Spatial distribution of the toxic dinoflagellate Alexandrium tamarense in summer in the Okhotsk Sea off Hokkaido, Japan

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Abstract: To investigate the mechanisms influencing the spatial distribution of the toxic dinoflagellate Alexandrium tamarense in the Okhotsk Sea off Hokkaido, Japan, intensive field surveys were conducted at 34–37 stations in late July every year from in 2002–2007. Alexandrium tamarense occurred every year. However, the abundance of A. tamarense fluctuated year by year, with extremely low cell densities of A. tamarense in 2005. High abundances of A. tamarense were found frequently in the oceanic area of the surface low-salinity water (LSW, salinity<32.5) and the mixed water (MW). Low abundances were found along the coastal area of the Soya Warm Current (SWC, salinity>33.6) and in the dichothermal water (DTW, temperature<2°C) in the layer of oceanic areas deeper than 30 m. The PO4-P concentration in each water mass was in the order DTW>MW>LSW>SWC and the lowest PO4-P concentration that occurred in the SWC is considered to be a potential limiting factor for the growth of A. tamarense. The reason for the low A. tamarense abundance in the DTW is considered to be the low water temperature and low light intensity. It is concluded that the water mass of LSW has favorable conditions for the growth of A. tamarense. The relative frequency of each water mass fluctuated every year and the results suggest that the frequency of occurrence of the LSW and MW is one of the most significant factors controlling the abundance of A. tamarense in the area.

Key words: Alexandrium tamarense, Hokkaido, Nutrients, Okhotsk Sea, Spatial distribution, Water mass

Introduction

Paralytic shellfish poisoning due to the toxic dinoflagellate Alexandrium tamarense (Lebour) Balech has caused extensive economic damage to the scallop aquaculture industry along the coast of Hokkaido in the Okhotsk Sea in summer every few years (Nishihama 1994, Shimada & Miyazono 2005). Prediction of the poisoning is very important for controlling the fishing plan for scallops. Nishihama et al. (1993) and Nishihama (1994) reported that A. tamarense occurred in the surface low-salinity water and the mixed water (MW). Low abundances were found along the coastal area of the Soya Warm Current (SWC, salinity>33.6) and in the dichothermal water (DTW, temperature<2°C) in the layer of oceanic areas deeper than 30 m. The PO4-P concentration in each water mass was in the order DTW>MW>LSW>SWC and the lowest PO4-P concentration that occurred in the SWC is considered to be a potential limiting factor for the growth of A. tamarense. The reason for the low A. tamarense abundance in the DTW is considered to be the low water temperature and low light intensity. It is concluded that the water mass of LSW has favorable conditions for the growth of A. tamarense. The relative frequency of each water mass fluctuated every year and the results suggest that the frequency of occurrence of the LSW and MW is one of the most significant factors controlling the abundance of A. tamarense in the area.

Russia, including the Okhotsk Sea. Therefore the distribution of A. tamarense in the oceanic area off Hokkaido is very important information for evaluation of the risk of the occurrence of paralytic shellfish poisoning in summer. However, there is a paucity of information on the spatial distribution of A. tamarense in the complicated water mass structure in this region, because the water sampling of Nishihama et al. (1993) and Nishihama (1994) was conducted from only surface layers (0 and 5 m).

Oceanographic structures of the Okhotsk Sea off Hokkaido are characterized by sea ice coverage in the winter season, and four distinct water masses in summer as follows (Aota 1975):

1. Soya Warm Current (SWC, salinity>33.6): Warm current generally flowing along the coast of Hokkaido in the direction from Wakkanai to Abashiri, and originating from the Tsushima Warm Current
2. Surface low-salinity water (LSW, water temperature ≥2°C, salinity>32.5): Low salinity water mass in the sur-
face layer of the oceanic area of the Okhotsk Sea

3. Dichothermal water (DTW, water temperature ≤2°C): Cold water mass under the LSW in the oceanic area

4. Mixed water (MW, water temperature >2°C, salinity >32.5 and <33.6): Water mass occurring from mixing among the above stated water masses (SWC, LSW and DTW)

Nishihama (1994) reported that *A. tamarense* occurred in the surface waters of the oceanic area in July 1989 at the same stations as in the present study. However, the spatial distribution of *A. tamarense* within the four water masses was not entirely elucidated. The aims of the present study are to clarify the relationship between spatial distribution and water mass structure and to get fundamental information for prediction of the paralytic shellfish toxin contamination of scallops along the coast of the Okhotsk Sea.

**Materials and Methods**

Map of the sampling stations and periods of surveys are shown in Fig. 1 and Table 1, respectively. Water samples were collected from each layer of 0, 10, 20, 30 and 40 m depth using Nansen bottles (1 L) at each station. 500 mL of each water sample was fixed with 2% formalin and concentrated to 1 mL by sedimentation for 6 h. The cells numbers of *Alexandrium tamarense* in 0.1 mL subsamples were counted under an epifluorescence microscope (Nikon, XF-EFD2) with UV excitation after calcofluor staining (Fritz & Triemer 1985). Identification of *A. tamarense* was performed on the basis of morphology after Balech (1995). Water temperature and salinity were measured using CTD instruments (Seabird, SBE-911plus). Secchi disk transparencies were measured during daytime observations. 230 mL of each surface water (0 m) sample was filtered through Whatman GF/F glass fiber filters. The filters were frozen at −20°C in situ, and the chlorophyll a concentrations were measured later using a fluorometer (Turner Design, 10-AU) after extraction by immersing the filter in 90% acetone for 6h in the laboratory. Nutrient concentrations (NO3-N and PO4-P) in 30 mL subsamples from all the sampled depths in 2004–2007 were analyzed using an autoanalyzer (Bran+Luebbe, Autoanalyzer II).

**Results**

The spatial distributions of water temperature and water masses and cell densities of *Alexandrium tamarense* are shown in Fig. 2a, b. Overall trends of the distributions of the water masses were interpreted as follows. The SWC flowed along the coast of Hokkaido, the LSW occurred in the surface layer shallower than 10 m depth, the DTW was observed in the layer deeper than 30 m depth, and the MW was found among the water masses every year in 2002–2007. *A. tamarense* was widely distributed in the oceanic area outside the SWC, varying with quantitative and spatial annual fluctuations while they were rarely sampled in 2005. Blooms of *A. tamarense* (>10³ cells L⁻¹) appeared near the frontal area between the LSW and the SWC or the MW outside the SWC in 2004 and 2006. High abundances of *A. tamarense* (>10² cells L⁻¹) occurred broadly in the LSW and the MW shallower than 20 m, except for in 2005. Howver, sporadic high abundances of *A. tamarense* were observed sometimes in the layer deeper than 30 m depth, and the MW was found among the water masses every year in 2002–2007. *A. tamarense* was widely distributed in the oceanic area outside the SWC, varying with quantitative and spatial annual fluctuations while they were rarely sampled in 2005. Blooms of *A. tamarense* (>10³ cells L⁻¹) appeared near the frontal area between the LSW and the SWC or the MW outside the SWC in 2004 and 2006. High abundances of *A. tamarense* (>10² cells L⁻¹) occurred broadly in the LSW and the MW shallower than 20 m, except for in 2005. However, sporadic high abundances of *A. tamarense* were observed sometimes in the layer deeper than 30 m in 2002, 2004 and 2007. Cell densities of *A. tamarense*, superimposed on temperature-salinity diagrams are shown in Fig. 3. Cell densities of *A. tamarense* in each water mass, and the water temperature and salinity values recorded for each bracket of *A. tamarense* abundance are given in Tables 2 and 3. As for the relationship between cell density of *A. tamarense* and water masses, the highest cell densities were found in the LSW and the second highest were in the MW. Low cell densities were found in the SWC and the DTW (Fig. 3). Concerning the vertical distribution, *A. tamarense* was frequently found in the surface layer shallower than

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**Table 1.** Periods of surveys, number of sampling stations and research vessels.

<table>
<thead>
<tr>
<th>Period of survey (m/d/y)</th>
<th>Number of stations</th>
<th>Research vessel*</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 22–25, 2002</td>
<td>37</td>
<td>Hokuyou Maru,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oyashio Maru</td>
</tr>
<tr>
<td>July 24–26, 2003</td>
<td>37</td>
<td>Oyashio Maru</td>
</tr>
<tr>
<td>July 23–25, 2004</td>
<td>35</td>
<td>Oyashio Maru</td>
</tr>
<tr>
<td>July 21–23, 2005</td>
<td>37</td>
<td>Oyashio Maru</td>
</tr>
<tr>
<td>July 25–27, 2006</td>
<td>34</td>
<td>Oyashio Maru</td>
</tr>
<tr>
<td>July 25–27, 2007</td>
<td>35</td>
<td>Oyashio Maru</td>
</tr>
</tbody>
</table>

* Hokuyou Maru: 214 ton, R/V of Hokkaido Wakkanai Fisheries Experiment Station  
Oyashio Maru: 178 ton, R/V of Hokkaido Central Fisheries Experiment Station
Fig. 2a. Spatial distribution of water temperature (contours), water masses (screentone) and cell density of *Alexandrium tamarense* (bubbles) in 2002–2004. Abbreviations of water masses are as follows. SWC: Soya Warm Current (salinity ≥ 33.6). LSW: Surface low-salinity water (salinity ≤ 32.5). MW: Mixed water (water temperature > 2°C, salinity > 32.5 and < 33.6). DTW: Dichothermal water (water temperature ≤ 2°C).
Fig. 2b. Spatial distribution of water temperature (contours), water masses (screentone) and cell density of *Alexandrium tamarense* (bubbles) in 2005–2007.
20 m depth, usually in the LSW and/or MW, but was rare in waters deeper than 30 m depth, where DTW prevailed (Fig. 2). Blooms of *A. tamarense* (10^3 cells L^-1) appeared at water temperatures of 5.9–14.4 °C and salinities of 31.9–32.5 (Fig. 3, Table 3).

Depths of the euphotic layer were estimated from transparencies and defined as the layer shallower than the isolum of 1% of the light intensity at sea surface using the formula of Poole & Atkins (1929): k = 1.7/d (k: extinction coefficient, d: transparency) and the formula: z = log 0.01/k (z: depth of the layer with 1% of the light intensity at the sea surface) and shown in Table 4. The euphotic layer depth was between 30 and 40 m in the LSW and MW, however the depth was deeper than 40 m in the SWC. Since the DTW existed below the LSW and MW, it could be estimated that the euphotic layer depth occurred between 30 and 40 m in the DTW, even without transparency observations. Therefore, the light intensity of the DTW deeper than 30 m was considered to be insufficient for photosynthesis.

Spatial distribution of nutrient concentrations and cell densities of *A. tamarense* in 2004–2007 are shown in Fig. 4a, b. Figure 5 shows the surface chlorophyll *a* concentration and maximum cell density of *A. tamarense* at each sta-

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**Table 2.** Cell density of *Alexandrium tamarense* in each water mass in 2002–2007. Abbreviations of water masses are as follows.

<table>
<thead>
<tr>
<th>Water mass</th>
<th>Number of samples</th>
<th>Cell density of <em>A. tamarense</em> (cells L^-1, mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWC</td>
<td>312</td>
<td>7.4±27.7</td>
</tr>
<tr>
<td>LSW</td>
<td>182</td>
<td>114.0±342.1</td>
</tr>
<tr>
<td>MW</td>
<td>262</td>
<td>40.8±127.5</td>
</tr>
<tr>
<td>DTW</td>
<td>183</td>
<td>3.7±29.2</td>
</tr>
</tbody>
</table>

**Table 3.** Water temperature and salinity in each bracket of *Alexandrium tamarense* cell density in 2002–2007.

<table>
<thead>
<tr>
<th>Range of <em>A. tamarense</em> cell density (cells L^-1)</th>
<th>Number of samples</th>
<th>Water temperature (°C, mean±SD [min–max])</th>
<th>Salinity (mean±SD [min–max])</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 0</td>
<td>730</td>
<td>8.1±5.8 [−1.3–19.6]</td>
<td>33.2±0.8 [31.5–34.1]</td>
</tr>
<tr>
<td>20–80 0 0</td>
<td>137</td>
<td>11.1±4.3 [0.7–17.0]</td>
<td>32.9±0.7 [31.6–34.0]</td>
</tr>
<tr>
<td>100–980 0 0</td>
<td>64</td>
<td>11.4±3.9 [1.9–16.9]</td>
<td>32.7±0.6 [31.7–3.9]</td>
</tr>
<tr>
<td>1000=</td>
<td>8</td>
<td>11.8±3.2 [5.9–14.4]</td>
<td>32.2±0.2 [31.9–32.5]</td>
</tr>
</tbody>
</table>

**Table 4.** Estimated euphotic layer depth from measurements of transparency during daytime observations in 2002–2007.

<table>
<thead>
<tr>
<th>Water mass</th>
<th>Number of observation</th>
<th>Transparency (m, mean±SD)</th>
<th>Euphotic layer depth* (m, mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWC</td>
<td>33</td>
<td>15.4±3.6</td>
<td>41.7±9.8</td>
</tr>
<tr>
<td>LSW</td>
<td>55</td>
<td>13.0±2.6</td>
<td>35.3±6.9</td>
</tr>
<tr>
<td>MW</td>
<td>36</td>
<td>11.1±2.6</td>
<td>30.2±6.9</td>
</tr>
<tr>
<td>DTW</td>
<td>0</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

* Euphotic layer depth is estimated from transparency as the layer where light intensity has decreased to 1% of that at the sea surface, using the formula of Poole & Atkins (1929): k = 1.7/d (k: extinction coefficient, d: transparency) and the formula: z = −log 0.01/k (z: depth of the layer with 1% of the light intensity at the sea surface).

20 m depth, usually in the LSW and/or MW, but was rare in waters deeper than 30 m depth, where DTW prevailed (Fig. 2). Blooms of *A. tamarense* (10^3 cells L^-1) appeared at water temperatures of 5.9–14.4°C and salinities of 31.9–32.5 (Fig. 3, Table 3).

Depths of the euphotic layer were estimated from transparencies and defined as the layer shallower than the isolum of 1% of the light intensity at sea surface using the formula of Poole & Atkins (1929): k = 1.7/d (k: extinction coefficient, d: transparency) and the formula: z = −log 0.01/k (z: depth of the layer with 1% of the light intensity at the sea surface) and shown in Table 4. The euphotic layer depth was between 30 and 40 m in the LSW and MW, however the depth was deeper than 40 m in the SWC. Since the DTW existed below the LSW and MW, it could be estimated that the euphotic layer depth occurred between 30 and 40 m in the DTW, even without transparency observations. Therefore, the light intensity of the DTW deeper than 30 m was considered to be insufficient for photosynthesis.

Spatial distribution of nutrient concentrations and cell densities of *A. tamarense* in 2004–2007 are shown in Fig. 4a, b. Figure 5 shows the surface chlorophyll *a* concentration and maximum cell density of *A. tamarense* at each sta-
Surface chlorophyll $a$ concentrations were patchily high near the cold water belt along the frontal area just outside the SWC (see Fig. 2a, b). Blooms of *A. tamarense* ($\geq 10^3$ cells L$^{-1}$) were found off the patch of high chlorophyll $a$ concentration in 2004 and 2006. The diatoms *Chaetoceros* spp. and *Thalassiosira* spp. were the dominant species in the patch of high chlorophyll $a$ concentration.

Table 5 summarizes nutrient concentrations in each water mass. Regarding the relationship between cell density of *A. tamarense* and nutrient concentrations, the higher cell densities were not always found in waters with higher nutrient concentrations. In particular, low cell densities were found in the DTW with the highest nutrient levels but lowest water temperatures. On the other hand, in water with low nutrients, such as the LSW and the SWC, *A. tamarense* was frequently found in the LSW but rarely found in the SWC.

Comparing nutrient concentrations of the LSW and the SWC, mean NO$_3$-N concentration was lower in the LSW, and mean PO$_4$-P concentration was lower in the SWC, respectively ($t$-test, $p<0.01$).

**Discussion**

Based on the results of the present study, a schematic diagram is presented regarding the spatial distribution of water masses and *Alexandrium tamarense* in Fig. 6. It was clearly shown that *A. tamarense* occurred most frequently in the LSW followed by the MW in the oceanic areas, while *A. tamarense* was rarer in the SWC along the coastal area and in the DTW beneath the LSW and the MW. The present study revealed the detailed spatial distribution of *A. tamarense* in all of the water masses, surpassing the report on the distribution only from the surface layer by Nish-
hama (1994). It has been reported in Funka Bay, southern Hokkaido, that the optimum water temperature for *A. tamarense* was 8–12°C (Nishihama 1982) and as 5–10°C (Shimada et al. 1996). In the present study, *A. tamarense* blooms were confirmed at temperatures of 5.9–14.4°C as is the case in Funka Bay.

Fukuyo (1982) reported that newly germinated cells of *A. tamarense* arising from cysts could not grow at the low water temperature of 5°C. It can be suggested that *A. tamarense* can not increase in the DTW under the low water temperature regime (see Fig. 3) combined with the reduced light intensity as found in the present study. Tarutani (1999) suggested that in comparison with the dominant diatom species, *A. tamarense* was unable to increase to dominance because of their larger half-saturation constant for PO$_4$-P uptake. Yamamoto & Tarutani (1999) reported that the PO$_4$-P concentration required for the maximum growth rate (0.54 day$^{-1}$) of the *A. tamarense* Hiroshima Bay strain was estimated to be 0.12 μM as estimated by a culture experiment. Shinada (2005) described that the PO$_4$-P concentration of the SWC was lower than 0.12 μM in most cases and insufficient to support growth of *A. tamarense*. In the present study, concentrations of PO$_4$-P lower than 0.12 μM occurred in 25% of all samples from the SWC. In summary, our results suggest that the lower PO$_4$-P concentrations in the SWC, below those found in the LSW, are not always sufficient for growth of *A. tamarense* (see Table 5).

Why can *A. tamarense* increase in the LSW even though NO$_3$-N concentrations are lower than in the other water masses? MacIntyre et al. (1997) suggested that *A. tamarense* sustains growth through nocturnal migrations to nutrient rich deeper layers for nitrogen uptake, based on a culture experiment using a laboratory water column (2.1 m height). Lewis et al. (2006) reported that the mean swim-
The swimming speed of *A. tamarense* was 108 mm s\(^{-1}\) (=3.89 m h\(^{-1}\)) at a temperature of 12°C in a laboratory experiment. In the present study, it is thought that *A. tamarense* could increase in the LSW with low NO\(_3\)-N concentrations through nocturnal migrations to the MW for NO\(_3\)-N uptake. On the other hand, concerning nutrients, Ogata et al. (1996) found that *A. tamarense* can grow through utilization of organic nitrogen such as in yeast extract. It is also supposed that *A. tamarense* might utilize organic nitrogen in the LSW.

The present study found that the LSW is the most favorable water mass for the growth of *A. tamarense*. Figure 7 depicted annual fluctuations in the relative frequencies of each water mass and the annual average abundances of *A. tamarense* detected in samples at the same depth layers at the 34 stations and the 10 coastal stations collected in 2002–2007. It was found through analysis of the fluctuations at the 34 stations that the frequency of water preferred by *A. tamarense* (LSW + MW), was lowest in 2005 (see Fig. 7 above). This result suggests that water mass structure is a significant factor controlling the abundance of *A. tamarense* in the Okhotsk Sea. Regarding the abundances of *A. tamarense* in the coastal areas, which were suspected to be directly coupled with the occurrences of paralytic shellfish poisoning, it was found that the abundances of *A. tamarense* were low in 2005 and 2006 when the amounts LSW and the MW occurring were low (Fig. 7, lower figure), mirroring the results for oceanic areas.

It has been reported that large abundances of toxic *Alexandrium* spp. resting cysts can be found in bottom sediments on the continental shelf off Hokkaido and Sakhalin Island in the Okhotsk Sea (Orlova et al. 2004, Shimada & Miyazono 2005). It was also reported that blooms (\(\geq 10^3\) cells L\(^{-1}\)) of *A. tamarense* have occurred in Aniva Bay, southern Sakhalin Island (Selina et al. 2006). These reports indicate that *A. tamarense* originates by germination of cysts on the continental shelf in the area. Thus, one of the reasons why *A. tamarense* was frequently found in oceanic

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**Table 5.** Nutrient concentrations in water masses in 2004–2007.

<table>
<thead>
<tr>
<th>Water mass</th>
<th>Number of samples</th>
<th>Nutrient concentrations ((\mu M), mean±SD)</th>
<th>Nutrient concentrations ((\mu M), mean±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWC</td>
<td>225</td>
<td>1.52±2.41</td>
<td>0.26±0.22</td>
</tr>
<tr>
<td>LSW</td>
<td>120</td>
<td>0.21±0.86</td>
<td>0.35±0.15</td>
</tr>
<tr>
<td>MW</td>
<td>156</td>
<td>3.51±4.55</td>
<td>0.59±0.37</td>
</tr>
<tr>
<td>DTW</td>
<td>125</td>
<td>15.79±4.53</td>
<td>1.55±0.29</td>
</tr>
</tbody>
</table>

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**Fig. 5.** Horizontal distribution of sea surface chlorophyll \(a\) concentration (contours) and maximum cell density of *Alexandrium tamarense* (bubbles) at each station in 2002–2007.
areas of the LSW is that *A. tamarense* cells, originating from cysts, grow in the LSW water mass due to the optimum conditions for growth.

The blooms ($\approx 10^3$ cells L$^{-1}$) of *A. tamarense* tended to be found near the frontal areas outside the SWC in the present study. It has been reported that the formation of a cold water belt through upwelling can often be detected in the frontal areas in summer (Nakata et al. 1996, Ishizu et al. 2006), and that diatom blooms frequently occur through utilization of the richer nutrients in the upwelling water (Watanabe 1990). In the present study, the blooms of *A. tamarense* existed not within the frontal area, but rather near the frontal area where the diatom blooms were also found (Figs. 2a, b, 5). These results suggest that phototrophic dinoflagellates such as *A. tamarense* can not compete with diatoms generally because of disadvantages concerning their nutrient uptake abilities (Tarutani 1999, Shimada 2000, Miyazono & Shimada 2000).

The SWC usually predominates along the coastal areas containing the culture grounds for scallops (Nishihama 1994). Paralytic shellfish poisoning might not have occurred if the SWC water mass had predominated continuously within the area, because the SWC contains only small numbers of *A. tamarense*. Our results suggest that paralytic shellfish poisoning occurs when the LSW containing *A. tamarense* extends to the coastal area from the oceanic area due to physical processes (e.g. temporal weakening of the SWC). Nishihama et al. (1993) pointed out that the extension of LSW might have a strong linkage with the occurrence of paralytic shellfish poisoning in the culture grounds of scallops in the Okhotsk Sea, according to the observations of *A. tamarense* occurrences linked to salinity decreases in the surface water during July 1986. Therefore, it is important to elucidate the physical processes enabling the extension of LSW to the culturing grounds of scallop, and to establish a forecasting system for the occurrences of paralytic shellfish poisoning in the near future. Therefore we are now trying to elucidate the relationship between the extension of LSW contaminated with *A. tamarense* to the coastal area and the temporal weakening of the SWC.

**Acknowledgments**

We are deeply grateful to Mrs. T. A. Mogilnikova and Mrs. I. V. Motilkova of the Sakhalin Scientific Research Institute of Fisheries and Oceanography (SakhNIRO) for valuable information about the distribution of *Alexandrium tamarense* and the occurrence of paralytic shellfish poisoning along the coast of Sakhalin Island. We are grateful to the captains and crews of R/V Hokuyo Maru and Oyashio Maru for kindly helping with sampling. We also thank colleagues at the Hokkaido Fishery Experiment Station for their helpful comments.

**References**


