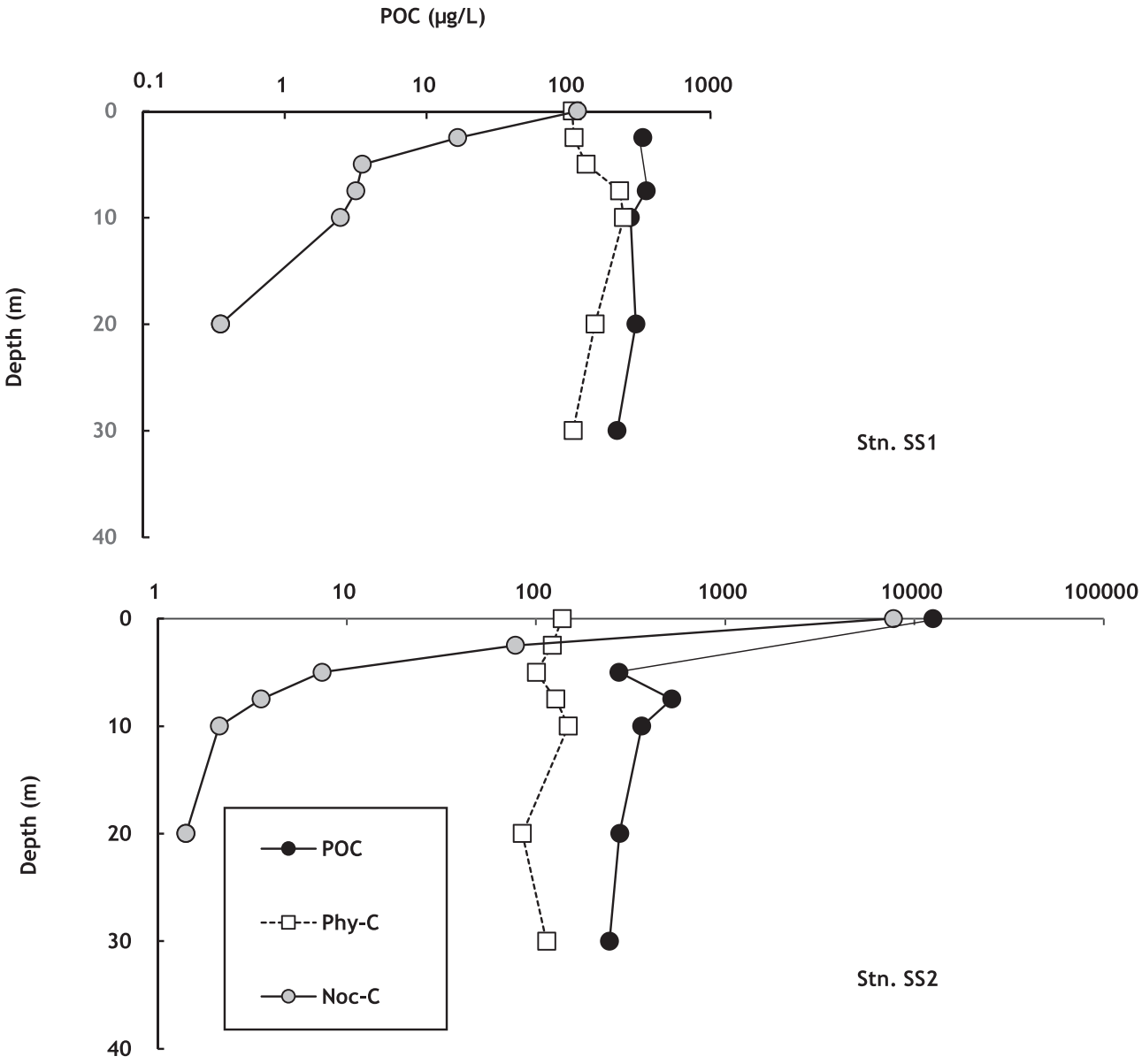

Figure 4 Vertical distributions of POC, estimated *Noctiluca scintillans* and phytoplankton carbon in the seawater at Stn. SS1 and Stn. SS2. Unfortunately, the samples taken at 0 and 5 m at Stn. SS1 and at 2.5 m at Stn. SS2 were lost.

10 m depth were higher than those at the 0 m depth, even if the *N. scintillans* feeding activity at the two stations was similar at the 0 and 10 m depth.

It was thought that the high cell density in surface layer was due to *N. scintillans* growth and accumulations by tidal current and wind. It has been reported that an increase in water temperature during the spring period enhanced the growth rate of *N. scintillans* in the Seto Inland Sea and led to high abundances observed in early summer (Tada et al., 2004). But although the high density of buoyant *Noctiluca* cells in the surface water was a result of their growth, the cells were not active, but they were at least alive.

Red discoloration is observed at *N. scintillans* density of 10,000 cells/L (Kuroda, 1990). However, these high cell densities are thought to be inactive. Furthermore, Tada et al. (2004) reported that the biomass of *N. scintillans* cannot be ignored, even in the absence of a red tide outbreak. Moreover, Nakamura (1998) suggested that *N. scintillas* was an important member of the mesozooplankton in terms of biomass and production in the Seto Inland Sea during summer. In addition, Kitatsuji et al. (2019) investigated the role of *N. scintillans* on a coastal ecosystem, and they reported that the active feeding ended a diatom bloom in the Seto Inland Sea. On the basis of these findings and the result of this study, it is thought that active *N. scintillans* cells in the subsurface layer with low cell density have a major predatory role in the coastal ecosystem, although high-density cells in the surface water are not active. A field study is needed to assess the activity of *N. scintillans* cells and ATP contents as well as *N. scintillans* cell density.

We determined vertical profiles of both *N. scintillans* cell density and ATP contents. *N. scintillans* cell densities were highest in the surface layer, and decreased with increasing depth. However, the ATP contents of the cells were highest

at middle layers with a maximum at a depth of 10 m, and were low in surface and lower layers. These results suggested that the low-active high-density cells of *N. scintillans* in surface seawater is probably due to the cells being in the stationary or death phase. Our results also suggest that active *N. scintillans* cells in subsurface layers with low density have an important role in the coastal ecosystem.

Acknowledgements

The authors thank Mr. Koji Kishimoto of the Aji Marine Station, Seto Inland Sea Regional Research Center, Kagawa University for his kind assistance during the field observations. We are grateful to Mr. Shuzo Sato of Kagawa University for his help in this study. Our gratitude is extended to all members of the Laboratory of Marine Sciences, Kagawa University. Finally, we thank my old friend, Mr. Brian Bigler for reading this manuscript and providing useful suggestions.

References

Ara, K., Nakamura, S., Takahashi, R., Shiomoto, A., Hiromi, J., 2013. Seasonal variability of the red tide forming heterotrophic dinoflagellate *Noctiluca scintillans* in the neritic area of Sagami Bay, Japan: its role in the nutrient-environment and aquatic ecosystem. Plankton Benthos Res. 8 (1), 9—30.

Brezonik, P.L., Browne, F.X., Fox, J.L., 1975. Application of ATP to plankton biomass and bioassay studies. Water Res. 9 (2), 155—162.

Bulleid, N.C., 1978. An improved method for the extraction of adenosine triphosphate from marine sediment and seawater. Limnol. Oceanogr. 23 (1), 174—178.

Elbrächter, M., Qi, Y-Z., 1998. Physiological Ecology of Harmful Algal Blooms. Springer, Berlin, 315—335.

Huang, C., Qi, Y., 1997. The abundance cycle and influence of factors on red tide phenomena of *Noctiluca miliaris* (Dinophyceae) in Dapeng Bay, the south China Sea. J. Plankton Res. 19 (3), 303—318, https://doi.org/10.1093/plankt/19.3.303.

Hobbie, J.E., Holm-Hansen, O., Packard, T.T., Pomeroy, L.R., Sheldon, R.W., Thomas, J.P., Wiebe, W.J., 1972. A study of the distribution and activity of microorganisms in ocean water. Limnol. Oceanogr. 17 (4), 544—555.

Holm-Hansen, O., 1970. ATP levels in algal cells as influenced by environmental conditions. Plant. Cell Physiol. 11 (5), 689—700, https://doi.org/10.1093/oxfordjournals.pcp.a074557.

Holm-Hansen, O., Booth, C.R., 1966. The measurement of adenosine triphosphate in the ocean and its ecological significance. Limnol. Oceanogr. 11 (4), 510—519.

Holm-Hansen, O., Lorenzen, C.J., Holmes, R.W., Strickland, J.D.H., 1965. Fluorometric determination of chlorophyll. J. Cons. — Cons. Int. Explor. Mer. 30 (1), 3—15.

Hyun, B., Cha, H-G., Lee, N., Yun, S., Baek, S.H., Shin, K., 2018. Development of an ATP assay for rapid onboard testing to detect living microorganisms in ballast water. J. Sea Res. 133, 73—80, http://dx.doi.org/10.1016/j-seares.2017.03003.

Kitatsuji, S., Yamaguchi, H., Asahi, T., Ichimi, K., Onizuka, G., Tada, K., 2019. Does *Noctiluca scintillans* end the diatom bloom in coastal water? J. Exp. Mar. Biol. Ecol. 510, 10—14, https://doi.org/10.1016/j.jembe.2018.09.006.

Kuroda, K., 1990. *Noctiluca scintillans* (MACARTNEY) EHRENBERG. In: Fukuyo, Y., Takano, H., Chihara, M., Matsuyoka, K. (Eds.), Red tide organisms in Japan — An Illustrated Taxonomic Guide. Uchida Rokakuho, Tokyo, 78—79 (in Japanese with English abstract).

Montani, S., Pithakpol, S., Tada, K., 1998. Nutrient regeneration in coastal seas by *Noctiluca scintillans*, a red tide-causing dinoflagellate. J. Mar. Biotechnol. 6, 224—228.

Nakamura, Y., 1998. Biomass, feeding and production of *N. scintillans* in the Seto Inland Sea. Japan. J. Plankton Res. 20 (11), 2213—2222, https://doi.org/10.1093/plankt/20.11.2213.

Parsons, T.R., Maita, Y., Lalli, C.M., 1984. Determination of Adenosine Triphosphate (ATP) in Particulate Material. A Manual of Chemical and Biological Methods for Seawater Analysis. Pergamon Press, Oxford, 80—84.

Patterson, J.W., Brezonik, P.L., Putnam, H.D., 1970. Measurement and significance of adenosine triphosphate in activated sludge. Envir. Sci. Technol. 4 (7), 569—575.

Pithakpol, S., Tada, K., Montani, S., 2000a. Nutrient regeneration during *Noctiluca scintillans* red tide in Harima Nada, the Seto Inland Sea, Japan. In: Proceedings of the 15th Ocean Engineering Symposium (ed. the Society of Naval Architects of Japan), 127—134.

Pithakpol, S., Tada, K., Montani, S., 2000b. Ammonium and phosphate pools of *Noctiluca scintillans* and their supplies to the water column in Harima Nada, the Seto Inland Sea. Japan. La mer 37 (4), 153—162.

Skjoldal, H.R., Båmstedt, U., 1975. Ecobiochemical studies on the deep-water pelagic community of Korsfjorden, Western Norway. Adenine nucleotides in Zooplankton. Mar. Biol. 42 (3), 197—211.

Tada, K., Morishita, M., 1997. The changes of environmental chemical conditions and biomass on lower trophic levels in a coastal bay. Tech. Bull. Fac. Agric. Kagawa Univ. 49, 35—47 (in Japanese with English abstract).

Tada, K., Pithakpol, S., Yano, R., Montani, S., 2000. Carbon and nitrogen content of *Noctiluca scintillans* in the Seto Inland Sea. Japan. J. Plankton Res. 22 (6), 1203—1211, https://doi.org/10.1093/plankt/22.6.1203.

Tada, K., Pithakpol, S., Montani, S., 2004. Seasonal variation in the abundance of *Noctiluca scintillans* in the Seto Inland Sea. Japan. Plankton Biol. Ecol. 51 (1), 7—14.

Uhling, G., Sahling, G., 1990. *Noctiluca scintillans*: Zeitliche Deuschen Bucht (Langzeitreihen 1970—1993). Ber. Biol. Anst. Hegoland 9, 1—127.