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Seasonal change of riverine nutrients and distribution of chlorophyll *a* in Ishikari Bay, subarctic oligotrophic coastal environment of Japan.

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Abstract: Nutrients and Chlorophyll *a* were measured in the Ishikari River Plume and Out-Plume area in Ishikari Bay. Ishikari Bay is considered as oligotrophic because of an inflow of Tsushima warm current despite in a subarctic region. However, it receives a nutrient flux from the Ishikari River, the second largest river in catchment area in Japan. In spring, when the riverine supply of nutrients was highest due to a spring thaw, however, average Chl *a* concentrations were not different between the Plume (2.3 mg m^{-3}) and the Out-Plume (2.4 mg m^{-3}).

In autumn, a high average concentration of Chl *a* , (7.4 mg m^{-3}) was observed in the Plume which were dominated by micro-sized Chl *a* (>10 μm). In the Out-Plume, pico- and nano-sized Chl *a* (2-10 μm) dominated throughout the season. Despite high nitrate concentrations in the Plume of spring, light attenuation coefficients were relatively high near the river mouth due to the influence of suspended solids from the river, and phosphate was depleted offshore of the Plume. Thus, either light or phosphate limitation was considered for the lower Chl *a* concentrations in the Plume of spring.

Keywords: *Nutrients, Size-fractionated Chl a, Estuary, Plume*

1 Introduction

Phytoplankton are commonly the most important primary producers in coastal ecosystems, strongly influence material cycles and support higher trophic organisms such as zooplankton and filter feeders (*e.g.* clams, krill, sponges). Extensive studies on phytoplankton biomass, productivity and nutrients fluxes have been carried out in oceanic and coastal waters around the world. As a result, it is recognized that phytoplankton size distributions are related to phytoplankton biomass and the structure of food chains in the ocean (KIORBOE *et al.* 1990), and that the growth of marine phytoplankton is directly dependent on their abilities to utilize nutrients and light in environments where neither nutrients nor light are optimal for these processes. Ambient nutrients concentrations are believed to be an important factor in regulating the size structure of a phytoplankton community (MAITA and ODATE, 1988; SHIOMOTO, 1997); Large–sized phytoplankton become dominant in eutrophic conditions (nutrient abundant) and small–sized ones in oligotrophic conditions. Also, while sizeable data on size fractionated phytoplankton has been obtained from open–ocean waters (*e.g.* PLATT *et al.*, 1983; JOINT *et al.*, 1992; JOCHEM and ZEITSCHEL 1993), only a few report has been known from coastal systems especially in the subarctic coastal waters.

In the western subarctic North Pacific, seasonal variation of primary production and size

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fractionated chlorophyll *a* has been reported during the KNOT (44° N, 155° E) time–series experiment (IMAI *et al.*, 2002). In the coastal subarctic, intensive research has been carried out in Funka Bay (KUDO and MATSUNAGA, 1999; KUDO *et al.*, 2000). However, few chemical and biological studies were carried out in Ishikari Bay (YOSHIDA *et al.*, 1977). Unlike the Pacific coastal region which is influenced by the subarctic ocean current (Oyashio) with high nutrients, Ishikari Bay receives a little nutrient fluxes from oligotrophic subtropical (Tsushima) warm water current (YOSHIDA *et al.*, 1977). Thus, the Ishikari Bay is characterized as oligotrophic subarctic coastal water with a considerable influence of riverine discharge from the Ishikari River.

The present study is the first attempt to document the dynamics of nutrients and phytoplankton biomass in Ishikari Bay. We used chlorophyll *a* (Chl *a*) as an indicator of phytoplankton biomass. Chl *a* is the prevailing photosynthetic pigment found in phytoplankton and has been extensively used to estimate phytoplankton biomass. Nutrient concentrations in a coastal water body are influenced by nutrient loadings, the degree of mixing with freshwater and seawater, and biological uptake. This study therefore assesses the spatio–temporal distribution of nutrients and phytoplankton biomass in relation to the oceanographic conditions in spring, summer and autumn, except for in winter, and the Plume and Out–Plumes area in Ishikari Bay.

2 Materials and Method

2.1 Study Area

The study area of approximately 4,370 km^2 lies between latitude 43° 10′ N to 44° 00′ N, and between longitude 140° 30′ E to 141° 22′ E. Twenty–six sampling stations were allocated in Ishikari Bay (Fig. 1). Six stations (representative stations of Plume and Out–Plume areas) were assigned for a detailed observation of nutrient and size fractionated phytoplankton biomass.

2.2 Sampling

Three cruises were carried out in spring (May 7–9), summer (August 18–20) and autumn (November 4–6) of 2006. Nutrients (NO_3, NO_2 NH_4, PO_4 and Si $(OH)_4$, phyto-

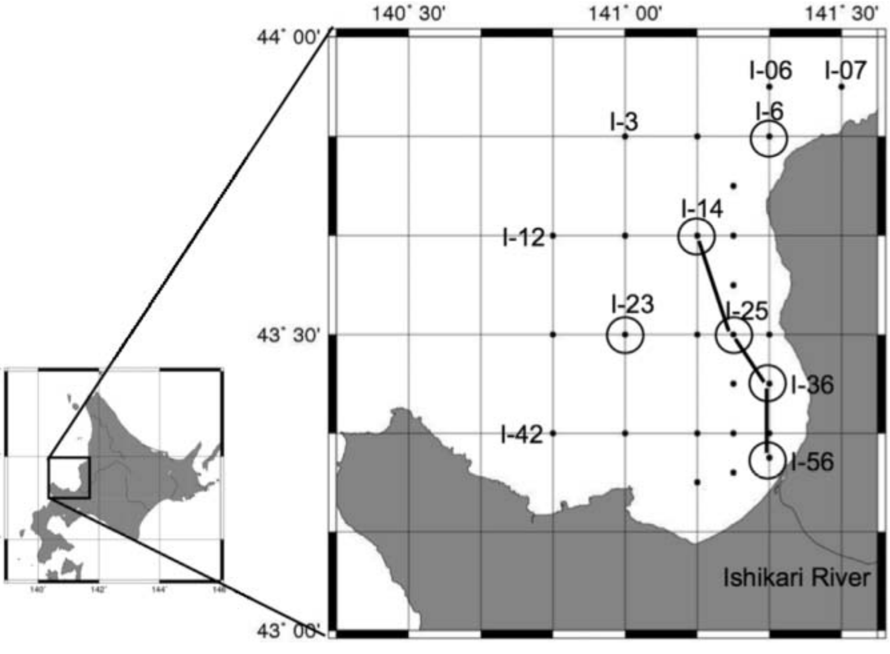

Fig. 1. Sampling stations in Ishikari Bay. Large circles indicate station where size- fractionated Chl *a* measurement was conducted. Bar indicates transect for contour plots.

plankton biomass (Chl *a*) and other related physical and chemical parameters were measured. Profiles of temperature, salinity, sigma *t* and Photosynthetic Active Radiation (PAR) were measured using a Sea Bird 911 CTD system equipped with a PAR sensor. The CTD was equipped with a carousel multi–sampler of 12 Niskin bottles (2.5–L) to collect discrete samples for macronutrients and Chl *a* in the water column (down to 5 m above the sea floor) at each sampling station.

2.3 Chlorophyll *a* and nutrient analyses

For total and size–fractionated Chl *a* , 140 ml of seawater were filtered, respectively, through Whatman GF/F (25 mm diameter, nominal pore size 0.7 μm), and 2 and 10 μm pore size Whatman Nucleopore polycarbonate filters respectively using parallel filtration. All filtrations were done under low vacuum pressure (<250 mm Hg) ; after filtration, filters were soaked in vials containing 6 mL of *N*, *N'*–dimethylformamide (DMF) and stored at −30 ℃ in the dark for 24 hr until analysis (SUZUKI and ISHIMARU, 1990). The concentration of the extracted Chl *a* and phaeo–pigment were measured fluorometrically using a HITACHI F2000 fluorescence spectrophotometer. The definitions for the size fractions were as follows: micro–sized (retained on a 10 μm filter), nano–sized (passing through 10 μm but retained by 2 μm), and pico–sized (passing through 2 μm but retained on 0.7 μm).

Water samples for nutrients were stored frozen at –30 ℃ until analysis in the laboratory. Concentrations of the dissolved inorganic nutrients were determined using an air–segmented continuous flow analyzer (QuAAtro, BRAN + LUBBE).

2.4 Underwater irradiance

Underwater irradiance was measured using a PAR sensor mounted on the Sea Bird 911 CTD system. The total diffuse attenuation coefficient, k_d for downward irradiance was determined from the equation:

$$E_d(z) = E_d(0)\, e^{-k_d z}$$

where $E_d(z)$ and $E_d(0)$ are the values of downward photosynthetically active radiation (PAR, 400–700 nm) at depth *z* (m) and just below the surface, respectively. The above equation is more satisfactory for monochromatic light but it can be used for a broad waveband as a useful approximation (KIRK, 1986). The average value of the attenuation coefficient $\overline{k}_d$ was used for calculating the euphotic zone ($Z_{1\%}$ of surface irradiance) (KIRK, 1986). Where station maximum depth is shallower than the euphotic depth, the maximum depth was computed as euphotic depth.

2.5 Statistical analyses

Physical water properties, nutrients concentration and Chl *a* (total and fractionated) concentrations were compared for spring, summer and autumn of 2006 using a two–way analysis of variance (ANOVA), whereas, Duncan multiple range test was used for separation of means. Intercorrelation of variables was investigated using the Pearson Product Moment Correlations coefficient.

Vertical profiles of nutrient concentrations in the water column were statistically compared for the different season (spring, summer and autumn) and area (Plume and Out–Plume) using ANOVA. This was achieved by grouping the water column as surface waters (0– 20 m) based on the average euphotic depth of Plume, subsurface waters (20–40 m) based on the average euphotic depth of Out–Plume and water column depths > 40 m as bottom waters (40–80 m). Nutrient standing stock was integrated to 50 m depth across seasons by trapezoidal integration. As NO_2 concentration was negligible in the samples, total concentration of dissolved inorganic nitrogen (DIN) is the sum of NO_3 and NH_4. Total and fractionated Chl *a* were integrated from 0 to 20 m.

3 Results

3.1 River discharge in Ishikari Bay.

To assess the spatial distribution of variables, especially nutrients and Chl *a* , Ishikari Bay was classified into river Plume (hereafter, Plume) and outside the Plume (Out–Plume) areas. Plume may be literally defined as an area in the sea under the influence of river water, identified by visual observation such as ocean color or using satellite images. In the

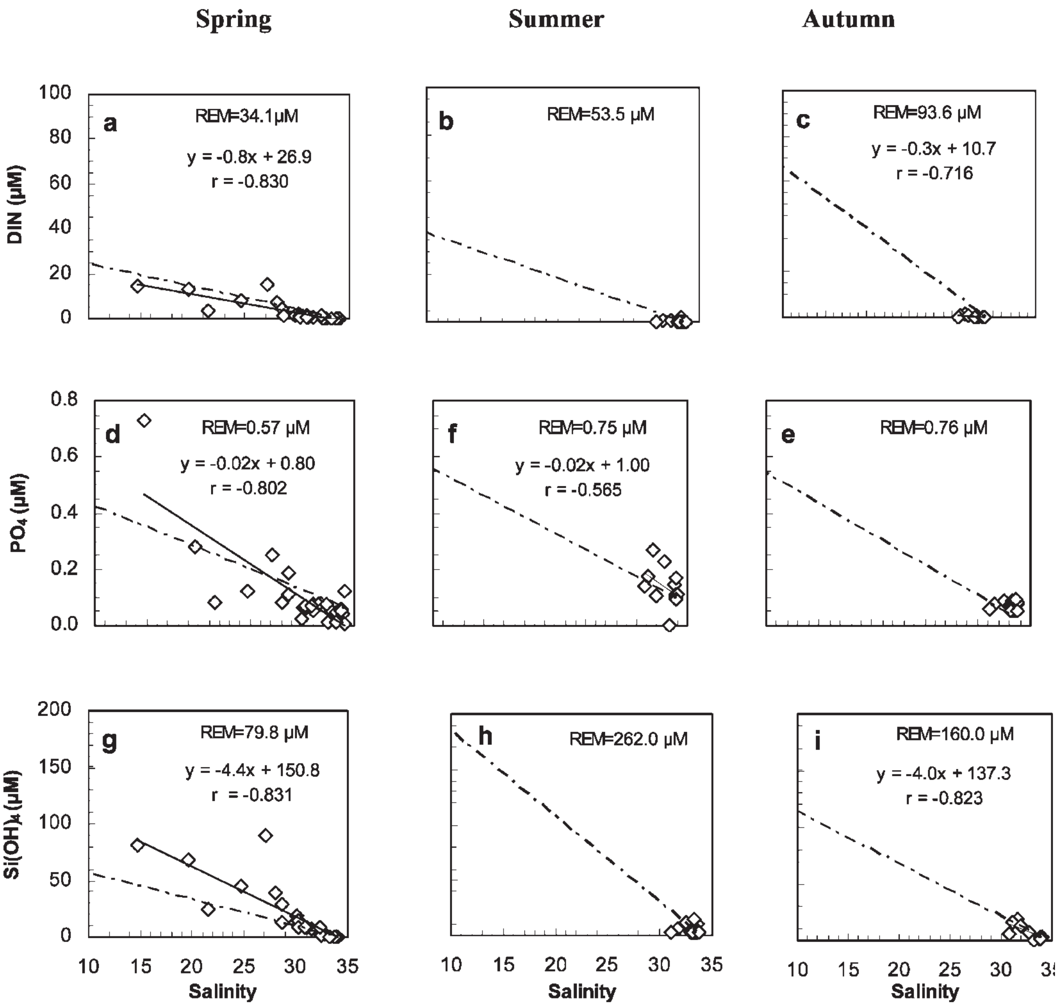

Fig. 2. Theoretical dilution lines (dash) and regression lines (solid) of salinity versus nutrients (DIN, PO_4, $Si(OH)_4$) during spring (a, d & g), summer (b, e & h) and autumn (c, f & i) in Ishikari Bay. The regression lines were indicated when regression was statistically significant ($p<0.05$). REM means river end-member which was indicated in Table 1..

Table 1 Surface nutrient concentration, relative ratio of nutrients, water discharge and suspended particulate matter (SPM) at the most downstream station (Ishikari O-hashi) where discharge monitoring was conducted in the Ishikari River.

Parameters	Spring	Summer	Autumn
DIN (μM)	34.1	53.5	93.6
PO_4 (μM)	0.57	0.76	0.75
Si $(OH)_4$ (μM)	79.8	262.0	160.0
Si:P	140.0	342.4	246.9
DIN:P	60.0	70.4	24.2
Si:DIN	2.3	4.9	1.7
Discharge (m^3s^{-1})	2250	400	750
SPM (mgl^{-1})	450	10.0	40.0

different seasons (spring, summer and autumn), the theoretical dilution lines of salinity gradient between the oceanic end-member (nutrient concentration of the most saline seawater) and riverine end-member (nutrient concentration of river water) were respectively obtained (Fig. 2). Since riverine end-member of nutrients changed seasonally (Table 1), a fixed criterion of salinity for distinguishing between the Plume and Out-Plume was not practical in considering the influence of riverine nutrients in this study. Thus, we calculated the salinity criteria for the Plume area, which was equivalent to 1 μM increase of DIN from the theoretical dilution lines in each season because DIN was potentially limiting nutrient in Ishikari Bay (this study). These salinity criteria for Plume boundary were 31.1, 32.9 and 33.6 for spring, summer and autumn seasons of 2006, respectively.

3.2 Physical parameters

Temperatures were lower in spring (Plume: 7.75 ± 0.46 ℃; Out-Plume: 7.05 ± 1.07) than summer (Plume: 20.81 ± 1.07 ℃; Out-Plume: 18.54 ± 2.56 ℃) and autumn (Plume: 14.01 ± 0.66 ℃; Out-Plume: 12.84 ± 2.16 ℃) in both areas and were always lower in the Out-Plume than Plume area (Table 2). Lower values were also observed for average sigma-t in the Plume (spring: 25.07 ± 1.36; summer: 23.37 ± 0.49 and autumn: 24.92 ± 0.45) than in the Out-Plume (spring: 26.61 ± 0.19; summer: 24.09 ± 0.71 and autumn: 25.38 ± 0.55).

In the Plume area the average $\overline{k}_d$ values were 0.28, 0.10 and 0.29 m^{-1} and the average $\overline{Z}_{1\%}$ values were 22, 16 and 30 m in spring, summer and autumn of 2006, respectively (Table 2). Although the average $\overline{k}_d$ values were similar in spring and autumn, standard deviation was much larger in spring (0.31) than in autumn (0.06). In the Out-Plume, the euphotic layer was thicker in the three seasons since the respective average values in spring, summer and autumn were for $\overline{k}_d$ 0.17, 0.08 and 0.10 m^{-1}, and for $\overline{Z}_{1\%}$ 33, 41 and 42 m, respectively.

3.2.1 Spatial and seasonal distribution of salinity

Due to a spring thaw, river discharge was highest in spring (Table 1). Low salinity water distributed wider in the bay in spring and its distribution biased to north-east side of the bay (Fig. 3a). The lowest salinity of 14.7 was observed at Stn. I-56 in spring. The Plume water was found only near the river mouse in summer (Fig. 3b). In autumn, the extension of the Plume water was larger than in summer, but smaller than in spring. Estimated Plume area cover was 2,562, 121.2 and 848.9 km^2 in spring, summer and autumn, respectively.

3.2.2 Water column salinity structure

A vertical section of salinity along the Plume transect indicated that Plume area was widest in spring, followed by autumn and lowest in summer (Fig. 4). Halocline was found between 5 -10 m in spring and autumn, whereas, it was above 5 m in summer. At Stn. I-25, salinity of 25 contour paths reappeared close to the surface water suggesting a heterogeneous distribution of salinity (Fig. 4a). On the contrary,

Table 2 Mean ±SD of water column physical parameters in Ishikari Bay of 2006.

Area	Plume			Out－Plume		
Parameter	Spring	Summer	Autumn	Spring	Summer	Autumn
Temperature (℃)	7.75 ± 0.46^{a}	20.81 ± 1.07^{b}	14.01 ± 0.66^{c}	7.05 ± 1.07^{a}	18.54 ± 2.56^{b}	12.84 ± 2.16^{c}
Salinity	32.15 ± 1.69^{a}	33.48 ± 0.40^{a}	33.37 ± 0.76^{a}	33.99 ± 0.12^{a}	33.80 ± 0.20^{a}	33.74 ± 0.45^{a}
Sigma t (mg cm^{-3})	25.07 ± 1.36^{a}	23.37 ± 0.49^{b}	24.92 ± 0.45^{b}	26.61 ± 0.19^{a}	24.09 ± 0.71^{b}	25.38 ± 0.55^{c}
Attenuation Coefficient $\overline{k}_d$ (m^{-1})	0.28 ± 0.31^{a}	0.11 ± 0.06^{a}	0.29 ± 0.08^{a}	0.17 ± 0.08^{b}	0.08 ± 0.04^{a}	0.10 ± 0.07^{a}
Euphotic zone $\overline{Z}_{1\%}$ (m)	22 ± 9.35^{ab}	26 ± 16.79^{b}	16 ± 8.69^{a}	33 ± 21.76^{a}	41 ± 20.56^{a}	42 ± 20.32^{a}

Values of respective area along the same row bearing the same superscripts are not statistically different at 5% probability level using the Duncan Multiple Range Test summer and autumn seasons were nearly homogenous ranging from 31 to 34 (Fig. 4b & c). However, the deeper halocline in autumn compared to summer suggests the Ishikari River discharge influence.

3.3 Spatial, vertical and seasonal distributions of nutrients

High concentrations of nutrients were found only in the Plume area in spring (Fig. 5), corresponded with the highest seasonal river discharge in spring (Table 1). Ishikari Bay showed a strong spatial and seasonal variation in dissolved inorganic nitrogen concentration (DIN, with ~79% of NO_3^-): with the highest average concentrations in the Plume area (1.15 μM) and lowest average concentrations in the Out–Plume area (0.07 μM) (Not shown). Seasonally, average DIN concentration in the Plume was highest in spring (1.15 μM) and lowest in summer (0.08 μM), whereas, in the Out–Plume, average DIN concentration was highest in autumn (0.72 μM) and lowest in summer (0.07 μM).

Nitrate concentration depended on the degree of the influence of the Ishikari River water, with the highest value recorded in the Plume area (Fig. 5), but in autumn, nitrate concentrations were less than 0.5 μM, not different between Plume and Out–Plume areas. Nitrate exhibited a strong inverse relationship with salinity in spring (Pearson correlation coefficient, $r = -0.831$; $P = 0.001$, $n = 26$) and autumn ($r = -0.727$ $P = 0.001$, $n = 19$). This indicated conservative mixing between nitrate rich freshwater and nitrate–drought coastal waters and the absence of significant sources or sink of nitrate in near river mouth area. Ammonium exhibited a strong inverse relationship with salinity (Pearson correlation coefficient, $r = -0.765$; $P = 0.001$, $n = 26$) in only spring (not shown in figure).

The spatial and seasonal changes in phosphate concentration in surface waters of Ishikari Bay were not very evident in the Out–Plume stations (Fig. 5). Summer average concentration in the Plume and Out–Plume was 0.07 μM. There was a significant inverse relationship between phosphate and salinity in spring ($r = -0.802$; $P = 0.001$, $n = 26$) and

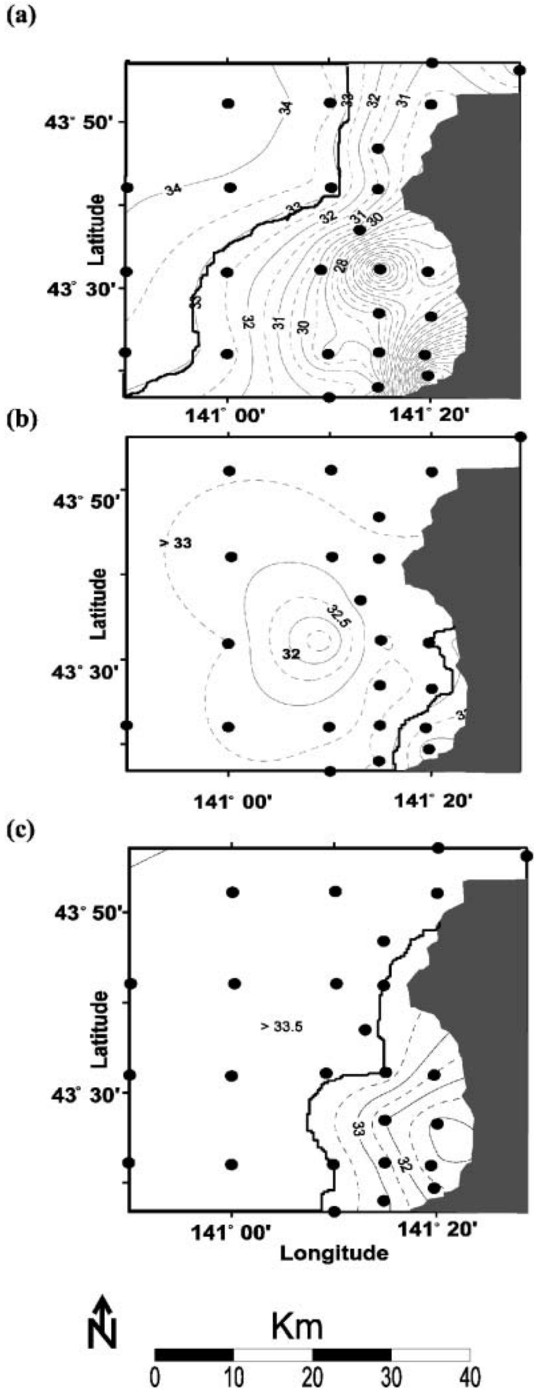

Fig. 3. Surface salinity distributions in spring (a), summer (b) and autumn (c) in Ishikari Bay. Solid lines indicate Plume boundary.

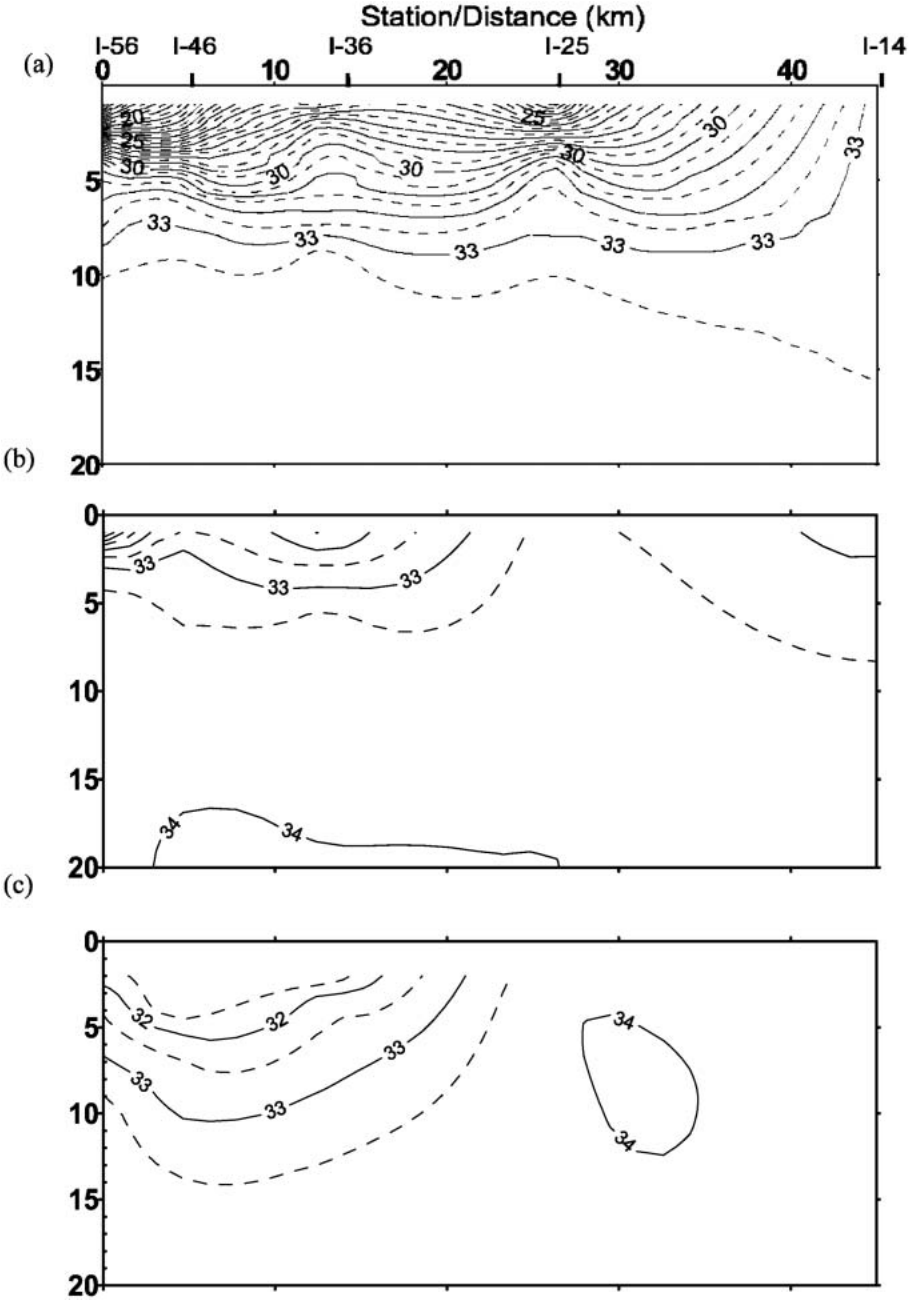

Fig. 4. Vertical section of salinity along the Plume transect in spring (a), summer (b) and autumn (c) in Ishikari Bay. Each contour was drawn based on 1 m pitch data (starting from 2 m).

autumn ($r = -0.565$; $P = 0.001$, $n = 19$) (Fig. 2).

Silicic acid concentration in Ishikari Bay exhibited clear spatial and seasonal variation with the highest average concentration in the Plume (6.9 μM) and the lowest at seaward boundary (Out–Plume, average of 0.6 μM) (Fig. 5). Silicic acid concentration in the Plume decreased from spring to summer, followed by an increase in autumn. Silicic acid also exhibited a strong inverse relationship with salinity

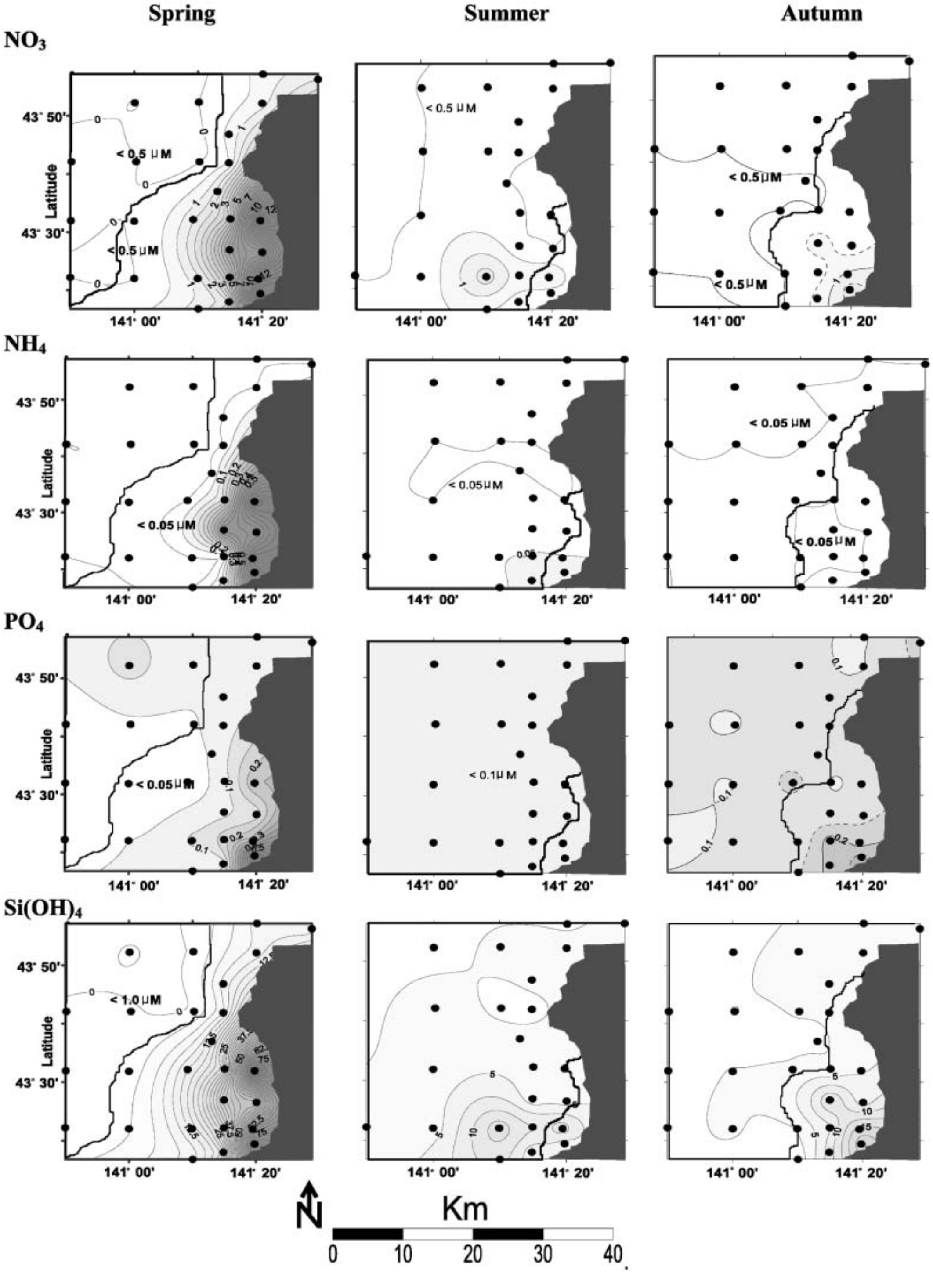

Fig. 5. Spatial distributions of surface nutrients in spring, summer and autumn in Ishikari Bay of 2006. Solid lines indicate Plume boundary.

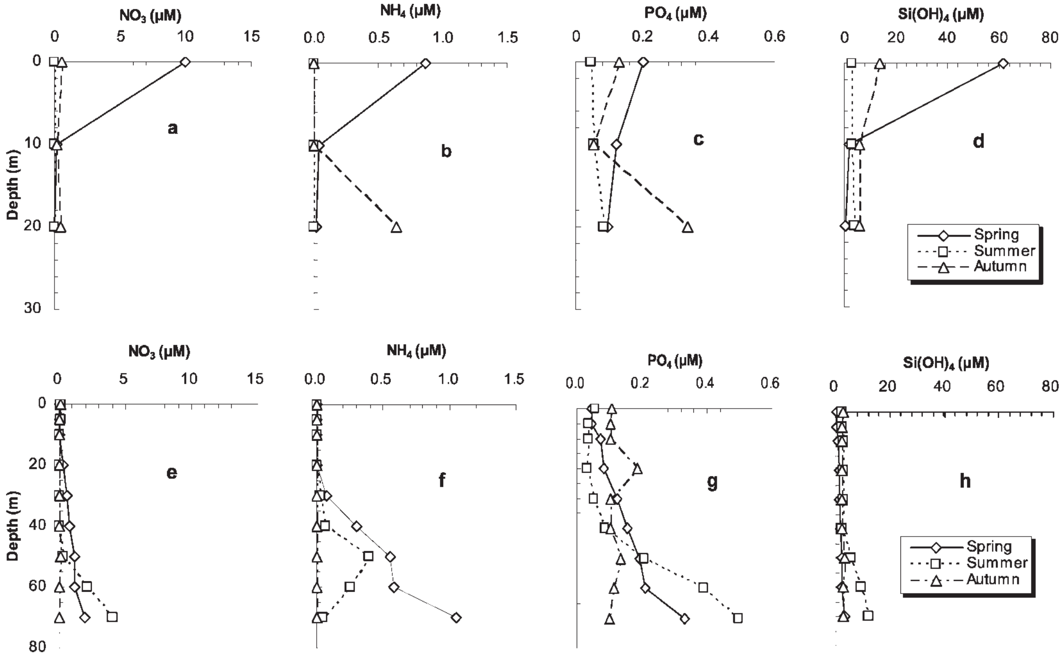

Fig. 6. Vertical profiles of nutrients at Stn. I-36 (Plume) (a, b, c, d) and at Stn. I-14 (Out-Plume) (e, f, g, h) during spring, summer and autumn seasons in Ishikari Bay of 2006.

in spring (r = –0.831; P = 0.001, n = 26) and autumn (r = –0.823; P = 0.001, n = 19), suggesting conservative mixing between silicic acid–rich river/estuarine waters and silicic acid–poor coastal water (Fig. 2). In the Plume, silicic acid concentrations exceeded those in the Out–Plume, in all seasons.

Vertical distributions of nitrate and silicic acid in the Plume (Stn. I–36) exhibited higher concentrations at surface especially in spring than at deeper layers (Fig. 6). Such high concentrations were not observed for phosphate. In the Out–Plume (Stn. I–14), concentrations of all nutrients increased with depth (Fig. 6). Surface depletion of nitrate and silicic acid was observed throughout the seasons while phosphate remained at c.a. 0.1 μM.

Nitrogen, particularly the oxidized form (nitrate) was generally depleted, but only in excess in spring. During summer and autumn, mean values for N:P ratio in surface water varied from 0.35 (Out–Plume) to 5.57 (Plume). However, in spring, N:P ratio was 29.6 (Plume), well above the REDFIELD Ratio of 16 during this period, phosphate appears to be limiting and this may suggest one of the possible reasons for the observed lower Chl *a* concentration compared with autumn.

3.4 Spatial and seasonal distribution of phytoplankton biomass

In spring, Chl *a* was nearly homogeneous at around 1–2 mg m^{-3} across the Plume and Out–Plume areas, whereas, in summer and especially autumn, higher Chl *a* values were more evident within the Plume area (Fig. 7)

Total Chl *a* concentration in Ishikari Bay (0–20 m depth average) showed maximum (7.36 mg m^{-3}) in the Plume of autumn and minimum (0.25 mg m^{-3}) in the Out–Plume of summer (Fig. 8). Micro–sized Chl *a* concentration in the Plume area was maximum (4.1 mg m^{-3}) in autumn and minimum (0.4 mg m^{-3}) in summer. In the Out–Plume area, nano–sized fraction was relatively high concentration (1.4 mg m^{-3}) in spring, but very low (<0.1 mg m^{-3}) in summer and autumn. Total Chl *a* exhibited strong inverse relationship with salinity (r = –0.927; P = 0.001, n = 19) in autumn, suggesting river influence on the total Chl *a* concentration (Fig.

9). No relationship was found in spring and summer seasons.

The relative contribution of micro–sized fraction accounted for more than 55% of the total Chl *a* in the Plume in spring and autumn (Fig. 8b). In summer, micro–sized fraction also accounted for 35.7% in the Plume area. The pico– and nano–sized fractions were predominated and accounted between 64 and 72% of the total Chl *a* in the Out–Plume area across the seasons. The relative contribution of micro–sized fraction ranged between 27 to 35.9%.

3.5 Intercorrelation of total and size–fractionated Chl a

The intercorrelation among total and each size–fractions of Chl *a* (Table 3) revealed a strong positive correlation of pico–sized ($r = 0.882$, $P = 0.001$) and micro–sized fractions ($r = 0.945$, $P = 0.001$) with total Chl *a* . The highly significant correlation of micro–sized fraction suggests its relative importance to the total Chl *a* in Ishikari Bay, especially in the Plume.

4 Discussion

The results of this study revealed Ishikari Bay as a dynamic heterogeneous water body with spatial and temporal variations in its hydrographic, physico–chemical and biological components. At the river/ocean interface where mixing between freshwater and seawater occurs, estuarine systems are characterized by drastic changes in physical and chemical conditions, which are primarily related to the salinity gradient. Salinity distributions in Ishikari Bay were significantly different ($P < 0.001$) by season, as a result of the change in river discharge. Observed lower average salinity value in spring (32.65 ± 1.69) revealed an influence of the snow thaw. In summer and autumn, average salinity increased to 33.48 ± 0.40 and 33.37 ± 0.76, respectively following the period of low river discharge. The influence of the spring thaw was also evident from the large Plume area (2,562 km^2) in spring, compared to summer (121.2 km^2) and autumn (848.9 km^2).

According to Harris (2001) and Anzecc/Armcanz (2000), nutrient impacts on coastal waterways vary as a function of both the

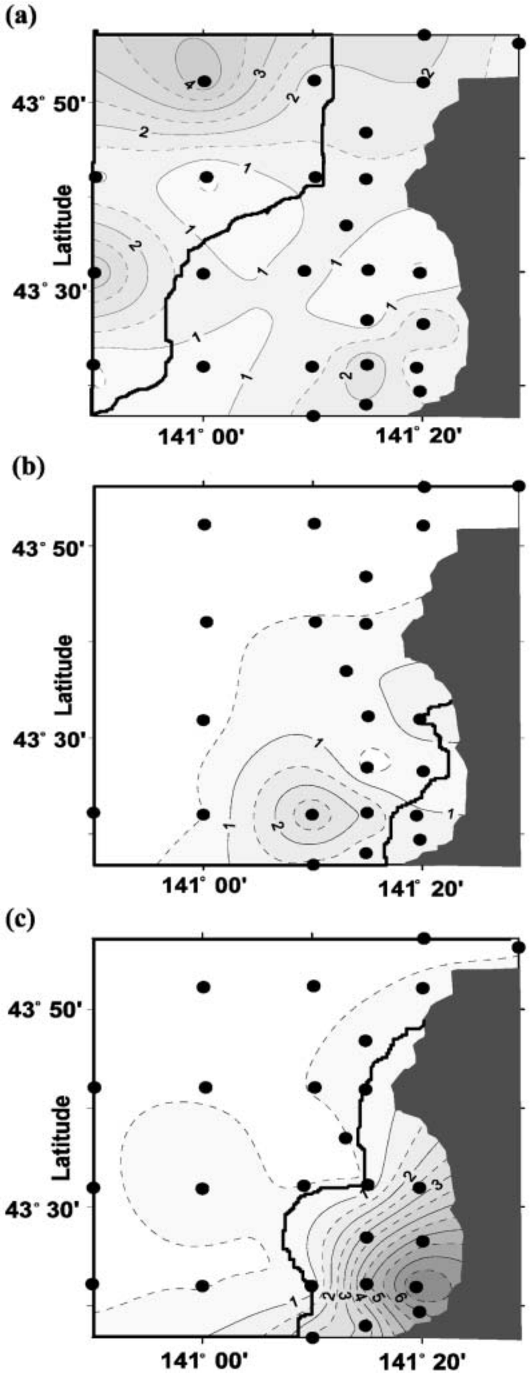

Fig. 7. Surface Chl *a* concentration (mg m^{-3}) in spring (a), summer (b) and autumn (c) in Ishikari Bay.

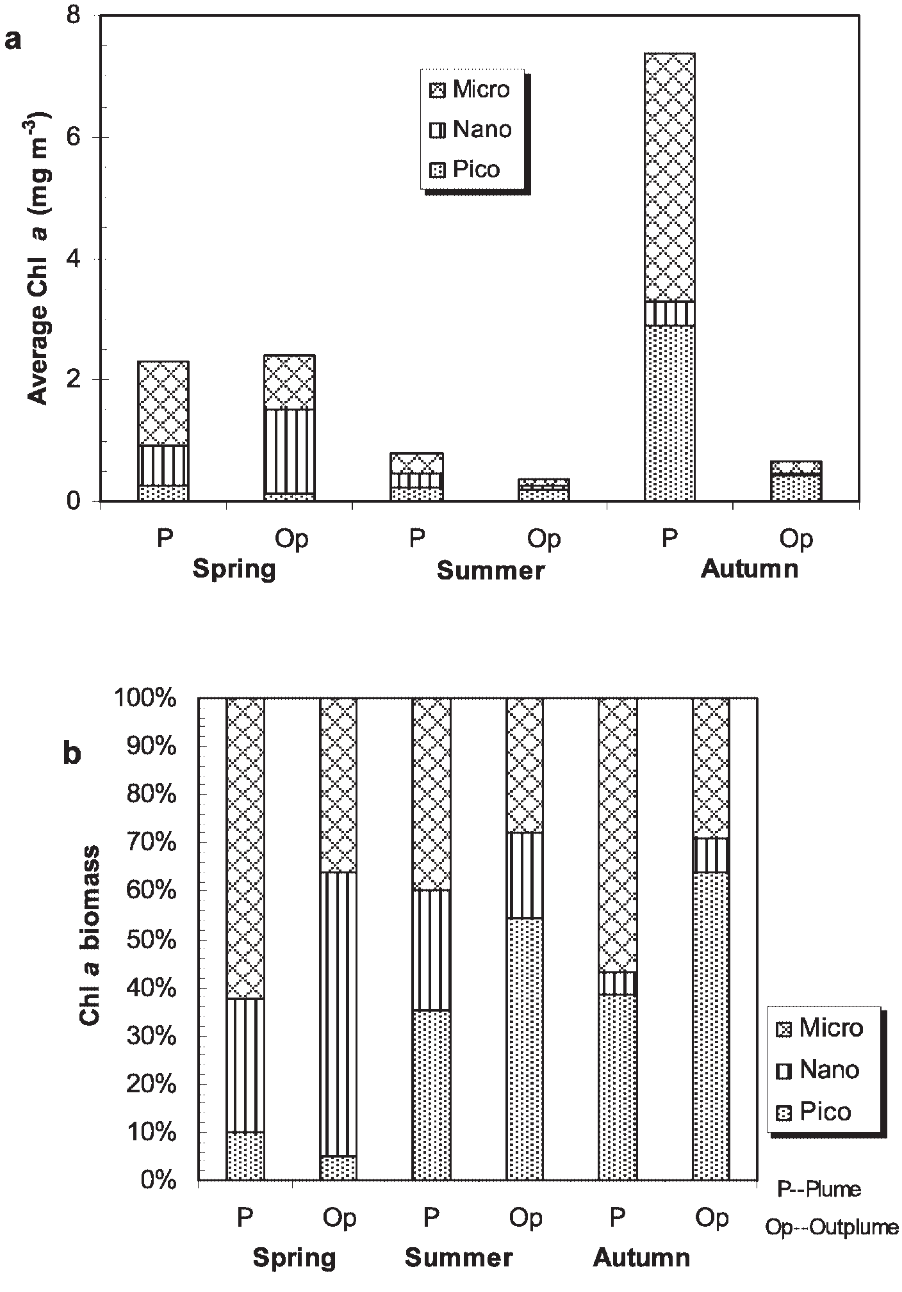

Fig. 8. Bar graph of size fractionated Chlorophyll *a* (mg m^{-3}) (a) and percentage contribution of each size fraction to the total Chl *a* (b).

loadings (fluxes) and bioavailability of the nutrients, and the extent to which hydrodynamic features (*e.g.* water volumes, residence times and extent of mixing) and turbidity levels modulate the stimulatory effects of nutrients on plants and algae. Nutrient concentrations decreased in Ishikari Bay from river–mouse (Plume) to marine influenced area (Out–Plume), reflecting the main nutrient discharge from the Ishikari River. The DIN exhibited a

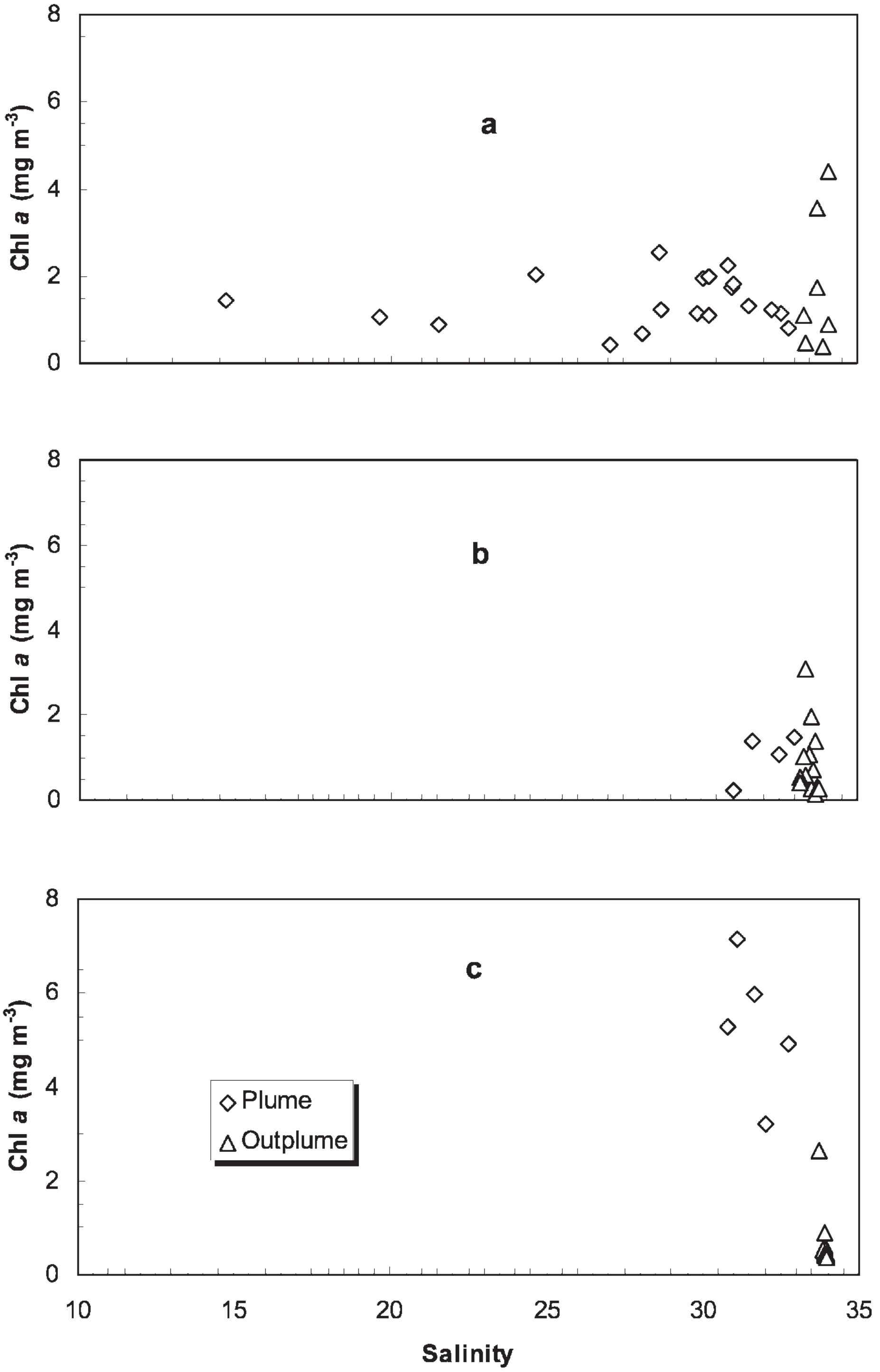

Fig. 9. Chl *a* distribution along salinity gradient in surface waters in spring (a), summer (b) and autumn (c) in Ishikari Bay.

Table 3 Pearson's correlation matrix of total and size fractionated Chl *a* biomass in Ishikari Bay.

Chl *a* biomass	Pico	Nano	Micro	Total	Temperature	Region	Season
Pico	1						
Nano	0.056	1					
Micro	0.812^{**}	0.179	1				
Total	0.882^{**}	0.392	0.945^{**}	1			
Temperature	−0.162	-0.627^{**}	-0.480^{*}	-0.506^{*}	1		
Region	−0.329	−0.048	-0.537^{*}	-0.452^{*}	0.243	1	
Season	0.499^{*}	-0.588^{**}	0.112	0.108	0.420	0.194	1

** Correlation is significant at the 0.01 level (2-tailed).

* Correlation is significant at the 0.05 level (2-tailed).

clear conservative mixing along the theoretical dilution line only in spring, indicating river as important nutrient source (Fig. 2). In autumn, an increase in the apparent utilization of DIN gave a corresponding increase in phytoplankton biomass (Chl *a*). The apparent utilization of DIN (ΔDIN) was calculated as the difference between the observed DIN and the theoretical DIN from the theoretical dilution line (see Fig.2). The ΔDIN versus Chl *a* relationship (Fig. 10c) fell on the reference lines (C:Chl *a* = 30–70), predicting the Chl *a* concentration if depleted DIN was converted entirely into phytoplankton biomass with a molar ratio of C:N = 6.6. These C:Chl *a* ratios were within the literature values (PARSONS *et al.*, 1977). In the same season, there was little or no apparent utilization of phosphate (Fig.11c) in relation to observed phytoplankton biomass, ruling out phosphate as a possible limiting nutrient. Also, strong inverse relationship ($r = -0.927$) between Chl *a* and salinity in autumn (Fig. 9c) suggests that DIN from the Ishikari River produced the phytoplankton biomass maximain autumn. We did not establish nutrient exchange or benthic–pelagic coupling resulting from the intense vertical mixing (estuarine circulation) in autumn between the bottom and the water column as co–fueling source of production in autumn, however, storm events prior to and during sampling in autumn may have possible implication on biomass production in Ishikari Bay.

In determining the spatial and temporal patterns of the total and fractionated phytoplankton biomass (Chl *a*), they were related to the prevailing oceanographic conditions. For example, in autumn when the contribution of micro–sized phytoplankton (57%) and pico–sized phytoplankton (64 %) to the total Chl *a* was highest for the Plume and Out–Plume areas, respectively, and the total Chl *a* was highest across seasons, there was highly significant inverse relationship ($P < 0.001$) with physical forcing such as temperature, salinity and sigma–*t*. Owing to the inverse relationship between temperature and nutrient, nutrient–temperature flux has influence on Chl *a* , thus, lower temperature area tend to have high Chl *a*. Also, lower salinity area may have high nutrient supply from freshwater discharge resulting in Chl *a* abundance. MAITA and ODATE (1988) showed that in subarctic coastal water, picoplankton (<2 μm) were more abundant than larger sized phytoplankton (>10 μm) in warm (10–20℃), nutrient–poor water in summer. This trend was also observed during the warm temperatures (10–21℃, mean value) in summer and autumn when pico–sized fraction (<2 μm) contributed more than half to the total Chl *a* biomass in nutrient–poor Out–Plume waters. At low total Chl *a* , phytoplankton variability depended on both the smallest (picophytoplankton) and the largest (microphytoplankton) size fractions, while at high total Chl *a* values the picophytoplankton reached an upper limit and phytoplankton variability depended on the variation of the largest fractions. The micro–sized phytoplankton was the most important fraction in the three seasons. In addition, the relative importance of micro–sized phytoplankton was higher in autumn than in the other two seasons. Lower (spring) or similar (summer and autumn) contributions of picophytoplankton were observed in the Plume than the Out–Plume areas. These

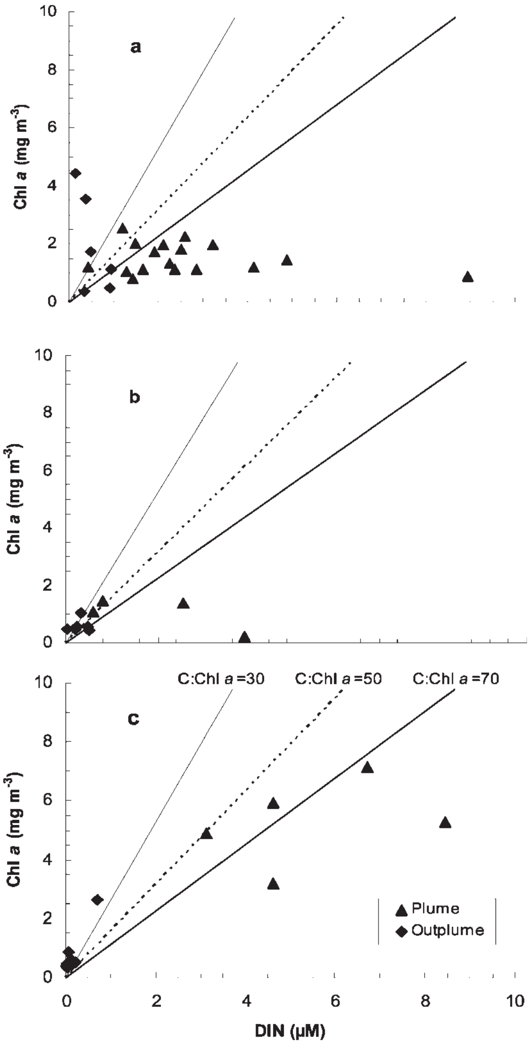

Fig. 10. Relationship between Chl *a* concentrations and apparent deviation in DIN concentration (ΔDIN) from the theoretical dilution line in spring (a), summer (b) and autumn (c) in Ishikari Bay. ΔDIN values less than zero were not plotted.

observations may be related to the fact that the combination of small–scale turbulence and nutrients would benefit the growth of large cells (MARGALEF, 1978; ARIN *et al.*, 2002), while small cells, due to their higher cell surface to volume ratios, are better competitors at low nutrient levels (KIORBOE 1993).

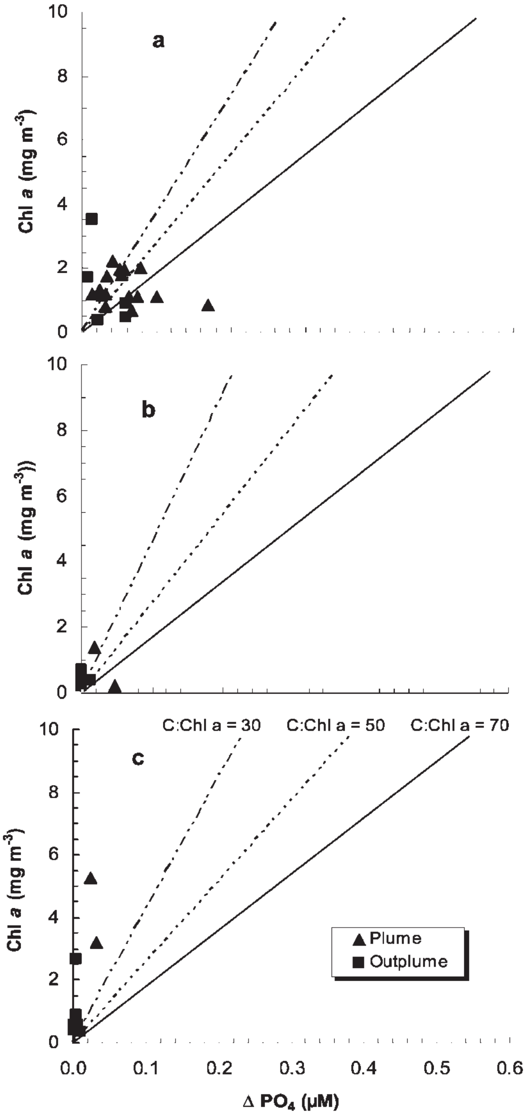

Fig. 11. Relationship between Chl *a* concentrations and apparent deviation in phosphate concentration (ΔPO_4) from the theoretical dilution line in spring (a), summer (b) and autumn in Ishikari Bay. ΔPO_4 values less than zero were not plotted.

The relative molar ratios among DIN, P and Si for phytoplankton growth are Si:N:P = 16:16:1 (REDFIELD *et al.*, 1963; BRZEZINSKI, 1985; RAHM *et al.*, 1996). These ratios are merely used to define resource availability (del AMO *et al.*, 1997) as a consequence of loading and biotic activity (REYNOLDS, 1999; SOMMER, 1999; LOPES *et.al.*, 2007). In Fig. 12, the molar quotients between the concentrations of potentially

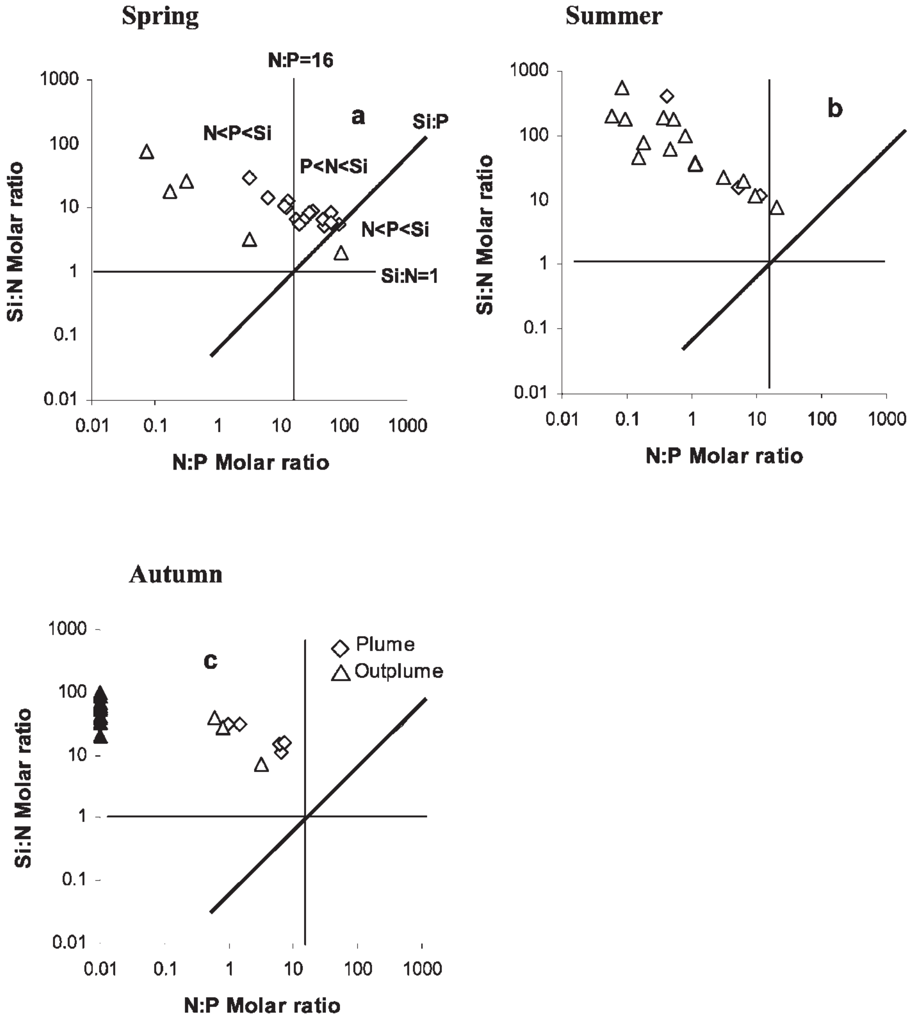

Fig. 12. Plots for relative molar ratio of Si:N:P in the surface water of Ishikari Bay in 2006. Open symbols indicate available nutrient remained. Filled symbols indicate nutrient depletion. (a) Spring (n = 26) (b) Summer (n = 21) (c) Autumn (n = 18). Vertical and horizontal lines: REDFIELD ratio (N:P) and Brzenzinski (1985) ratio (N:Si), respectively. Diagonal line: aggregated ratio (Si:N:P = 16:16:1).

limiting nutrients are delimited in the logarithmic plot (log N:P vs log Si:N) by the Si:N =1; N:P =16 and Si:P =16 lines. These define three different areas within the plot with each one characterized by the potentially limiting nutrients in Ishikari Bay in 2006 (Fig.12a). Here, the molar ratios of available nutrients evidence the limiting effect of phosphate in spring (Fig.11a and 12a) and the depletion effect of DIN (N) in autumn (Fig.10c and 12c) on phytoplankton biomass productions. Organic forms of N and P may also be present but were not reported in this study, as they are not considered to be immediately available for plant growth (ANTIA *et al.*, 1991).

Against the expected spring maximum phytoplankton biomass when riverine nutrients fluxes and nutrients were the highest in the Plume, the lower phytoplankton biomass of spring than autumn was possibly due to either light or phosphate limitation effect. At the near river mouth stations, light attenuation coefficient, K_d was highest at 1.42 m^{-1}, five times higher than the mean value for the

Plume area (0.28 m^{-1}). As light attenuation gradually decreased towards offshore the Plume, phosphate also decreased to less than 0.05 μM (Fig. 5), suggesting phosphate limitation. The relatively high light attenuation (K_d) across the Plume area in spring corresponded with highest SPM value from the Ishikari River (Table 1) compared to summer and autumn. Also, when there was apparent utilization of phosphate corresponding to C:Chl a = 50 and 70 reference lines (Fig.11a), there was no appreciable increase in phytoplankton biomass. Most of P–removal from the water column takes place through sedimentation of organic matter (BERNER *et al.*, 1993), and since the highest river discharge occurred in spring, one may conclude that, tide–dominated coastal waterways are generally turbid, and light attenuation caused by suspended sediment is a major control on phytoplankton production and biomass (HINGA *et al.*, 1995; CLOERN, 1987; MONBET, 1992).

The present study demonstrate that Ishikari Bay ecosystem could not be characterized as being at steady state because of the spatial and temporal heterogeneity in the magnitude and patterns of the examined nutrients and phytoplankton biomass. In this non–steady state environment, the space and time structure of phytoplankton biomass and nutrients is subject to fluctuations in concentration and dominance which characterizes temperate and coastal waters. While either light or phosphate limitation effect was considered major limiting factors in spring, generally, water mixing, biological consumption and possible vertical convection were the three major factors controlling the nutrient distribution across the seasons: water mixing which explains the gradual decrease of nutrient content offshore, biological consumption which leads to noticeable removal of nutrients across the Plume water and possible vertical convection which carries nutrients released from bottom upward to the surface waters.

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