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A method for predicting the occurrence of paralytic shellfish poisoning along the coast of Hokkaido in the Okhotsk Sea in summer

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Abstract To design a method for predicting outbreaks of paralytic shellfish poisoning (PSP) in scallop fishing grounds, the relationship between the distribution of the toxic dinoflagellate, *Alexandrium tamarense* and the dynamics of the Soya Warm Current (SWC) was examined in the Okhotsk Sea off Hokkaido. Surveys were conducted from May to June to clarify the transportation mechanism of *A. tamarense* from the oceanic area to the coastal area. The sea-level difference (SLD) between Wakkanai and Abashiri was monitored as an index of the strength of the SWC southeastward flow in an alongshore belt to examine the possible occurrence of *A. tamarense* in the coastal area during temporal weakening of the SWC. A bottom-mounted ADCP was used for direct observations of the SWC. The results indicated that PSP occurred when the low salinity water contaminated with *A. tamarense* extended to the coast during the temporal weakening of the SWC due to a decrease of the SLD. Our results strongly indicate that predictions can be realized by monitoring the decrease of SLD as an index of the temporal weakening of the SWC after surveys of the distribution of *A. tamarense* in the oceanic area before the period of PSP occurrence.

Keywords: *Alexandrium tamarense*, Okhotsk Sea, Paralytic shellfish poisoning, Prediction, Sea-level difference, Soya Warm Current

Introduction

The coastal area of Hokkaido in the Okhotsk Sea is one of the major scallop *Mizuhopecten yessoensis* (Jay) aquaculture areas in the world, with annual yields of ca. $2x10^8$ kg [1, 2]. Paralytic shellfish poisoning (PSP) caused by the toxic dinoflagellate *Alexandrium tamarense* (Lebour) Balech has led to extensive economic damage to the fishery and the scallop aquaculture industry in summer along the Okhotsk Sea coast of Hokkaido with outbreaks occurring once every few years (Fig. 1) [1, 3]. The ability to predict PSP outbreaks would have important advantages for harvesting and shipping plans for scallops (e.g., early shipment of scallops before PSP occurrence). However, there are very few reports about practical prediction methods for PSP occurrence caused by *A. tamarense*, although there are a number of published reports about predictive models for PSP occurrence caused by *A. fundyense* along the east coast of the United States [4-11].

As the result of a survey carried out in 1985-1990, Nishihama et al. [12] pointed out that *A. tamarense* occurred simultaneously with the decrease of surface salinity in the scallop fishing ground around July. Shimada et al. [13] reported that *A. tamarense* mostly occurred in the low salinity water (salinity $\leqq$ 32.5) and rarely occurred in waters of the Soya Warm Current (SWC, salinity $\geqq$ 33.6) that flows southeastwards in an alongshore belt or in the dichothermal water layer (DTW,

temperature $\leqq 2^{o}$C) in the oceanic area deeper than 30m due to the low concentrations of nutrients in the SWC or the low temperature in the DTW. It was also suggested that the blooms of *A. tamarense* originate by germination of cysts on the continental shelf from Hokkaido to Sakhalin Island [13]. These reports imply that a weakening of the SWC due to some physical process is possibly related to the occurrence of PSP because *A. tamarense* is only rarely found in the SWC which forms the water mass usually predominating in the scallop fishing grounds along the coastal area. The SWC is driven by the sea-level difference (SLD) between the Sea of Japan and the Okhotsk Sea, and there is a high correlation between the current velocity of the SWC and the SLD [14-16]. It was also reported that the SWC mostly consists of barotrophic components in summer, and the ratio of barotrophic components is 85-87 % [17]. From these reports, a hypothetical scenario can be supposed as "PSP occurs when the low salinity water contaminated with *A. tamarense* extends to the coastal area in relation to the weakening of the SWC due to a decrease of the SLD" (Fig. 2). In the present study, detailed oceanographic surveys and retrospective analysis were conducted to test this hypothesis as a means of predicting the occurrence of PSP using the decrease of SLD as an index of the weakening of the SWC.

Materials and methods

Horizontal distribution of *A. tamarense* in the oceanic area

Area-wide surveys were conducted four times in the oceanic area in 2004, 2007 and 2008, to clarify the horizontal distribution of *A. tamarense* before and after occurrences of PSP (Table 1, Fig. 3). The surveys in late May or early June were carried out before PSP occurrences and the others in late July after PSP occurrences. Water samples were collected from each layer using Nansen bottles (1 L) at each station. Water temperature and salinity were measured using CTD instruments (SBE-911plus, Sea-Bird Electronics, Washington, USA). Five hundred ml of each water sample was fixed with 2% formalin (final concentration) and concentrated to 1 ml by sedimentation for 6 h. The number of cells of *A. tamarense* in 0.1 ml subsamples were counted under an epifluorescence microscope (XF-EFD2, Nikon, Tokyo, Japan) with UV excitation after calcofluor staining [18]. Identification of *A. tamarense* was performed on the basis of morphology after Balech [19].

The time series relationship between the occurrence of *A. tamarense* and PSP toxicity at the coastal stations

Surveys at the three coastal stations were conducted two or three times every month from June to July, 2004, 2007 and 2008, to clarify the relationship between the occurrence of *A. tamarense* and PSP toxicity in the scallop fishing grounds (Table 2, Fig. 3). Water samples were collected from each layer (see Table 2) using Van Dorn bottles (6 liter) at each station. Water temperature and salinity were measured using logging STD profilers (AST-1000, Alec Electronics, Kobe, Japan). A mooring system with data loggers (TBI32-05+37, Onset, Massachusetts, USA) was used for the continuous observation of water temperature at Stn N. Methods for fixation and concentration of water samples and cell count of *A. tamarense* were the same as stated above in the previous paragraph. Data on the PSP toxicity of scallops by mouse bioassay came from monitoring results of the Hokkaido Government and fishery cooperatives around the coast of Abashiri Subprefecture. Toxicities were described as "mouse units (MU) per 1 g whole meat or 1 g digestive diverticula".

The time series relationship between current velocity of the SWC and SLD

To analyze the relationship between the occurrences of *A. tamarense* in the coastal stations and the dynamics of the SWC, an ADCP (WH-Sentinel 300kHz, RD Instruments, California, USA) housed in a trawl-resistant bottom mount (AL-200, Flotation Technologies, Texas, USA) was used two times in 2004-2008 for direct

observations of the SWC (Table 3, Fig. 3). Hourly data of current velocity were used for the analysis after excluding error values and removing tidal signals using 24-hour tide-killer filter [20]. The analyses of the SWC were done in seven (11, 23, 35, 47, 59, 71 and 83 m) of the nineteen layers (see Table 3) at Stn H and four (10, 22, 34, 46 m) of the nineteen layers (see Table 3) at Stn S. Alongshore velocity, 140 degrees from the north was used as the velocity of the SWC for the analysis. The hourly data of SLD from June to July were calculated using hourly sea-level data of Wakkanai and Abashiri from the database of the Japan Meteorological Agency after removing tidal signals using the 24-hour tide-killer filter, while the values of the sea-level were described in Tokyo Peil (T.P.; mean sea level of Tokyo Bay). An obvious decrease in the SLD was defined as the minimum and second minimum peaks of SLD after June before the PSP toxicity increased. Time-lag correlations were analyzed for statistical tests of the correlation coefficient between velocity of the SWC and SLD in time series. The time-lag was assumed to correspond to the highest R^2 value obtained.

Retrospective analysis of PSP toxicity and SLD in time series

To investigate the relationship between the PSP occurrence and changes in the SLD, we performed a retrospective analysis to determine whether PSP had occurred simultaneously with a SLD decrease before the present study from June to August since

1996 including typical PSP occurrence years (1996, 2001 and 2002) when the PSP toxicity increased over the quarantine level (4 MU/g whole meat) and there were sufficient data records of the PSP toxicity and SLD to perform the analysis. Data on the PSP toxicity of scallops by mouse bioassay came from monitoring results of the Hokkaido Government and fishery cooperatives around the coast of Abashiri Subprefecture. The hourly data of SLD from June to August were calculated using hourly sea-level data of Wakkanai and Abashiri from the database of the Japan Oceanographic Data Center (1996-2001) or the Japan Meteorological Agency (2002, 2003, 2005 and 2006) after data processing as stated.

Results

Horizontal distribution of *A. tamarense* in the oceanic area

The horizontal distribution of water temperature, water masses (the low salinity water and SWC water) on the sea surface and cell densities of *A. tamarense* in the oceanic area are shown in Fig. 4 with cell densities of *A. tamarense* indicating the maximum value at each station. In late July 2004, *A. tamarense* occurred widely in the low salinity water and the maximum cell density (2520 cells/l) was found near the frontal area with the SWC. In early June 2007, the maximum cell density (2280 cells/l) was observed in the low salinity water near the frontal area. In late July 2007, *A. tamarense* was widely distributed in the low salinity water and the maximum cell density (860 cells/l) was found near the frontal area. In late May 2008, *A. tamarense* occurred at only one station with a low cell density (20 cells/l).

The time series relationship between the occurrence of *A. tamarense* and PSP toxicity at the coastal stations

The surface water-temperature at Stn N, changes in the vertical distribution of water temperature and salinity at Stn T, the cell density of *A. tamarense* at three stations

and PSP toxicity of scallops along the coast off Abashiri in 2004, 2007 and 2008 are shown in Fig. 5. In 2004, a cell density of 240 cells/l of *A. tamarense* was found at the surface at Stn T on July 13 concurrently with a decrease in temperature and surface salinity (Fig. 5(a)). Maximum PSP toxicity (3.8 MU/g whole meat) was detected on July 26. In 2007, a cell density of 500 cells/l of *A. tamarense* also occurred at the surface of Stn T on July 9 concurrently with a decrease in temperature and surface salinity (Fig. 5(b)), and a maximum PSP toxicity (4.2 MU/g whole meat) was detected on July 16 exceeding the quarantine level (4 MU/g whole meat). In 2008, *A. tamarense* was found during one survey in the 10m depth layer at Stn A on June 17 with a low cell density (20 cells/l)(Fig. 5(c)), and the PSP toxicities were continuously lower than the detection limit (2 MU/g whole meat).

The time series relationship between current velocity of the SWC and SLD

Changes of the current profiles within the seven layers and the alongshore velocity (140 degrees from the north, average of seven or four layers) obtained using the ADCP and SLD are shown in Fig. 6. The correlations between velocity of the SWC and SLD in time series were highly significant ($p < 0.01$) in all three years. The time lag between SLD and the SWC was 0 hour in 2007 and 2008 when the data were obtained at Stn S (Fig. 6(b), (c)), while the time lag was 13 hours in 2004 when the data were

obtained at Stn H (Fig. 6(a)). The reason for the larger time lag observed at Stn H may be that Stn H is located in the lower reaches of the SWC than Stn S. In 2004, the SWC became remarkably weak on July 3 and 12 when the obvious decreases of the SLD were observed. In 2007, the SWC got weak remarkably on July 1 and 10 concurrently with the obvious decreases of SLD. In 2008, the SWC became weak on June 15 and 26 simultaneously with the obvious decreases of SLD.

Retrospective analysis of PSP toxicity and SLD in time series

Table 4 shows dates of three events in each year related to the PSP occurrence since 1996 excluding the present study (2004, 2007 and 2008). Obvious decreases of SLD were found every year before the first detection of PSP toxicity excluding 1999, 2000, 2005 and 2006 (no detection of PSP toxicity in whole meat of scallop). Changes of SLD and PSP toxicity from June to August in the typical PSP occurrence years, 1996, 2001 and 2002 are shown in Fig. 7 where the arrows indicate obvious decreases of the sea-level difference. In 1996, the PSP toxicity began to increase in mid-July after July 8 and 10 when the obvious decreases of SLD were observed. PSP toxicities over the quarantine level (4 MU/g whole meat) were detected in early August and a maximum PSP toxicity (55 MU/g digestive diverticula) was recorded on August 6 (Fig. 7(a)). In 2001, PSP toxicity started increasing in mid-July after June 21 and July 2 when obvious

decreases of SLD were observed. PSP toxicities over the quarantine level were detected in mid-July and a maximum PSP toxicity (88 MU/g digestive diverticula) was recorded on July 19 (Fig. 7(b)). In 2002, the PSP toxicity began to increase in late June after June 10 and 24 when the obvious decreases of SLD were observed. PSP toxicities over the quarantine level were detected in early July and a maximum PSP toxicity (454 MU/g digestive diverticula) was recorded in July 8 (Fig. 7(c)).

Discussion

The maximum cell density of *A. tamarense* in the oceanic area during five events related to the PSP occurrences in 2004, 2007 and 2008 are shown in Table 5. The results in 2004 and 2007 show that significant PSP toxicities were detected after moderately-high cell densities of *A. tamarense* were found in the coastal area concurrently with an obvious decrease of SLD (i.e. the weakening of the SWC) and the inshore influx of *A. tamarense* cells with the low salinity surface waters in the offshore oceanic region. On the other hand, the result in 2008 suggests that no PSP toxicity was detected despite the obvious decrease of SLD also observed because of the very low abundance of *A. tamarense* in the oceanic area. Considering the retrospective analysis (Table 4), our results indicate that the peak of PSP toxicity was observed 2-4 weeks after the obvious decrease of SLD (i.e. the weakening of the SWC) in each year. These results support our hypothesis that "PSP occurs when the low salinity water contaminated with *A. tamarense* extends to the coastal area as the result of a weakening of the SWC due to a decrease in the SLD" (Fig. 2). Therefore, it is suggested that predictions of PSP occurrence can be made by identification of the obvious decreases in the SLD as an index of the temporal weakening of the SWC after surveys of the distribution and abundance of *A. tamarense* in the oceanic area in late May or early June.

A method for predicting PSP occurrence

Our results revealed that PSP contamination occurred when the following two conditions were satisfied:

Condition A: A bloom of *A. tamarense* occurs in the surface of the oceanic area

Condition B: Oceanic surface waters containing *A. tamarense* cells extend into the coastal area due to an obvious decrease in the SLD

The PSP prediction method can be operated using the flowchart shown in Fig. 8, since the results in the present study showed that PSP occurred a few weeks after the two conditions were satisfied. To cover the seasonal change of the distribution of *A. tamarense*, the surveys in the oceanic area and the monitorings of the SLD are planned twice-yearly from late May to early September. It has been reported that the maximum cell division rate of *A. tamarense* is ca. 0.4 division /day at 10^{o}C in laboratory [21] and blooms of *A. tamarense* were observed from May to July in Funka Bay [22, 23] and Aniva Bay [24], and almost disappeared by August due to the warm conditions (> 15^{o}C). Thus the seasonal peak of cell density in the oceanic area of the Okhotsk Sea is supposed to appear in June - August (ca. 1 month later than the coastal area) due to the lower (1-2^{o}C) sea surface temperature and the bloom may disappear by September. Therefore, the period of the surveys and the monitoring in Fig. 8 is reasonable to

perform the prediction. Needless to say, it is necessary to operate the prediction method of PSP occurrence in parallel with routine monitoring of occurrence of *A. tamarense* and PSP toxicity in the coastal area.

For the operation of the prediction method, the following standards should be applied to judge whether the two conditions are satisfied. Regarding **condition A**, PSP toxicities over the quarantine level were caused by ca. $1x10^2$ cells/1 density of *A. tamarense* in Funka Bay, southwestern Hokkaido [22] and Kure Bay, Hiroshima Prefecture [25]. It should be judged that the criteria for a "bloom of *A. tamarense*" as "occurrence of *A. tamarense* with higher cell density than 10^2 cells/1". In exceptional cases such as low cell densities of *A. tamarense* (< 10^2 cells/l) widely occur in late May or early June so that judgment of the **condition A** remains difficult, optional sampling should be done in the oceanic area in late June or early July as needed. Concerning **condition B**, it should be judged the criteria for "the obvious decrease of SLD" as "SLD decrease from usual value, ca. 20 cm, to unusual value, ca. 10 cm, based on the present study. It might be necessary to determine flexibly "the obvious decrease of SLD" as in an exceptional case as 1996 when the SLD remained around the 10 cm level from June to July (see Fig. 7). In 1996, the water temperature was markedly lower than any other year after 1990 around the coast of the Okhotsk Sea and the Sea of Japan from winter to summer [26]. Decrease of sea surface salinity due to weakening of the SWC also seems to be useful to determine "the obvious decrease of SLD" in the routine monitoring in the

coastal area.

If we can predict the weather conditions promoting “the obvious decrease of SLD”, the prediction method of PSP occurrence will be more useful. Ebuchi et al. [27] suggested that the sea-level along the west coast of Hokkaido decreased and the sea-level along the east coast of Sakhalin Island increased under the influence of northerly winds, causing a decrease of SLD. Thus changes in the SLD may be estimated by changes in the occurrence of northerly winds.

Regarding the horizontal distribution of *A. tamarense* in the oceanic area, the blooms tended to be found near the frontal areas outside the SWC. It has been reported that the formation of a cold water belt through upwelling can often be detected in the frontal areas in summer [28, 29], and that diatom blooms frequently occur through utilization of the richer nutrients in the upwelling water [30]. It is suggested that a bloom of *A. tamarense* often occurs near the frontal areas through utilization of the richer nutrients, competing with dominant diatom species (e.g., *Chaetoceros* spp., *Thalassiosira* spp.) [13].

Remaining subjects

The following two subjects remain in relation to the prediction method of PSP occurrence.

Subject 1: “What is the reason for annual fluctuation of the timing and abundance of the bloom of *A. tamarense*?”

Since *A. tamarense* prefers the low salinity water in the Okhotsk Sea [13], one of the necessary conditions for formation of the bloom may be the predominance of the low salinity water on the surface of the oceanic area. Since *A. tamarense* generally occurs in the temperature range of 5-15^{o}C from spring to summer along the coastal area of Hokkaido [31], if this temperature range occurs earlier in the low salinity water, the bloom may also appear earlier in the Okhotsk Sea. So it can be supposed that higher temperatures in spring enhance the bloom of *A. tamarense* and the predominance of the low salinity water in summer enlarges the distribution of the bloom. It has been reported that large concentrations of toxic *Alexandrium* spp. resting cysts can be found in sediment on the continental shelf off Hokkaido and Sakhalin Island in the Okhotsk Sea [3, 32]. It was also reported that blooms (> 10^3 cells/l) of *A. tamarense* have occurred from June to August in Aniva Bay, southern Sakhalin Island [24, 33, 34]. So the information about the distribution of resting cysts and vegetative cells in the Okhotsk Sea including the Russian area is important for risk management of bloom occurrence. Considering the seasonal occurrences of *A. tamarense* bloom in Aniva Bay, it is reasonable to start monitoring the bloom of *A. tamarense* in late May or early June in the oceanic area off Hokkaido. On the other hand, a decrease of sea ice and warming of intermediate water has been reported as warming phenomena [35, 36] and it can be

supposed that the formation of blooms of *A. tamarense* possibly may occur earlier in the Okhotsk Sea in the future. Therefore, for the operation of the PSP prediction method, it is important to monitor the distribution of *A. tamarense* in the oceanic area before the PSP occurrence, paying attention to annual fluctuations of the timing and abundance of the bloom of *A. tamarense*.

Subject 2: "Why did the maximum PSP toxicities vary so markedly between years?"

It can be supposed that the variation of the cellular toxin content of *A. tamarense* may influence the maximum PSP toxicities from year to year in addition to the fluctuation in the abundance of the bloom of *A. tamarense* in the oceanic area of the Okhotsk Sea. Our information about the abundance of the bloom in the oceanic area (maximum cell density in late July 2002: 360 cells/1) [13] and the occurrence of *A. tamarense* in the coastal area (maximum cell density in early July 2002: 140 cells/1, data source: Hokkaido Government) cannot explain the highest PSP toxicity (454 MU/g digestive diverticula, Fig. 7) experienced in 2002. We found that the cellular toxin contents of cultured strains of *A. tamarense* isolated in the coastal area around Hokkaido varied remarkably in the range 1-1128 fmol/cell [37]. It was also reported that the cellular toxin contents of cultured strains of *A. tamarense* isolated in the coastal area of Iwate, northeastern Japan also varied in the range of 20-160 fmol/cell [38]. Therefore it is necessary to check the cellular toxin content of *A. tamarense in situ* by sampling using plankton net in the oceanic area before the PSP occurrence for the operation of the

PSP prediction method.

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Fig. 1 Paralytic shellfish poison toxicity in whole meat of scallop along the coast off Hokkaido in the Okhotsk Sea since 1980 (Data source: Hokkaido Government). Broken line indicates quarantine level of PSP toxicity (4 mouse unit /g). Toxicity value of “ND” means “not detected (toxicity < 2 mouse unit /g)”
∗: PSP occurrence over quarantine level

Fig. 2 Schematic path of the Soya Warm Current (salinity ≧ 33.6) and location of the scallop fishing grounds and the low salinity water (salinity ≦ 32.5) contaminated with *Alexandrium tamarense* in summer by the hypothetical scenario.
(a) usual condition, (b) paralytic shellfish poisoning occurrence

Fig. 3 Map showing the 37 oceanic sampling stations (•), 3 coastal sampling stations (■) and 2 stations (▲) for observation of the Soya Warm Current using the bottom-mounted ADCP

Fig. 4 Results of observation in the oceanic area. Horizontal distribution of water temperature (contours), water masses (screentone) on sea surface and cell densities of *Alexandrium tamarense* (bubbles) in the oceanic area during each sampling period. Cell densities are the maximum value at each station

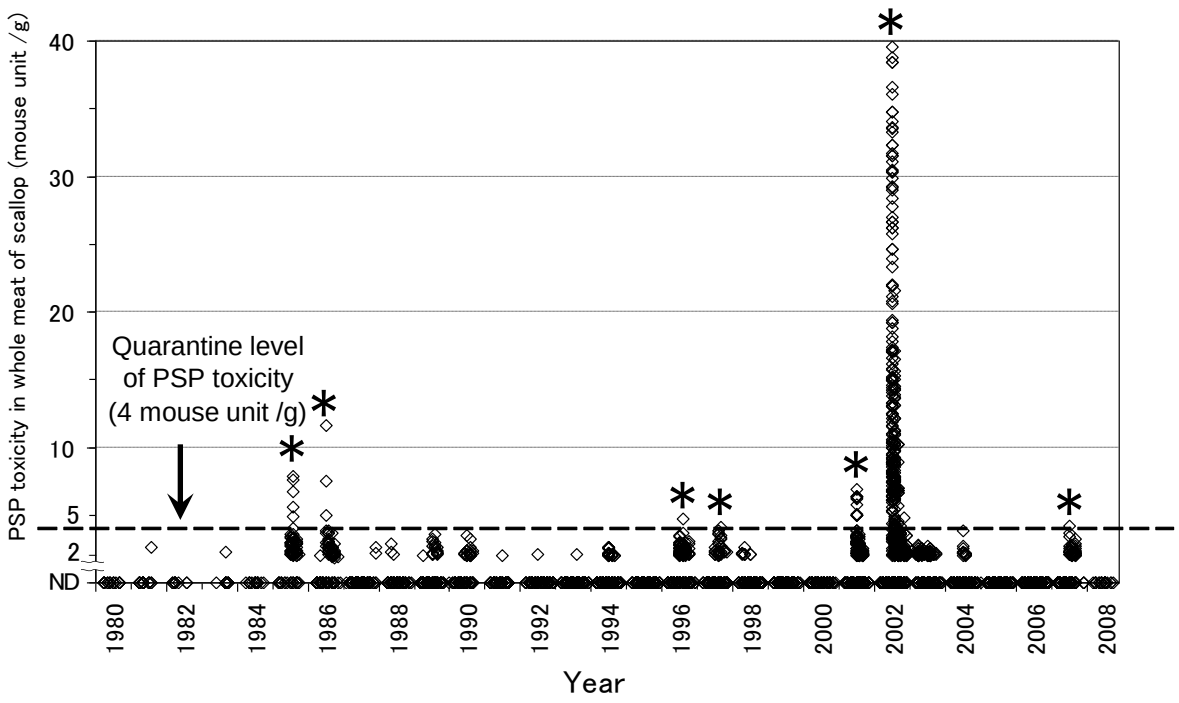
Fig. 5 Results of observation at the coastal stations in 2004 (a), 2007 (b) and 2008 (c). Water-temperature time series at Stn N (line chart), changes of vertical distribution of water temperature and salinity (contours) at Stn T in 2004 and 2007 and at Stn A in 2008, cell density of *Alexandrium tamarense* (bubbles) at all stations and PSP toxicity of scallop along the coast off Abashiri (two axes scatterplot) in June – July. Toxicity value of “ND” means “not detected (toxicity < 2 mouse unit /g)”

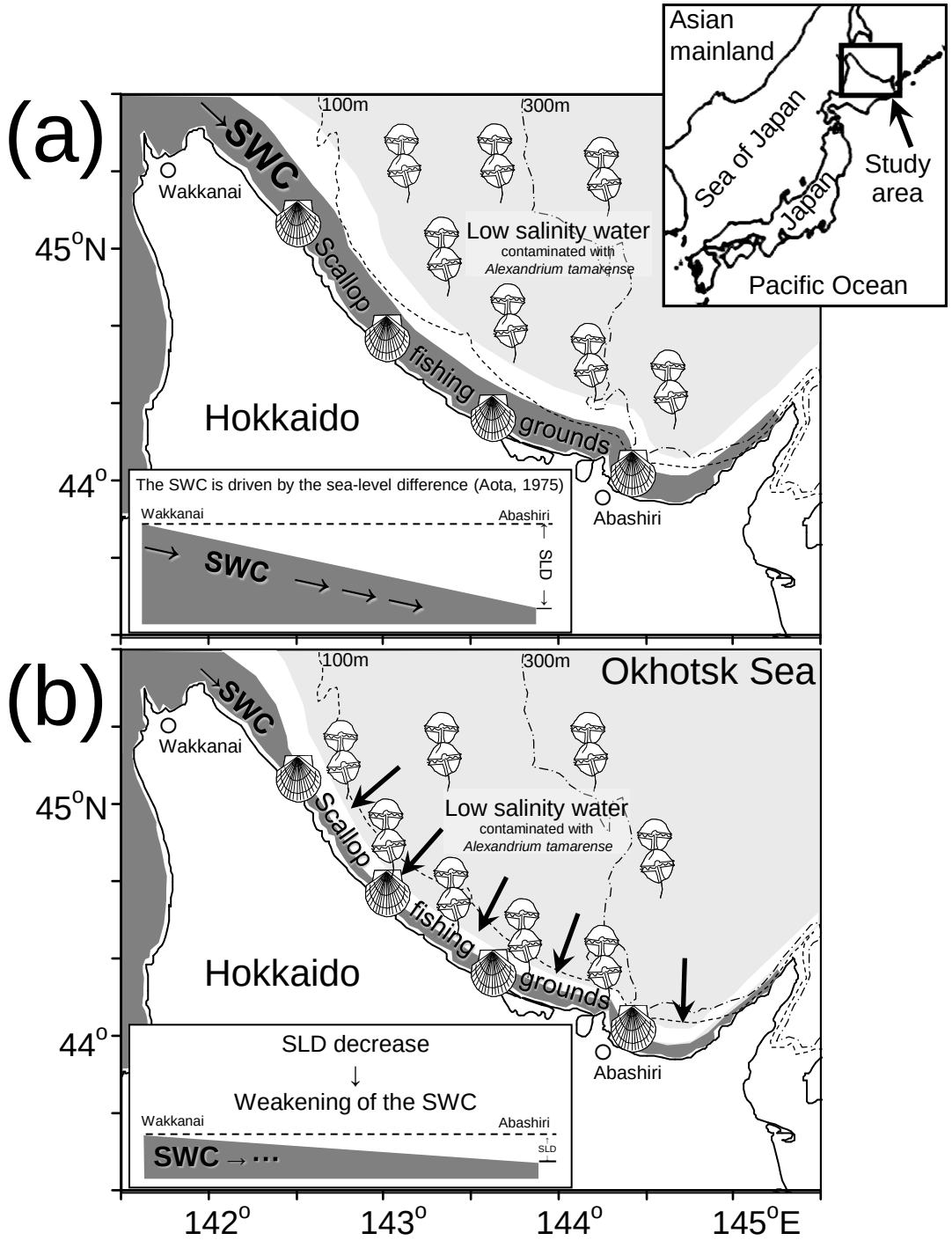
Fig. 6 Results of observation of current velocity of the Soya Warm Current and the sea-level difference in time series in 2004 (a), 2007 (b) and 2008 (c). Current profile in seven layers (vector plot) at Stn H in 2004 (depth of layer: 11, 23, 35, 47, 59, 71 and 83 m, thickness of layer: 4 m) and at Stn S in 2007 and 2008 (depth of layer: 10,22,34 and 46 m, thickness of layer: 2 m), alongshore velocity (solid line, average of seven layers) and the sea-level difference (dashed line) with maximum R-square of time lag correlation in June – July (R-square with two asterisk means significant correlation, $p < 0.01$). The arrows indicate obvious decreases of the sea-level difference. Obvious decrease of the sea-level difference is defined as the minimum and second minimum peaks of the sea-level difference after June before increase of PSP toxicity

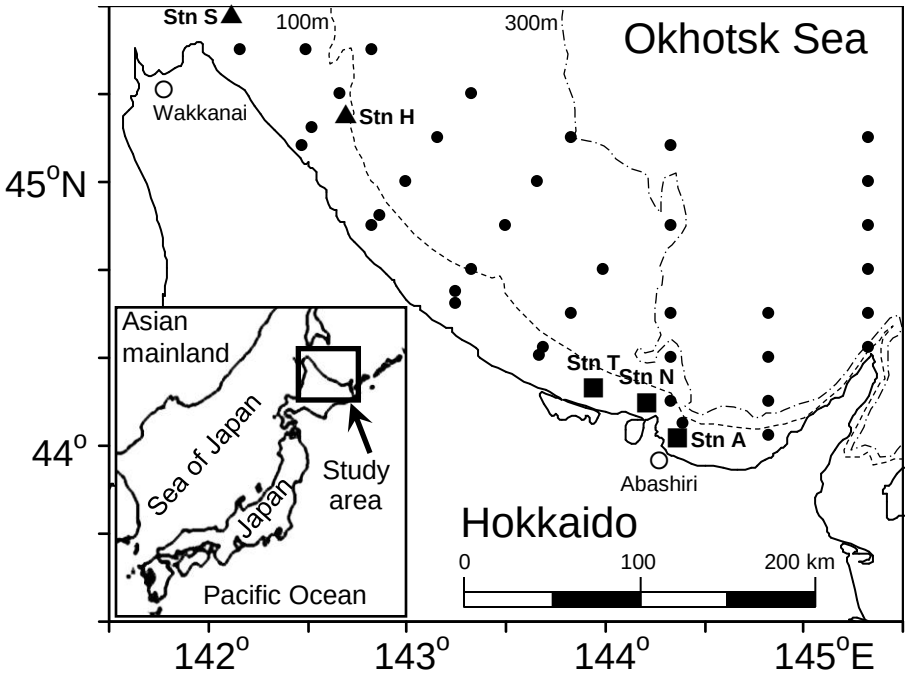
Fig. 7 Results of retrospective analysis. Changes of the sea-level difference and PSP toxicity in June – August 1996 (a), 2001 (b) and 2002 (c). The arrows indicate obvious decreases of the sea-level difference. Obvious decrease of the sea-level difference is defined as the minimum and second minimum peaks of the sea-level difference after June before increase of PSP toxicity. Toxicity value of “ND” means “not detected (toxicity < 2 mouse unit /g)”

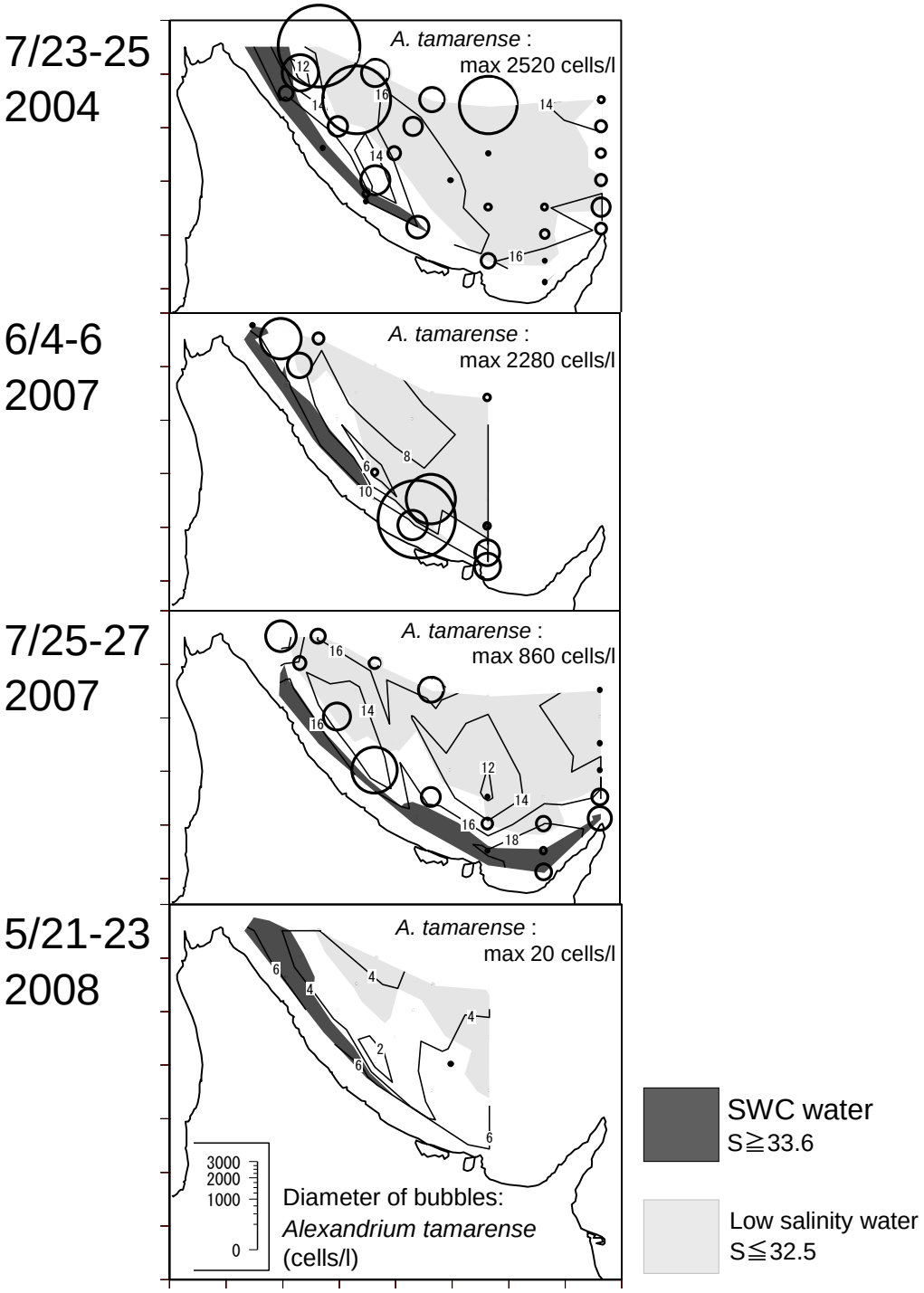
Fig. 8 Flowchart of prediction of PSP occurrence concluded by the present study

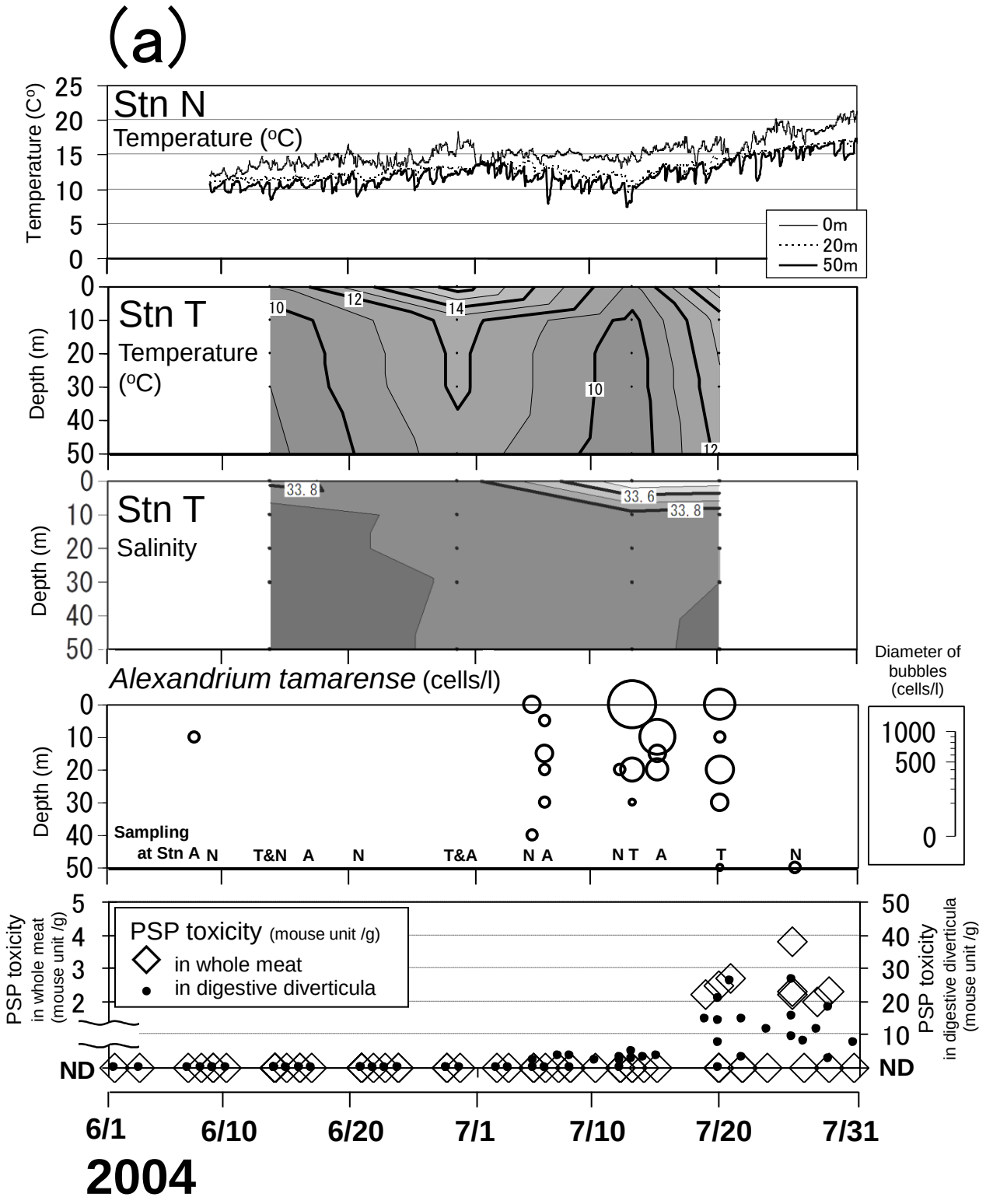
Fig. 1

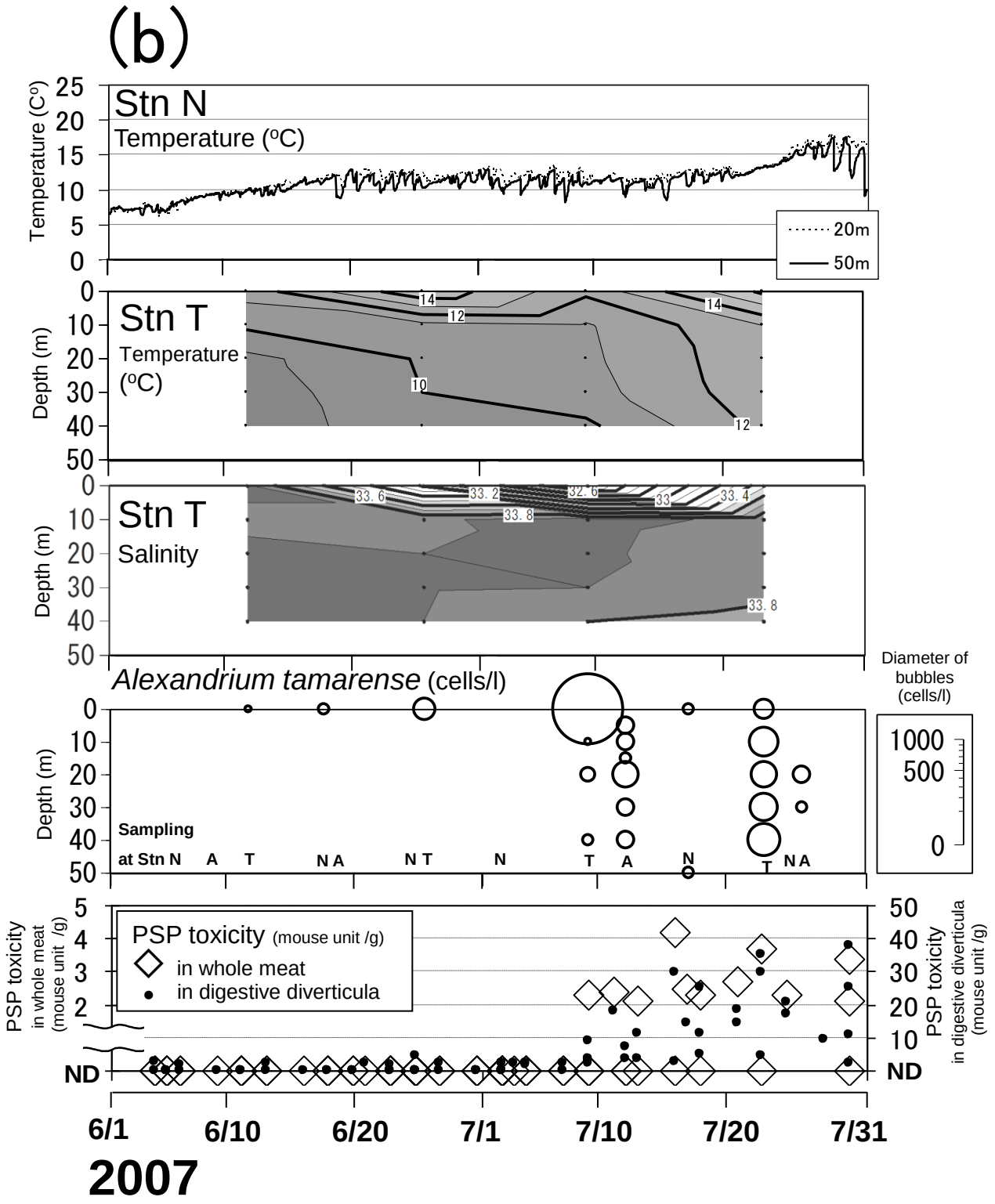

PSP toxicity in whole meat of scallop (mouse unit /g)
40
30
20
10
5
2
ND
Quarantine level
of PSP toxicity
(4 mouse unit /g)
1980
1982
1984
1986
1988
1990
1992
1994
1996
1998
2000
2002
2004
2006
2008
Year

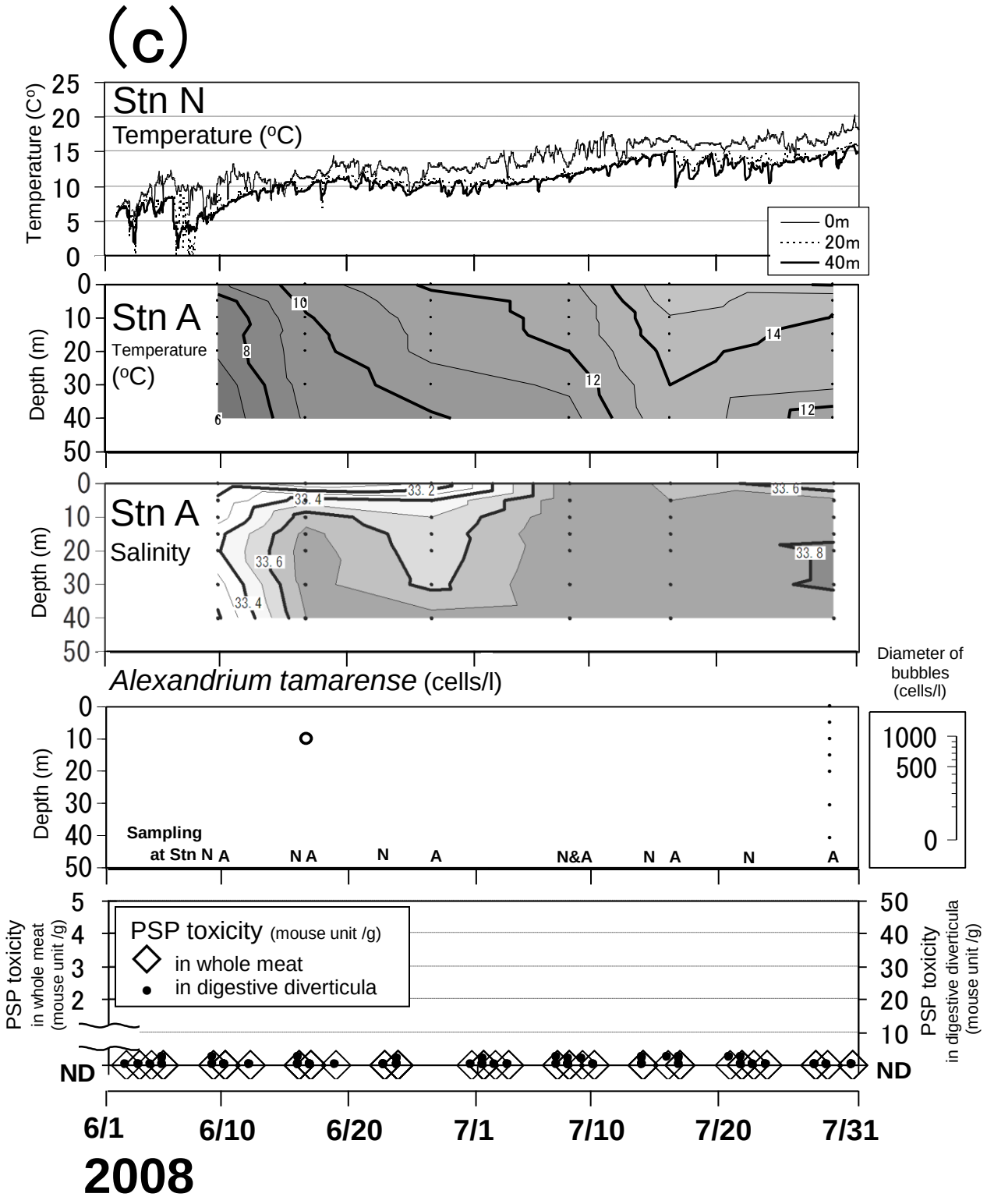

(a)
SWC
Wakkanai
100m
300m
Low salinity water
contaminated with
Alexandrium tamarense
Scallop
fishing
grounds
Hokkaido
45°N
44°
The SWC is driven by the sea-level difference (Aota, 1975)
Wakkanai
Abashiri
SLD
SWC
Abashiri
Asian
mainland
Sea of Japan
Japan
Study
area
Pacific Ocean
(b)
SWC
Wakkanai
100m
300m
Okhotsk Sea
Low salinity water
contaminated with
Alexandrium tamarense
Scallop
fishing
grounds
Hokkaido
45°N
44°
SLD decrease
↓
Weakening of the SWC
Wakkanai
Abashiri
SLD
SWC → ···
Abashiri
142°
143°
144°
145°E

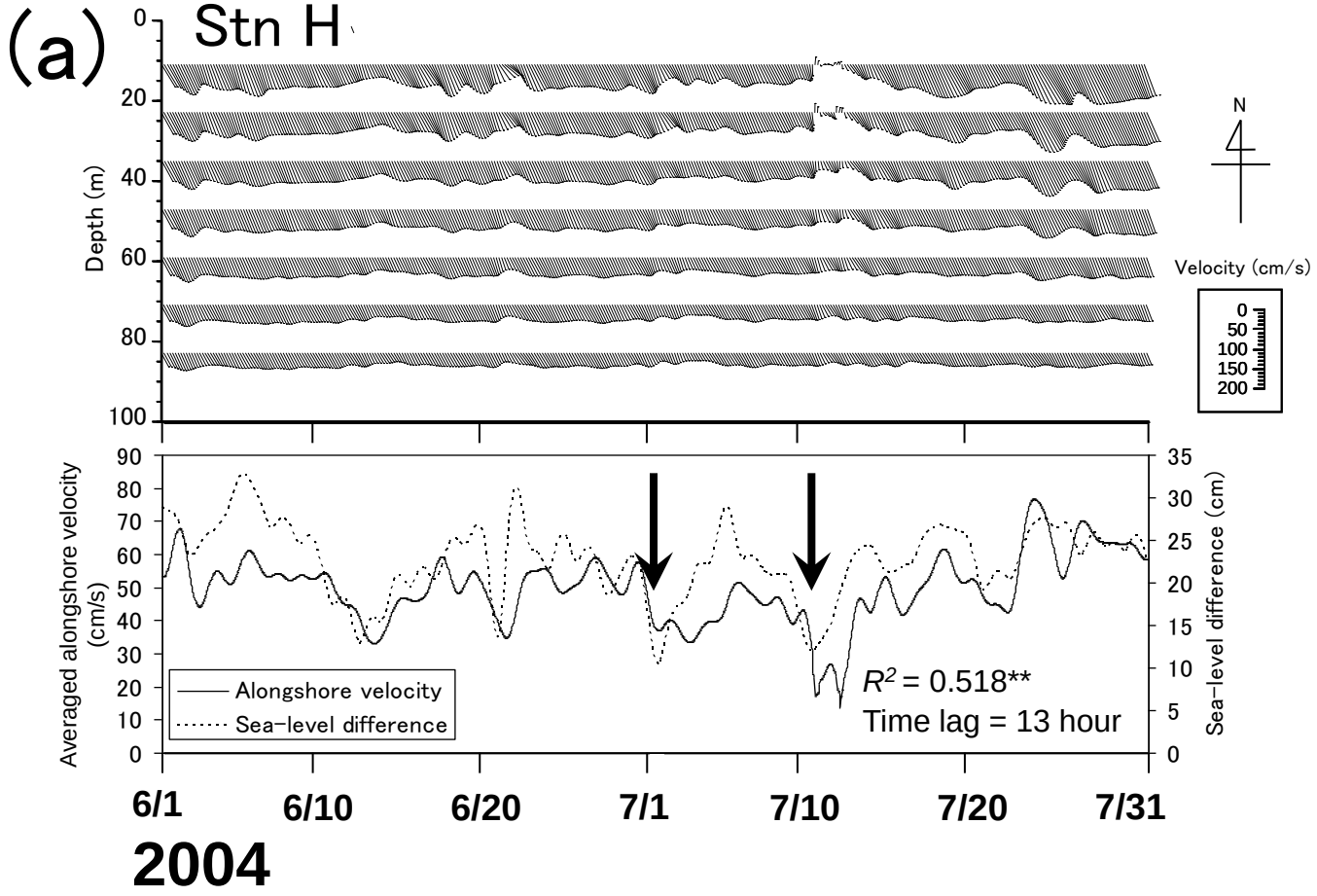

Stn S
100m
300m
Okhotsk Sea
Wakkanai
Stn H
45°N
Asian
mainland
Stn T
Stn N
Stn A
Sea of Japan
Study
area
Japan
Abashiri
Hokkaido
0
100
200 km
Pacific Ocean
44°
142°
143°
144°
145°E

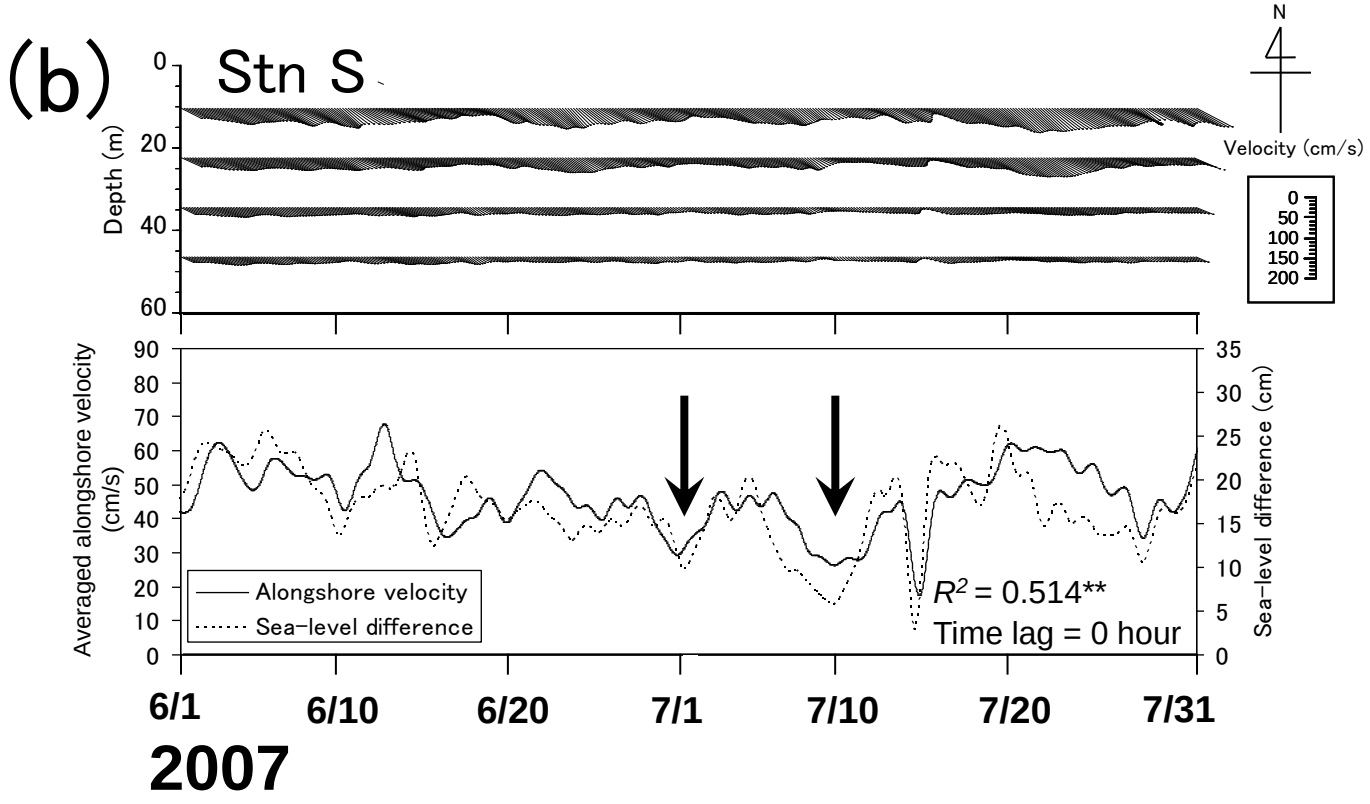

7/23-25
2004
A. tamarense :
max 2520 cells/l
6/4-6
2007
A. tamarense :
max 2280 cells/l
7/25-27
2007
A. tamarense :
max 860 cells/l
5/21-23
2008
A. tamarense :
max 20 cells/l
3000
2000
1000
0
Diameter of bubbles:
Alexandrium tamarense
(cells/l)
SWC water
S≧33.6
Low salinity water
S≦32.5


(a)
Stn N
Temperature (°C)
Temperature (C°)
0m
20m
50m
Stn T
Temperature (°C)
Depth (m)
Stn T
Salinity
Depth (m)
Alexandrium tamarense (cells/l)
Diameter of bubbles (cells/l)
1000
500
0
Depth (m)
Sampling at Stn A
N
T&N
A
N
T&A
N A
N T A
T
N
PSP toxicity (mouse unit /g)
in whole meat
in digestive diverticula
PSP toxicity in whole meat (mouse unit /g)
PSP toxicity in digestive diverticula (mouse unit /g)
ND
ND
6/1
6/10
6/20
7/1
7/10
7/20
7/31
2004


(b)
Stn N
Temperature (ºC)
Temperature (Cº)
20m
50m
Stn T
Temperature (ºC)
Depth (m)
Stn T
Salinity
Depth (m)
Alexandrium tamarense (cells/l)
Diameter of bubbles (cells/l)
1000
500
0
Depth (m)
Sampling at Stn N A T N A N T N T A N T N A
PSP toxicity (mouse unit /g)
in whole meat
in digestive diverticula
PSP toxicity in whole meat (mouse unit /g)
PSP toxicity in digestive diverticula (mouse unit /g)
ND
6/1
6/10
6/20
7/1
7/10
7/20
7/31
2007


(c)
Stn N
Temperature (°C)
Temperature (C°)
0m
20m
40m
Stn A
Temperature
(°C)
Depth (m)
Stn A
Salinity
Depth (m)
Alexandrium tamarense (cells/l)
Diameter of bubbles (cells/l)
1000
500
0
Depth (m)
Sampling at Stn N A N A N A N&A N A N A
PSP toxicity (mouse unit /g)
in whole meat
in digestive diverticula
PSP toxicity in whole meat (mouse unit /g)
PSP toxicity in digestive diverticula (mouse unit /g)
ND
ND
6/1
6/10
6/20
7/1
7/10
7/20
7/31
2008


(a)
Stn H
Depth (m)
0
20
40
60
80
100
N
Velocity (cm/s)
0
50
100
150
200
Averaged alongshore velocity (cm/s)
Sea-level difference (cm)
Alongshore velocity
Sea-level difference
R^2 = 0.518**
Time lag = 13 hour
6/1
6/10
6/20
7/1
7/10
7/20
7/31
2004


(b)
Stn S
N
Velocity (cm/s)
0
50
100
150
200
Depth (m)
0
20
40
60
Averaged alongshore velocity (cm/s)
Sea-level difference (cm)
Alongshore velocity
Sea-level difference
R^2 = 0.514**
Time lag = 0 hour
6/1
6/10
6/20
7/1
7/10
7/20
7/31
2007

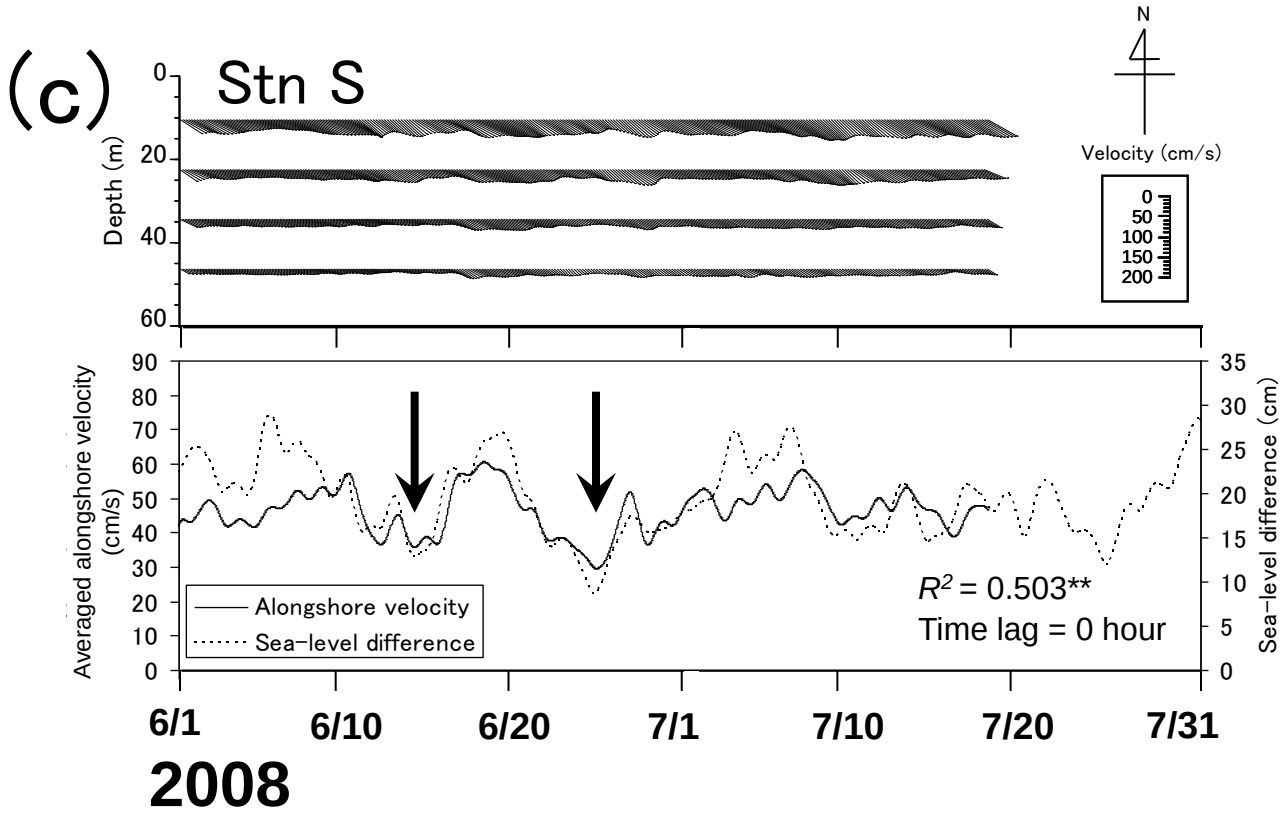

(c)
Stn S
Depth (m)
0
20
40
60
N
Velocity (cm/s)
0
50
100
150
200
Averaged alongshore velocity (cm/s)
Sea-level difference (cm)
Alongshore velocity
Sea-level difference
R^2 = 0.503**
Time lag = 0 hour
6/1
6/10
6/20
7/1
7/10
7/20
7/31
2008

Fig. 7

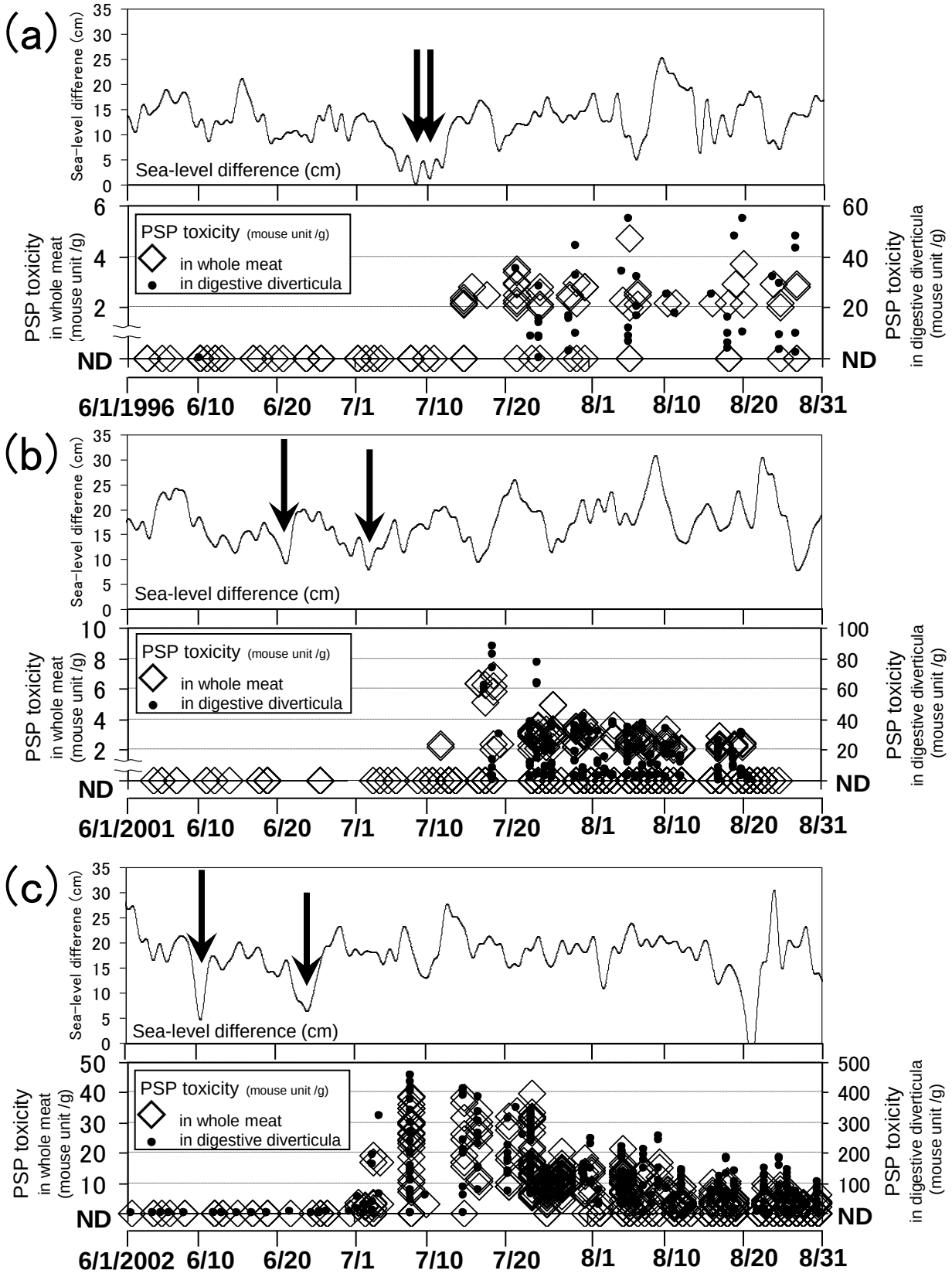

(a)
Sea-level differene (cm)
Sea-level difference (cm)
PSP toxicity (mouse unit /g)
in whole meat
in digestive diverticula
PSP toxicity in whole meat (mouse unit /g)
PSP toxicity in digestive diverticula (mouse unit /g)
ND
6/1/1996 6/10 6/20 7/1 7/10 7/20 8/1 8/10 8/20 8/31
(b)
Sea-level differene (cm)
Sea-level difference (cm)
PSP toxicity (mouse unit /g)
in whole meat
in digestive diverticula
PSP toxicity in whole meat (mouse unit /g)
PSP toxicity in digestive diverticula (mouse unit /g)
ND
6/1/2001 6/10 6/20 7/1 7/10 7/20 8/1 8/10 8/20 8/31
(c)
Sea-level differene (cm)
Sea-level difference (cm)
PSP toxicity (mouse unit /g)
in whole meat
in digestive diverticula
PSP toxicity in whole meat (mouse unit /g)
PSP toxicity in digestive diverticula (mouse unit /g)
ND
6/1/2002 6/10 6/20 7/1 7/10 7/20 8/1 8/10 8/20 8/31

Fig. 8

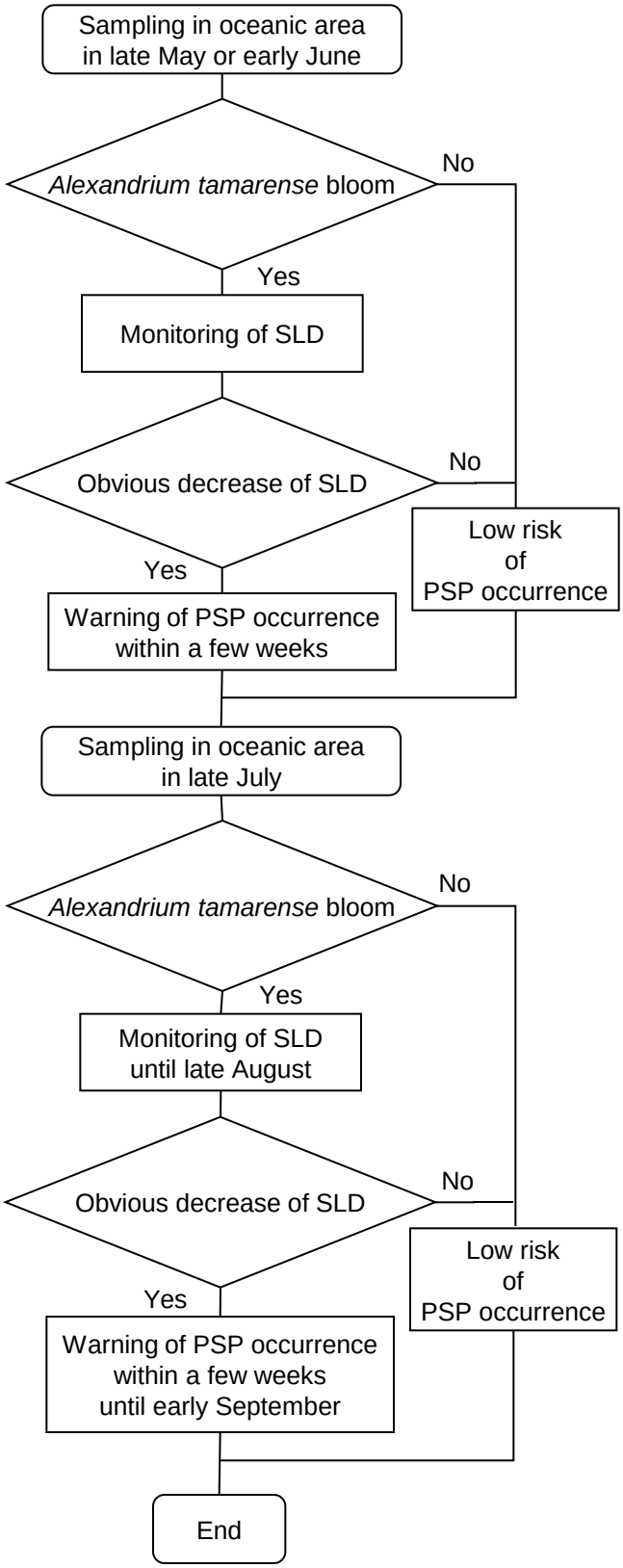

Sampling in oceanic area in late May or early June
Alexandrium tamarense bloom
No
Yes
Monitoring of SLD
Obvious decrease of SLD
No
Low risk of PSP occurrence
Yes
Warning of PSP occurrence within a few weeks
Sampling in oceanic area in late July
Alexandrium tamarense bloom
No
Yes
Monitoring of SLD until late August
Obvious decrease of SLD
No
Low risk of PSP occurrence
Yes
Warning of PSP occurrence within a few weeks until early September
End

Table 1

List of periods the surveys were carried out in the oceanic area, number of stations ("●" on Fig. 3), depth for water sampling and research vessels.

Period of survey (mo/d/yr)	Number of stations	Sampling depth (m)	Research vessel
7/23-25/2004	35	0, 10, 20, 30, 40	Oyashio Maru[a]
6/4-6/2007	27	0	Hokuyou Maru[b]
7/25-27/2007	35	0, 10, 20, 30, 40	Oyashio Maru
5/21-23/2008	23	0	Hokuyou Maru

[a] Oyashio Maru: 178 ton, R/V of Hokkaido Central Fisheries Research Institute

[b] Hokuyou Maru: 214 ton, R/V of Hokkaido Wakkanai Fisheries Research Institute

Table 2

List of the three coastal stations for water sampling and oceanographic observation ("■" on Fig. 3), date and depth of sampling.

Station and Position	Sampling depth (m)	Sampling date					
		2004		2007		2008	
		June	July	June	July	June	July
Stn T (off Tokoro) 143°59.2'E, 44°14.3'N	0, 10, 20, 30, 40 (50[a])	14, 29	13, 20	12, 26	9, 23	-	-
Stn N (off Notoro) 144°10.6'E, 44°09.3'N	0, 5, 10, 20, 40, 50	9, 14, 21	5, 12, 26	6, 18, 25	2, 17, 25	9, 16, 23	8, 14, 22
Stn A (off Abashiri) 144°21.1'E, 44°02.4'N	0, 5, 10, 15, 20, 30, 40	8, 17, 29	6, 15	9, 19	12, 26	10, 17, 27	8, 16, 29
Depth of temperature logger at Stn N (m)		0, 20, 50		20, 50		0, 20, 40	
Sampling interval		1 hour		1 hour		1 hour	

[a] Samplings from 50 m deep at Stn T were conducted in 2004

Table 3

List of the two stations for observations of the Soya Warm Current using the bottom-mounted ADCP ("▲" on Fig. 3) and duration of observation

Station and position	Depth of bottom (m)	Sampling layers	Duration of observation (mo/d/yr)		Sampling interval (hour)
			Start	End	
Stn H (off Hama-tonbetsu) 142°39.9'E, 45°15.5'N	90	19 layers from 11m to 83 m at 4 m intervals	5/25/2004	5/24/2005	1
Stn S (off Soya-misaki) 142°04.9'E, 45°38.1'N	51	19 layers from 10 m to 46 m at 2 m intervals	9/23/2006	7/24/2008	1

Table 4

Dates of three events related to the occurrence of paralytic shellfish poisoning in 1996-2003, 2005 and 2006

Year	Date of events related to PSP occurrence (mo/d)		
	Obvious decrease of the sea-level difference [a]	First detection of PSP toxicity in whole meat of scallop	Peak of PSP toxicity in whole meat of scallop
1996 [b]	7/8 and 10	7/15	8/6
1997 [b]	6/28 and 29	7/22	8/25
1998	6/3 and 21	7/24	7/24 and 27
1999	6/7 and 22	not detected	not observed
2000	no data	not detected	not observed
2001 [b]	6/21 and 7/2	7/12	7/19
2002 [b]	6/10 and 24	7/1	7/8
2003	6/10 and 7/15	3/26 [c]	7/28
2005	6/30 and 7/27	not detected	not observed
2006	6/3 and 7/3	not detected	not observed

[a] Obvious decrease of the sea-level difference is defined as the minimum and second minimum peaks of the sea-level difference after June before increase of PSP toxicity

[b] PSP occurrence year (maximum PSP toxicity $\geqq$ 4 mouse unit / g)

[c] Detected toxicity in scallop probably due to the residual toxin accumulated in 2002 summer

Table 5

Maximum cell density of *Alexandrium tamarense* in the oceanic area and dates of five events related to the occurrence of paralytic shellfish poisoning in 2004, 2007 and 2008

Year	Maximum cell density of *Alexandrium tamarense* in oceanic area (cells/l)		Date of events related to PSP occurrence (mo/d)				
	Late May or early June	Late July	Obvious decrease of the sea-level difference [a]	Weakening of the Soya Warm Current	Peak of *Alexandrium tamarense* occurrence at coastal stations	First detection of PSP toxicity in whole meat of scallop	Peak of PSP toxicity in whole meat of scallop
2004	-	2520	7/1 and 11	7/3 and 12	7/13	7/19	7/26
2007	2280	860	7/1 and 10	6/30 and 7/10	7/9	7/9	7/16
2008	20	-	6/15 and 26	6/15 and 26	not observed	not detected	not observed

[a] Obvious decrease of the sea-level difference is defined as the minimum and second minimum peaks of the sea-level difference after June before increase of PSP toxicity