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Seasonal dynamics and spatial distribution of epiphytic dinoflagellates in Peter the Great Bay (Sea of Japan) with special emphasis on *Ostreopsis* species

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ABSTRACT

Studies of epiphytic dinoflagellates in Peter the Great Bay, Sea of Japan in 2008–2011 revealed the presence of 13 species. Five of the species are known as potentially toxic: *Amphidinium carterae*, *A. operculatum*, *Ostreopsis* cf. *ovata*, *O.* cf. *siamensis* and *Prorocentrum lima*. The maximum species richness and abundance of epiphytic dinoflagellates were observed in autumn (from September to October). *Ostreopsis* spp. were most widely distributed and predominated, amounting to 99% of the total density of dinoflagellates. Multi-year seasonal dynamics of *Ostreopsis* spp. in Peter the Great Bay showed that these cells appear as epiphyton in August after maximum warming of surface waters (22–24 °C) and disappear in early November, when the water temperature decreases below 7 °C. *Ostreopsis* spp. proliferation occurred in September, when the water temperature was 17.2–21.0 °C. The highest densities of *Ostreopsis* spp. were recorded on September 9, 2010 on the rhodophyte *Neorhodomela aculeata* – 230×10^3 cells g^{-1} DW or 52×10^3 cells g^{-1} FW. The spatial distribution of epiphytic dinoflagellates was investigated in the near-shore areas of Peter the Great Bay during the second half of September 2010 to evaluate the role of hydrodynamic conditions. Epiphytic dinoflagellates were not found in sheltered sites having weak mixing hydrodynamics. However, the abundances of *Ostreopsis* spp. were significantly higher at sites having moderate turbulence compared to biotopes experiencing strong wave action. Densities of *Ostreopsis* spp. were not significantly different on macrophytes with branched thallus of all taxonomic divisions. However, the average cell densities of *Ostreopsis* spp. on green algae with branched thallus were significantly higher than on green algae having laminar thallus.

1. Introduction

The ecological aspects of benthic dinoflagellates have received considerably less attention than their planktonic counterparts. The most diversified groups of benthic dinoflagellates are the psammophilous, which inhabit the interstitial space in near-shore sands, and epiphytic dinoflagellates. The second group is mainly represented by the genera *Gambierdiscus*, *Ostreopsis*, *Coolia*, *Amphidinium* and *Prorocentrum*. Until recently, members of the first three genera were considered endemic to tropical and subtropical areas, where their appearance has been associated with widespread ciguatera poisoning. This human poisoning syndrome stems from consuming reef fish that have accumulated the algal toxins, and its symptoms include a number of gastrointestinal, neurological and cardio-vascular disorders. High abundances of some representatives of these genera have been reported in temperate seas over the last decade, suggesting their broad distribution in the World Ocean (Aligizaki and Nikolaidis, 2006; Shears and Ross, 2009; Aligizaki, 2010; Zingone, 2010; Litaker et al., 2010; Rhodes, 2011; Kim et al., 2011; Mangialajo et al., 2011).

Studies of psammophilous dinoflagellates in the near-shore coastal waters of the northwestern Sea of Japan began in the last decade and have shown the presence of a considerable diversity of species (Selina and Hoppenrath, 2004, 2008; Hoppenrath et al., 2007). The first preliminary investigations of epiphytic dinoflagellate assemblages on macrophytes in a cove of Peter the Great Bay have revealed 13 species of 8 benthic dinoflagellate genera during the summer and fall (Selina and Levchenko, 2011), including the first observations for the seas of Russia of representatives of the

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genera *Cabra*, *Ostreopsis* and *Prorocentrum fukuyoi* Murray et Patterson. The species *O. ovata* and *O. siamensis*, which have spread to temperate environments over the last decade (Vila et al., 2001; Monti et al., 2007; Mangialajo et al., 2008, 2011; Shears and Ross, 2009; Totti et al., 2010), were found in seas with winter water temperatures below zero for the first time (Selina and Orlova, 2010). In addition, the potentially toxic species *Amphidinium carterae* Hulburt, *A. operculatum* Claparede et Lachmann and *Prorocentrum lima* (Ehrenberg) Dodge were found in the epiphytic assemblages.

These new observations raise the question of whether the presence of these benthic dinoflagellates has resulted from northward range expansion, or if their low-level presence has escaped previous detection. Here, we examine the seasonal and many-year changes in the species composition, density, and spatial distribution of epiphytic dinoflagellates in Peter the Great Bay.

2. Materials and methods

2.1. Sampling area

Epiphytic dinoflagellates were collected from macrophytes and soft and hard bottom substrata at stations (1–40) situated predominantly in three largest second-order bays (Posyet Bay, Amursky Bay, Ussuriisky Bay) of Peter the Great Bay in the northwestern Sea of Japan (Fig. 1 and Table 1). This area has a monsoon climate with prevailing south-easterly winds during summer and early fall. In general, waters of Peter the Great Bay have the characteristics of open sea waters. The salinity of the near-shore surface water varies from 20 to 34. At the top of the bays (areas E2 and E3) and in smaller sheltered bays (E1) salinity decreases to 7–12 due to river runoff. Amursky Bay is influenced by fresh waters to the greatest degree, because the Razdolnaya River with an average water discharge of 2.46 km^3/year flows into its innermost northern part. However, at high floods during spring and summer the entire Amursky Bay may become an estuarine zone. Along with municipal waste waters from several cities, the Razdolnaya River transports runoff waters from forestlands agricultural fields. Nutrient transport via the river waters makes up 2/3 of the total annual load on Amursky Bay. The remaining one-third is contributed by the waste waters from Vladivostok, the largest city on the coast of Peter the Great Bay with a population of more than 600,000. The northern Amursky Bay receives 70% of the total nutrient inputs in the bay. The highest loads occur in near-shore waters, where the municipal waste waters and the runoff waters from the Razdolnay River are directly discharged (Zvalinskiy et al., 2012). The river runoff, which provides the input of fresh water into Ussuriisky Bay, does not exceed 10–12% of the Razdolnaya River discharge. In Posyet Bay, considerable freshening is observed in its western shallow-water part (area E1), into which flow numerous small rivers. Because this area communicates with the open part of the bay via a narrow strait, the water exchange in it is highly limited. The south-western area of open near-shore waters of Posyet Bay is periodically influenced by the East Korean Current and waters of the Tumen River (volume of discharge is 5.6 km^3/year) in period of summer monsoon winds but salinity never lowers below 30 (Vyshkvartsev and Lebedev, 1997).

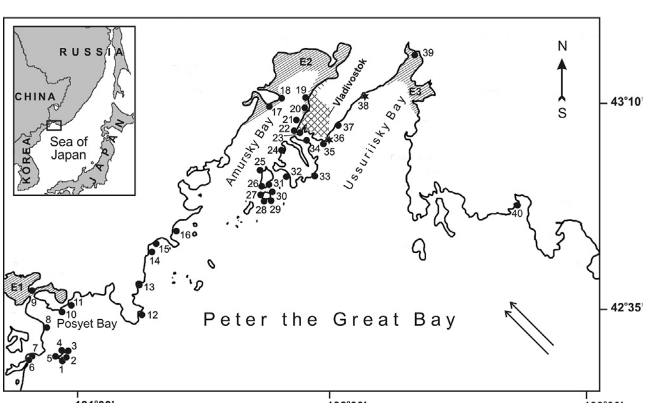

Fig. 1. Study area and station sampled. Stars – monitoring stations, underlined are stations, where the relationship of epiphytic dinoflagellate density on macrophytes and water turbulence was assessed statistically. Arrows – the main direction of the wind in the summer and early fall. Shaded areas of E1–E3 – eutrophic freshened areas.

The eutrophication level of coastal waters is defined by chlorophyll *a* concentration in the range of 8–25 $\mu g\,L^{-1}$ and phosphate concentration in the range of 35–100 $\mu g\,L^{-1}$ (Prepas and Charette, 2005). By these criteria, only some small areas in the inner part of Amursky Bay (E2), Ussuriisky Bay (E3), and Posyet Bay (E1) can be classified as being eutrophic. This eutrophication level is observed only for 2–3 weeks of proliferation of phytoplankton. Otherwise, all waters are classified as mesotrophic (Shulkin and Semykina, 2012).

Near-shore water temperature drops to −1.5 °C in winter and reaches 22.0–24.0 in summer. Many small sheltered bays along the coast are covered by ice from November through March. At a monitoring station (stn. 36), surface water temperature during the year varies from 1.5 to 22.3 °C and salinity varies from 30.0 to 34.0. Sludge ice is observed here during the ice freezing period.

2.2. Sampling methods

Samples of macrophytes were collected using scuba at 0.5–3 m depths every month during the winter–spring period (except January) and 1–3 times a month during the summer–fall period. Generally, six species of algae from different systematic divisions were sampled during each collection event. Macrophyte specimens were placed in tightly sealed plastic jars while still underwater. In the laboratory, the jars containing algae were vigorously shaken for 1 min to dislodge the dinoflagellates from the macrophyte surface. The remaining epiphytes were dislodged from the macrophytes by thoroughly rinsing with filtered seawater. The cell suspensions were sieved through a 120-μm screen and then concentrated on a 20-μm sieve. The samples were fixed with Lugol's iodine solution and kept in a refrigerator. Cell numbers were counted in a 1-ml Sedgewick-Rafter counting cell. Some planktonic dinoflagellate species common to the plankton of Peter the Great Bay were retained during the sampling process but were disregarded in the present study.

Morphological characterizations of the epiphytic dinoflagellates were carried out according to methods described earlier (Selina and Orlova, 2010; Selina and Levchenko, 2011). The macrophyte thalli were slightly dried on filter paper, then dried at 60 °C for 24 h and weighed. Cell densities of dinoflagellates were recalculated per dry weight (DW) of the host macrophyte. For comparison with literature data, the average fresh weights to DW ratios were determined for a subset of samples.

A total of 364 samples were collected, including 31 macrophyte species (Table 2) belonging to Rhodophyta (18), Ochrophyta – Phaeophyceae (10) and Chlorophyta (3). Of these, the most frequent macrophytes were the rhodophytes *Neorhodomela aculeata*, *Tichocarpus crinitus*, the ochrophyte *Sargassum pallidum*, and the chlorophytes *Codium fragile* and *Ulva lactuca*.

In order to study the seasonal and long-term dynamics of epiphytic dinoflagellates, samples were taken from macroalgae (Rhodophyta, Ochrophyta and Chlorophyta) in Sobol Bay (stn. 36) from September 2008 to November 2009 and from June to