

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

Hiroshi Shimada¹, Mayumi Sawada¹, Takanori Kuribayashi¹, Akifumi Nakata¹, Akira Miyazono¹ and Hiroki Asami²

¹ Hokkaido Central Fisheries Experiment Station, Yoichi, Hokkaido, Japan
E-mail: shimadah@fishexp.pref.hokkaido.jp

² Hokkaido Wakkanai Fisheries Experiment Station, Wakkanai, Hokkaido, Japan

Abstract

Spatial distributions of the toxic dinoflagellate, *Alexandrium tamarense*, were examined in the Okhotsk Sea off Hokkaido in summer to clarify the relationship between its distribution and water mass structure. Surveys were conducted at 37 stations in late July in 2002–2007. Water samples for cell counts of *A. tamarense* were collected from each layer of 0, 10, 20, 30 and 40 m depth at each station. Nutrient concentrations of each water sample in 2004–2007 were analyzed. Results show that *A. tamarense* appeared every year although its abundance fluctuated year by year, and it rarely appeared in 2005. A high abundance of *A. tamarense* tended to be found frequently in the oceanic area of surface low-salinity water (LSW, salinity ≤ 32.5) and in mixed water (MW) among the water masses, while a low abundance was found along the coastal area of the Soya Warm Current (SWC, salinity ≥ 33.6) and in the dichothermal water (DTW, water temperature $\leq 2^\circ\text{C}$) layer depth > 30 m. $\text{PO}_4\text{-P}$ concentrations in each water mass were in the order $\text{DTW} > \text{LSW} > \text{SWC}$. The lowest $\text{PO}_4\text{-P}$ concentration in the SWC might be limiting factor for growth of *A. tamarense*. On the other hand, the reason for low *A. tamarense* abundance in the DTW might be due to the low water temperature. The results suggest that the LSW is the optimum water mass for growth of *A. tamarense*.

Introduction

Paralytic shellfish poisoning caused by the toxic dinoflagellate, *Alexandrium tamarense*, accounts for much economic damage to the scallop culturing fishery along the coast of Hokkaido in the Okhotsk Sea once every few years (Nishihama, 1994; Shimada and Miyazono, 2005). Prediction of the poisoning is very important for fishing and shipping plans for scallops.

The Okhotsk Sea off Hokkaido is known for its very characteristic oceanographic structures, such as sea ice in winter (Aota, 1975). The following four water masses generally distribute in the area in summer (Aota, 1975).

1. Soya Warm Current (SWC, salinity ≥ 33.6): Warm current flowing along the coast of Hokkaido in the direction from Wakkanai to Abashiri, originating the Tsushima Warm Current;
2. Surface low-salinity water (LSW, water temperature $> 2^\circ\text{C}$, salinity ≤ 32.5): Low salinity

water mass in the surface layer of the oceanic area;

3. Dichothermal water (DTW, water temperature $\leq 2^\circ\text{C}$): Cold water mass under the LSW of the oceanic area;
4. Mixed water (MW): Water mass existing among the above, but not belonging to the above.

Nishihama (1994) reported that *A. tamarense* appeared in the surface waters of the oceanic area in July 1989 at the same stations as in the present study. However, the sampling was conducted only in the surface layer so that the spatial distribution of *A. tamarense* in the four water masses had not been revealed. The present study was carried out to clearly detail the relationship between spatial distribution and water mass structure and to get fundamental information for the prediction of shellfish poisoning.

Materials and Methods

A map of sampling stations and survey periods are shown in Figure 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 liter) at each station. The 500-ml water samples were fixed with 2% formalin and concentrated to 1 ml by sedimentation for 6 h. The 0.1 ml subsamples stained with one drop of fluorescent brightener 28 (Sigma) saturated solution were used for cell counting of *A. tamarensis* under an epi-fluorescence microscope (Nikon, XF-EFD2) with UV excitation. Water temperature and salinity were measured using CTD instruments (Seabird, SBE-911plus). The 230-ml surface water samples were filtered with Whatman GF/F. The filters were frozen at -20°C *in situ*, and chlorophyll-*a* concentrations were measured using a fluorometer (Turner Design, 10-AU) after 6 h extracting with 90% acetone in the laboratory. Nutrient concentrations ($\text{NO}_3\text{-N}$ and $\text{PO}_4\text{-P}$) of all the 30-ml water samples in 2004–2007 were analyzed using an autoanalyzer (Bran + Luebbe, Autoanalyzer II).

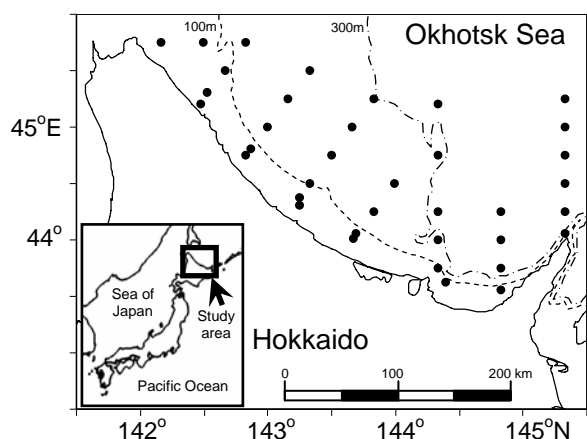


Fig. 1 Map showing 37 sampling stations in the Okhotsk Sea off Hokkaido.

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

Period of survey	No. stations	Research vessel*
July 22–25, 2002	37	<i>Hokuyou Maru</i> , <i>Oyashio Maru</i>
July 24–26, 2003	37	<i>Oyashio Maru</i>
July 23–25, 2004	35	<i>Oyashio Maru</i>
July 21–23, 2005	37	<i>Oyashio Maru</i>
July 25–27, 2006	34	<i>Oyashio Maru</i>
July 25–27, 2007	35	<i>Oyashio Maru</i>

* *Hokuyou Maru*: 214 ton, R/V of Hokkaido Wakkanai Fisheries Experiment Station.

Oyashio Maru: 178 ton, R/V of Hokkaido Central Fisheries Experiment Station.

Results

The spatial distribution of water temperature, water masses, and cell density of *A. tamarensis* are shown in Figures 2a and b. The SWC flowed along the coast of Hokkaido, the LSW was found in the surface shallower than 10 m deep, the DTW was observed in the layer lower than 30 m deep, and the MW was found among the water masses every year from 2002–2007. *Alexandrium tamarensis* was widely found in the oceanic area outside the SWC, fluctuating annually both quantitatively and spatially although it was rarely found in 2005. Blooms of *A. tamarensis* ($\geq 10^3$ cells l^{-1}) appeared near the front area outside the SWC in 2004 and 2006.

Cell density of *A. tamarensis* superimposed on a temperature–salinity diagram is shown in Figure 3. Cell density of *A. tamarensis* in each water mass is shown in Table 2 and water temperature and salinity in each range of *A. tamarensis* cell density is shown in Table 3. Table 2 shows that the highest cell densities were found in the LSW followed by the MW. However, cell densities were low in the SWC and the DTW. As to the vertical distribution, *A. tamarensis* was frequently found in the surface layer shallower than 20 m deep occupied by the LSW and the MW, but rarely found in the layer deeper than 30 m, occupied by the DTW (Fig. 2). Blooms of *A. tamarensis* ($\geq 10^3$ cells l^{-1}) appeared at a water temperature of $5.9\text{--}14.4^{\circ}\text{C}$ with a salinity of $31.9\text{--}32.5$ (Table 3).

Nutrient concentrations in each water mass are shown in Table 4. Higher cell densities did not tend to be found in water with higher nutrients. Especially, the lowest densities were found in the DTW with highest nutrients, but lowest water temperature. On the other hand, in water of the lower nutrients, such as the LSW and the SWC, *A. tamarensis* was frequently found in the LSW and rarely found in the SWC. Comparing nutrient concentrations of the LSW and the SWC, mean $\text{NO}_3\text{-N}$ concentration was lower in the LSW, and mean $\text{PO}_4\text{-P}$ concentration was lower in the SWC, respectively (t-test, $p < 0.01$).

Discussion

A schematic diagram of the spatial distribution of water masses and *A. tamarensis* from the results of present study is shown in Figure 4. It is seen that *A. tamarensis* appear frequently in the surface layer

of the LSW and the MW in the oceanic area while they rarely appear in the SWC along the coastal area and in the DTW under the LSW and the MW. The present study reveals the detailed spatial distribution of *A. tamarensis* in all the water masses in addition to the report on the distribution only in the surface layer by Nishihama (1994). Studies have shown that

the optimum water temperature for *A. tamarensis* is 8–12°C (Nishihama, 1982) or 5–10°C (Shimada *et al.*, 1996) in Funka Bay, southern Hokkaido. In the present study the water temperature for *A. tamarensis* blooms, 5.9–14.4°C, was almost same as that reported for Funka Bay.

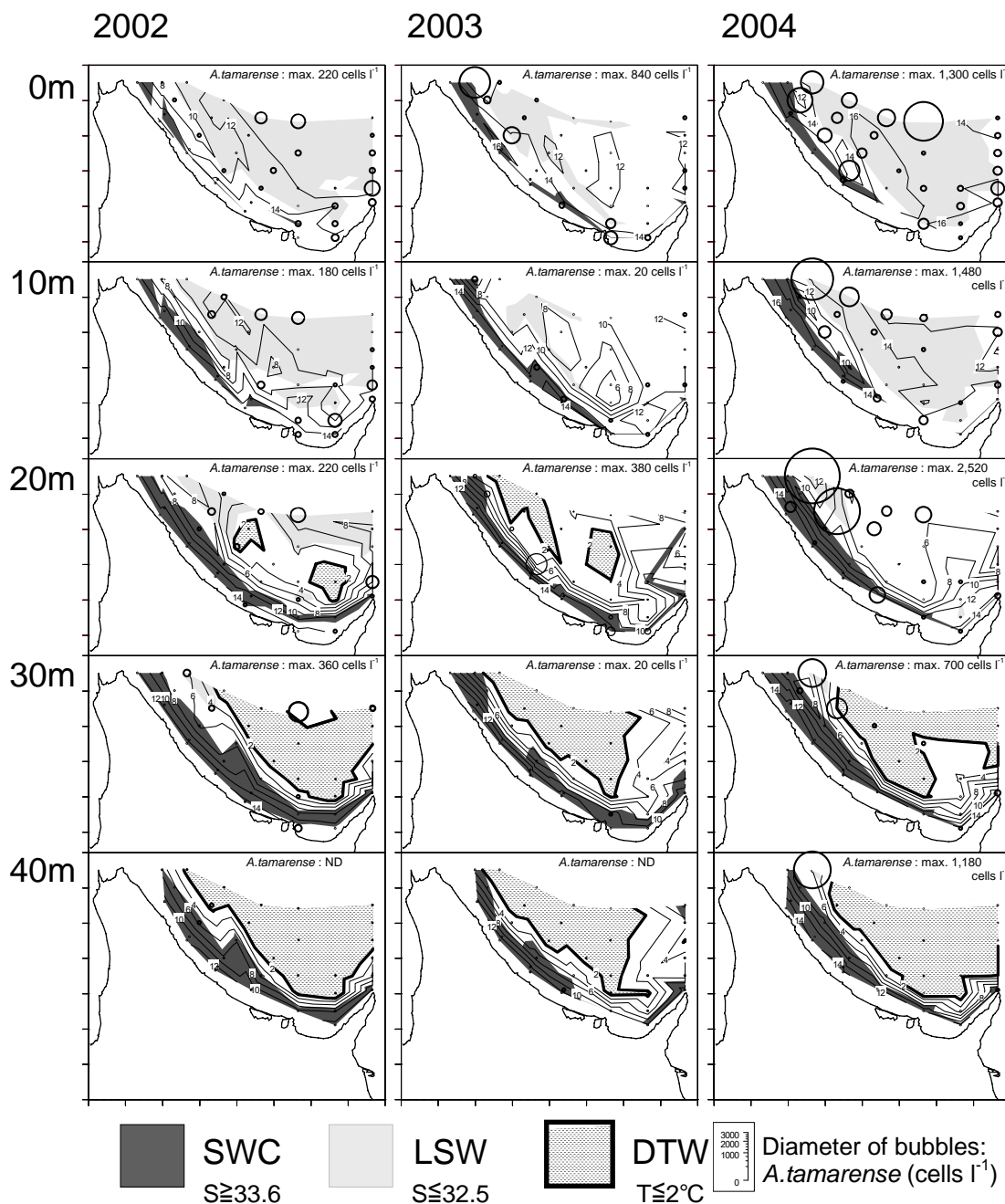


Fig. 2a Spatial distribution of water temperature (contour), water masses (screened) and cell density of *A. tamarensis* (bubbles) from 2002–2004. SWC = Soya Warm Current, LSW = Low Salinity Water, DTW = Dichothermal Water, ND = No detection.

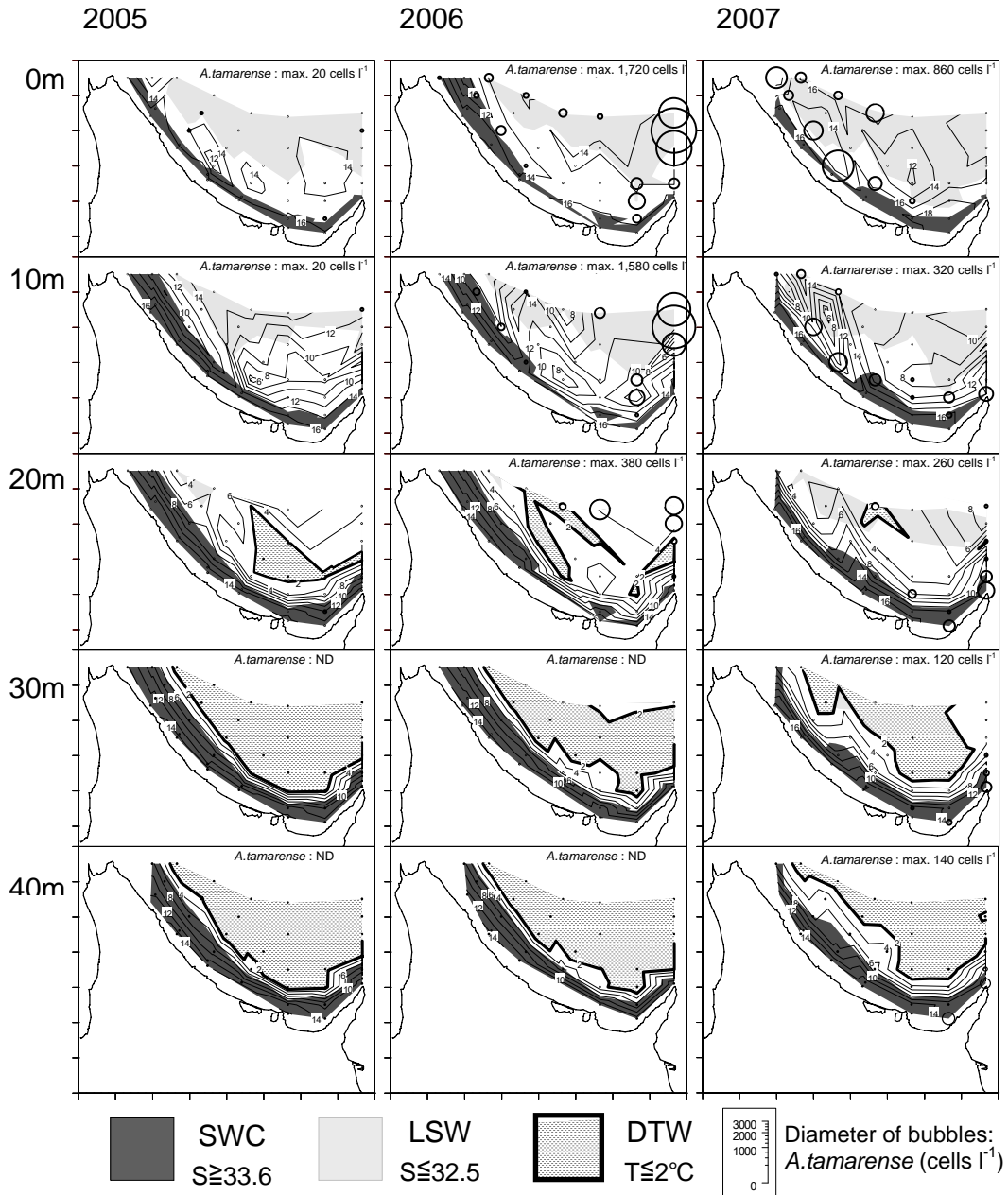


Fig. 2b Spatial distribution of water temperature (contour), water masses (screen-tone) and cell density of *A. tamarensis* (bubbles) from 2005–2007. SWC = Soya Warm Current, LSW = Low Salinity Water, DTW = Dichothermal Water, ND = No detection.

Fukuyo (1982) noted that germinated cells of *A. tamarensis* could not grow at low water temperatures less than 5°C . Tarutani (1999) and Yamamoto and Tarutani (1999) suggested that *A. tamarensis* was difficult to increase to dominant species because of their larger half-saturation constant compared to that of dominant diatom

species for $\text{PO}_4\text{-P}$ uptake. Shinada (2005) suggested that $\text{PO}_4\text{-P}$ concentration of the SWC was too low for growth of *A. tamarensis*. From these reports, and the present study, it is suggested that *A. tamarensis* cannot increase in the DTW having low water temperature and cannot increase in the SWC having low $\text{PO}_4\text{-P}$ concentrations.

MacIntire *et al.* (1997) suggested that *A. tamarensis* could sustain growth through nocturnal migrations to a nutrient-rich deeper layer for nitrogen uptake. In the present study, it is suggested that *A. tamarensis* could increase in the LSW of low NO₃-N concentration through the nocturnal migrations to the MW mixed with the DTW for NO₃-N uptake. Ogata *et al.* (1996) reported that *A. tamarensis* could increase through utilization of organic nitrogen. It is also supposed that *A. tamarensis* might utilize organic nitrogen in the LSW in the present study.

Therefore, it can be concluded that LSW is the optimum water mass for growth of *A. tamarensis*. Annual fluctuations of relative frequency of each water mass of samples on the same layers at common stations are shown in Figure 5. It can be found in the fluctuations that for the sum of the LSW and the MW, the frequency of preferable water for *A. tamarensis* was lowest in 2005. The result suggests that the frequency, that is, the volume of

each water mass is one of the important factors to control the abundance of *A. tamarensis* in the area.

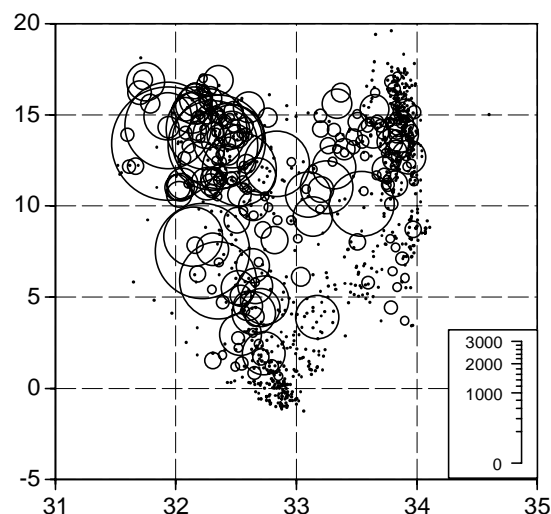


Fig. 3 Cell density of *A. tamarensis* (bubbles) on a temperature-salinity diagram in 2002-2007.

Table 2 Cell density of *A. tamarensis* in each water mass in 2002-2007.

Water mass	Number of samples	Cell density of <i>A. tamarensis</i> (cells l ⁻¹ , mean ± SD)
SWC	312	7.4 ± 27.7
LSW	182	114.0 ± 342.1
MW	262	40.8 ± 127.5
DTW	183	3.7 ± 29.2

SWC = Soya Warm Current, LSW = Low Salinity Water, MW = Mixed Water, DTW = Dichothermal Water

Table 3 Water temperature and salinity in each range of *A. tamarensis* cell density in 2002-2007.

Range of <i>A. tamarensis</i> cell density (cells l ⁻¹)	Number of samples	Water temperature (°C, mean ± SD, [min - max])	Salinity (mean ± SD, [min - max])
0	730	8.1 ± 5.8 [-1.3 - 19.6]	33.2 ± 0.8 [31.5 - 34.1]
20-80	137	11.1 ± 4.3 [0.7 - 17.0]	32.9 ± 0.7 [31.6 - 34.0]
100-980	64	11.4 ± 3.9 [1.9 - 16.9]	32.7 ± 0.6 [31.7 - 33.9]
1000≤	8	11.8 ± 3.2 [5.9 - 14.4]	32.2 ± 0.2 [31.9 - 32.5]

Table 4 Nutrient concentrations in each water mass in 2004-2007.

Water mass	Number of samples	Nutrient concentration	
		NO ₃ -N (μM l ⁻¹ , mean ± SD)	PO ₄ -P (μM l ⁻¹ , mean ± SD)
SWC	225	1.52 ± 2.41	0.26 ± 0.22
LSW	120	0.21 ± 0.86	0.35 ± 0.15
MW	156	3.51 ± 4.55	0.59 ± 0.37
DTW	125	15.79 ± 4.53	1.55 ± 0.29

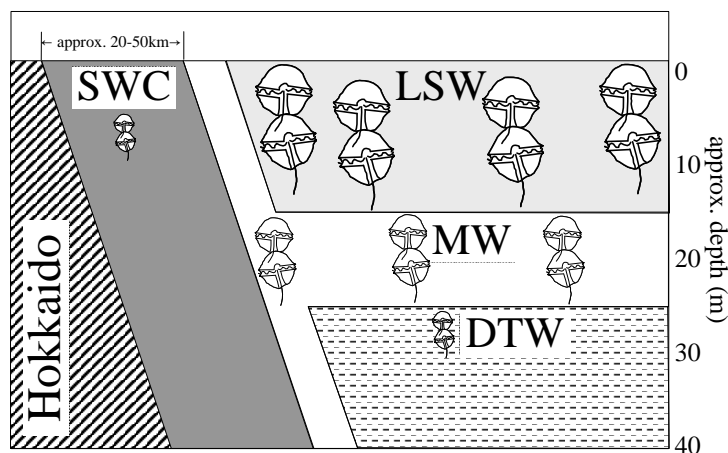


Fig. 4 Schematic diagram of spatial distribution of water masses and *A. tamarensis* in vertical section.

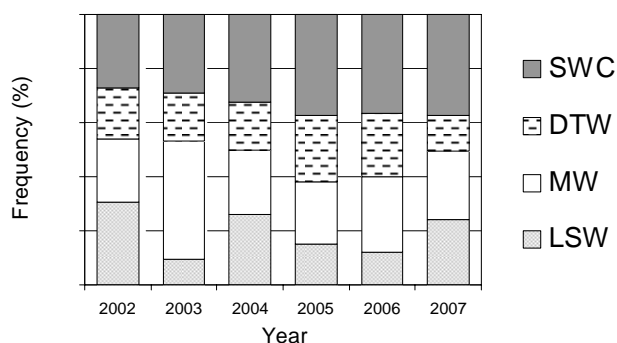


Fig. 5 Relative frequency of each water mass of samples on the same layers at 34 common stations in 2002–2007.

Acknowledgement

We are grateful to the captains and crews of R/Vs *Hokuyo Maru* and *Oyashio Maru* for kindly helping with sampling *in situ*. We also thank colleagues at the Hokkaido Fishery Experiment Station for helpful comments.

References

- Aota, M. 1975. Studies on the Soya Warm Current. *Low Temp. Sci.* **A33**: 151–172 (in Japanese with English summary).
- Fukuyo, Y. 1982. Taxonomical and ecological studies on *Protogonyaulax* occurring in Japanese coastal waters. Ph.D. thesis, University of Tokyo, Tokyo (in Japanese).
- MacIntire, J.G., Cullen, J.J. and Cembella, A.D. 1997. Vertical migration, nutrition and toxicity in the dinoflagellate *Alexandrium tamarensis*. *Mar. Ecol. Prog. Ser.* **148**: 201–216.
- Nishihama, Y. 1982. Seasonal abundance of

- Protogonyaulax* sp. causing paralytic shellfish poisoning in Funka Bay. pp. 319–327. In *Proceedings of North Pacific Aquaculture Symposium*, Anchorage, AK.
- Nishihama, Y. 1994. Paralytic shellfish poisoning. In *Scallop Fishery in the Okhotsk Sea Coast of Hokkaido*. Hokkaido University Press, Sapporo, pp. 170–180 (in Japanese).
- Ogata, T., Koike, K., Nomura, S. and Kodama, S. 1996. Utilization of organic substances for growth and toxin production by *Alexandrium tamarensis*. pp. 343–346 in *Harmful and Toxic Algal Blooms edited by T. Yasumoto, Y. Oshima and Y. Fukuyo*, UNESCO, Sendai.
- Shimada, H., Hayashi, T. and Mizushima, T. 1996. Spatial distribution of *Alexandrium tamarensis* in Funka Bay, Southwestern Hokkaido, Japan. pp. 219–221 in *Harmful and Toxic Algal Blooms edited by T. Yasumoto, Y. Oshima and Y. Fukuyo*, UNESCO, Sendai.
- Shimada, H. and Miyazono, A. 2005. Horizontal distribution of toxic *Alexandrium* spp. (Dinophyceae) resting cysts around Hokkaido, Japan. *Plankton Biol. Ecol.* **52**: 76–84.
- Shinada, A. 2005. Limiting factor for growth of *Alexandrium tamarensis* in the coastal water, northeastern part of Hokkaido, Japan in summer. *Sci. Rep. Hokkaido Fish. Exp. Stn.* **69**: 117–121 (in Japanese with English abstract).
- Tarutani, K. 1999. Ecophysiological studies on the population dynamics of toxic dinoflagellate *Alexandrium tamarensis*. *Bull. Fish. Environ. Inland Sea* **1**: 63–96 (in Japanese with English abstract).
- Yamamoto, T. and Tarutani, K. 1999. Growth and phosphate uptake kinetics of the toxic dinoflagellate *Alexandrium tamarensis* from Hiroshima Bay in the Seto Inland Sea. *Japan. Phycol. Res.* **47**: 27–3.