Effect of Nutrient Inputs on Water Quality Change and Phytoplankton Growth in Atsumi Bay

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Abstract. Eutrophication in an estuary occurs as an effect of the enrichment of nutrient inputs from rivers. This condition has become one of the most common environmental issues experienced around the globe and especially in Japan. Atsumi Bay is a eutrophic coastal area in Japan. The objective of this research was to analyze the influences of nutrient inputs from the Umeda River into Atsumi Bay on pre- and post-rainfall water quality conditions. This study was conducted from July to October 2010. The results showed a decrease of surface salinity after rainfall indicating that huge freshwater inputs had overlaid the surface layer of Atsumi Bay rather than the bottom layer. Moreover, post-rainfall conditions showed an increase of chlorophyll *a* as an effect of phytoplankton growth, followed by an increase of particulate nutrients. On the other hand, dissolved nutrients decreased due to uptake by phytoplankton and dilution by freshwater.

Keywords: *Atsumi Bay*; *chlorophyll a; eutrophication; nitrogen; phosphorus; rainfall; Umeda River.*

1 Introduction

Nowadays, the increase of the human population, economic development, industrial growth, and advanced application of fertilizers in agricultural activities cause significant anthropogenic impacts including eutrophication in lakes, rivers, and coastal environments. Especially in coastal areas, nutrient enrichment caused by riverine inputs is the most widespread and globally significant anthropogenic impact [1]. Enrichment of nutrients in estuaries

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produces huge effects in marine ecosystems, such as increased biomass of marine phytoplankton and epiphytic algae, shifts in phytoplankton species composition, changes in macroalgal production, biomass, and species composition, reduced water clarity, dissolved oxygen depletion in the water column, increased probability of deaths of recreationally and commercially important animal species, etc. [2].

As in many countries around the globe, agricultural areas in Japan are one of the major non-point sources of nutrients [3]. In Japan, especially in Atsumi Bay, red tide and hypoxia have occurred for a significant number of years due to riverine nutrient inputs mainly originating from the surrounding agricultural areas. Several rivers, such as the Toyo, Umeda, and Shio Rivers, are connected and flow into Atsumi Bay. These rivers are the main suppliers of freshwater and nutrients to the bay. The highest input of nutrients into the bay comes from the Umeda River Watershed [4]. The Umeda Watershed covers approximately 134 km^2 and comprises of agricultural area (45.7%), urban area (33.9%), forest area (6.5%), and other areas (13.9%) [5]. It was reported by Bodergat, *et al.* [6] that Mikawa Bay, including Atsumi Bay, has become highly eutrophic due to the increase of nutrient inputs from livestock farms and municipalities as well as from rapid industrial development since the mid-1960s.

Enrichment of nutrients around Umeda Watershed and Atsumi Bay generally reaches high values during the growing season, from spring to summer. This coincides with increasing fertilizer application in agricultural fields around the Umeda Watershed. The application of fertilizer containing nitrogen (N) and phosphorus (P) continuously diffuses huge concentrations of nutrients into the soil, which later results in accumulation in the soil body and becomes one of the source nutrients of nearby water bodies through leaching, erosion, and run-off [7]. Moreover, there is a significant relationship between rainfall and the transport of nutrients. Rainfall has an important influence on water quality and the hydrographic characteristics of riverine and estuarine environments. Xia, *et al.* [8] explained that rainfall acts as a nutrient driver and source, which is expected to have significant consequences for river nutrient dynamics. During rainfall, precipitation drives run-off on flushing nutrients from the land around the watershed into the river, which is a channel that delivers the nutrients throughout the estuary and to the sea. Eventually, nutrient enrichment in estuaries leads to harmful algal blooming, also called red tide [9], and subsequently causes oxygen depletion, also known as hypoxia. The occurrence of hypoxia is a response to algal blooming and decomposition of organic matter that consumes the availability of oxygen in waterbodies [10].

Water quality changes in Atsumi Bay have been studied by Rasul, *et al.* [5,11,12], who focused on the effects of nutrient enrichment and hypoxia after a typhoon event, where source of nutrient inputs come from three major rivers, i.e. the Toyo, Umeda, and Shio Rivers. Their evaluation was based on extreme meteorological conditions with very heavy rainfall and strong wind-induced water bodies, which resulted in salinity dilution and significant changes in nutrient concentrations from the surface to the bottom layer. In the current study, attention was paid to the effect of the Umeda River inputs on nutrient concentration changes and phytoplankton growth on pre- and post-rainfall water quality conditions in Atsumi Bay, especially around the Umeda transects. Here, the pre- and post-rainfall conditions were observed under normal meteorological conditions with heavy rainfall and gentle wind-induced waterbodies, where salinity and concentration alteration mostly occurred at the surface layer.

The purpose of the current research was to evaluate the influence of nutrient inputs from the Umeda River to Atsumi Bay. Our evaluation of water quality conditions was for the period of peak phytoplankton bloom in the summer of 2010. The evaluation consisted of a comparison between pre-rainfall conditions on a fine day, when river nutrient inputs to the estuary is relatively normal, and post-rainfall conditions, when huge riverine inputs during rainfall affects greater alteration of estuarine salinity and nutrient concentrations, followed by high phytoplankton growth. The structure of our evaluation contains several points about the water quality conditions in Umeda River and Atsumi Bay during the summer of 2010, including riverine nutrient concentration conditions during rainfall, the influence of freshwater inputs on salinity alteration pre and post rainfall, and Chl *a* and nutrient alterations pre and post rainfall. Several conditions are discussed, such as the effect of freshwater on the salinity gradient, proportion of nutrient concentrations, and ratio of phytoplankton and nutrients. The results of this research can be used in watershed management for suppressing red tide and hypoxia.

2 Material and Methods

2.1 Study Area

Atsumi Bay lies in the eastern part of Mikawa Bay in the Aichi Prefecture, Japan. Atsumi Bay is a partially mixed estuary, where river discharge and tidal forces dominantly influence the circulation and stratification of the water bodies. Its topographical condition is shallow and flat with a mean depth of about 10 m. The total area of the bay is approximately 180.5 km^2 (Figure 1). The area has a temperate climate with average precipitation was around 1,516 mm/year in 2010. During the summer of 2010, rainfall was recorded 39 times with amounts varying from 0.5 mm/day to 81 mm/day.

2.2 Field Sampling and Measurement

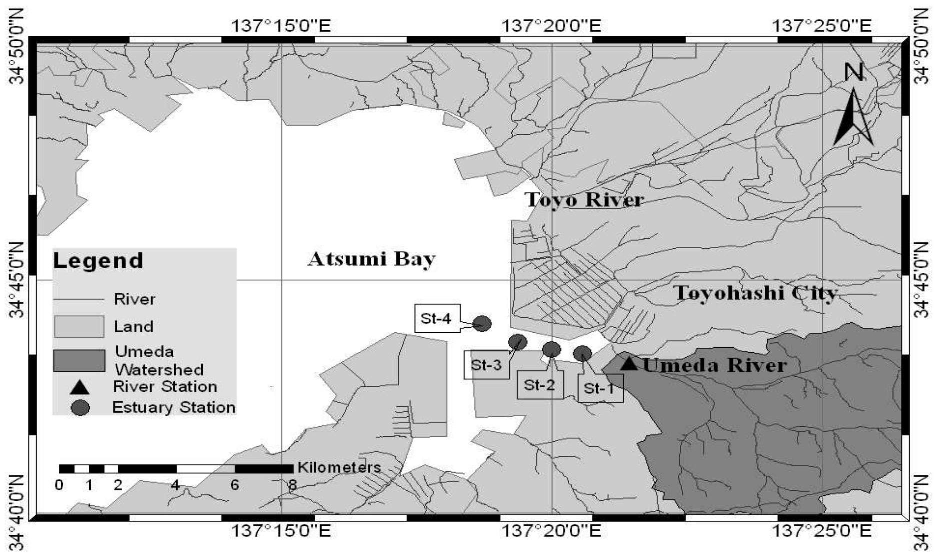

Figure 1 Atsumi Bay Area and sampling points.

Samples were collected at the Umeda River Station (Hatakeda Bridge) and four stations along the Atsumi Bay in the summer of 2010 (Figure 1). At the Umeda River Station, observations were conducted between July and October 2010 during rainfall and on fine days with a total of 99 samples collected. For each observation, water samples were taken from 8 to 24 times at hourly intervals. Samples were collected from when the flood started until the moment the flood subsided. All samples were collected at the surface layer using an auto sampler (6700, Teledyne ISCO, USA) and were then dispensed into 500-mL polypropylene bottles.

Moreover, salinity, nutrients and Chl *a* observations along the Umeda River transect were conducted ten times, with intervals of eight to ten days, from July to October 2010. At the estuary stations, 80 samples were collected at 1 m below the surface (surface layer) and 1 m above the bottom (bottom layer), and then dispensed into 500-mL polypropylene bottles with a peristaltic pump. To measure vertical salinity, we used conductivity-temperature-depth and a DO sensor (Rinko Profiler, JFE Advantech Co. Ltd, Kobe, Japan). This instrument measured vertical profiles at 0.1 m depth intervals.

2.3 Sample Analysis

Nutrient analyses were conducted at the laboratory immediately after sampling. An aliquot of each sample was filtered through a GF/F glass-fiber filter (Whatman International Ltd., Maidstone, UK) for chlorophyll *a* (Chl *a*) analysis. The filtrate samples were used for analysis of the dissolved nitrogen (DN) and dissolved phosphorus (DP), while unfiltered samples were used for analysis of total nitrogen (TN) and total phosphorus (TP). The amounts of particulate nitrogen (PN) and particulate phosphorus (PP) were calculated by subtracting total and dissolved nutrients. In order to minimize volatilization and biodegradation, the samples were stored in a dark freezer until analysis was done in the following days.

Nutrient analyses were conducted by adjusting the water characteristics of the sample locations under the measurement procedure of the American Public Health Association [13]. Analysis of the river samples was undertaken using Milli-Q water. In the case of the estuary samples, artificial seawater was created to adjust the density and ion concentration of the water by integrating 28.5 g of NaCl, 6.82 g of $Mg.SO_4.7H_2O$, 5.16 g of $Mg.Cl_2.6H_2O$, and 1.47 g of $CaCl_2.2H_2O$ to 1 L of Milli-Q water. In the analysis of Chl *a*, methanol extraction was used in accordance with the protocol of Otsuki, *et al.* [14], where the sample residue on the GF/F filters was extracted using 100% methanol.

3 Results and Discussion

3.1 Water Quality Condition at River Station

Rainfall is one of the driving factors in increasing nutrient release to river and marine environments. High precipitation intensity drives run-off, flushing nutrients and soil from the watershed to the river, and elevating river water levels and discharge while also delivering high input to the estuary. Riverine inputs are among the main circulation and nutrient inputs into estuaries, especially in Atsumi Bay, and indeed phytoplankton growth in the estuary is closely related to the amount of nutrient inputs from the river [15].

Our study location in Atsumi Bay has been experiencing eutrophication for the last four decades as a result of the rapid development occurring around this area, which has contributed to the release of nutrients into the bay [16]. In order to analyze the effects of nutrient concentration changes and phytoplankton growth pre and post rainfall in Atsumi Bay, it is necessary to know the conditions in the Umeda River during rainfall and how much the concentrations, in the form of nitrogen (N) and phosphorus (P), are delivered. During the study period, three typhoons and thirty-nine occurrences of rainfall were recorded around the

Umeda Watershed and Atsumi Bay, with precipitation ranging between 0.5 mm/day to 81 mm/day.

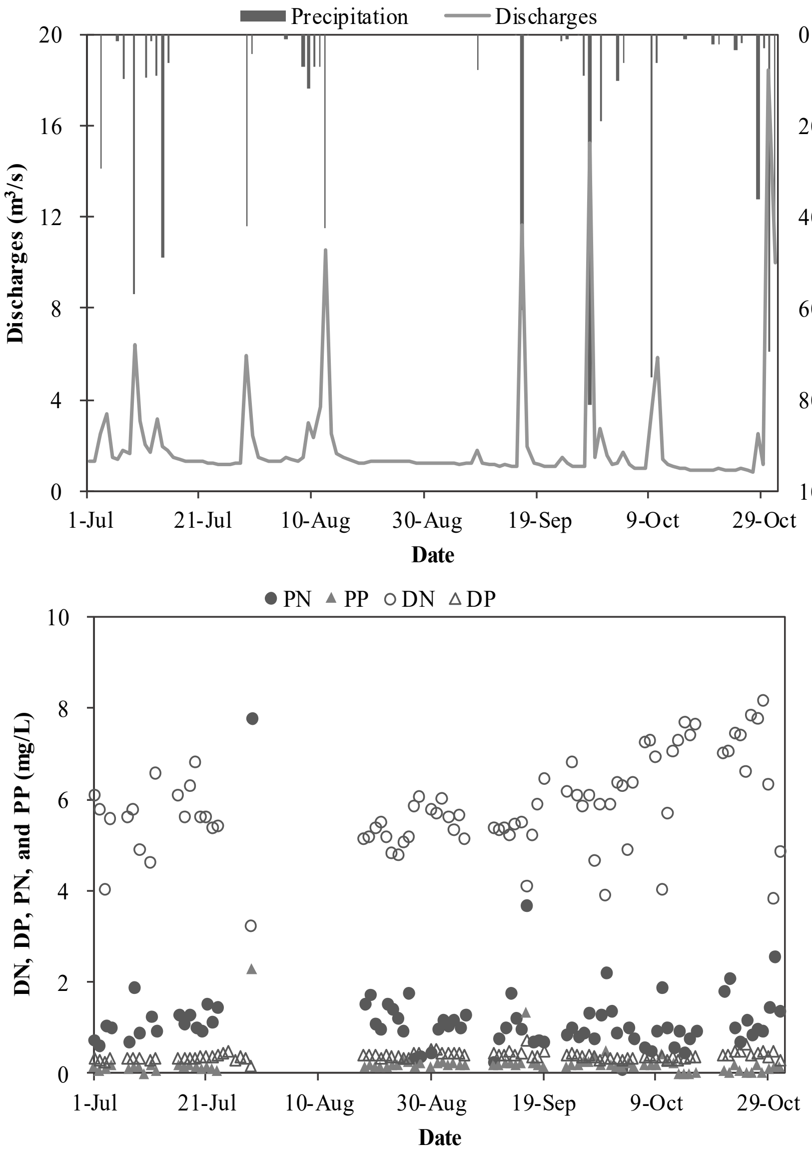

Figure 2 Precipitation data of Toyohashi rain gage station (operated by Japan Meteorological Agency), and discharges, DN, DP, PN, and PP of Umeda River conditions in the summer of 2010.

Figure 2 shows that dissolved nutrients were generally higher than particulate nutrients on both fine and rainy days. In contrast, at one rainfall event, on the 29th July, the concentrations of particulate nutrients became higher than the dissolved nutrients. The cause of the increase of particulate nutrients was high-intensity precipitation, which significantly effected in elevating run-offs, discharge levels, and particularly soil erosion which is the main source of particulate nutrients released into the river.

In this study, our focus was on the 1st August conditions when phytoplankton growth in Atsumi Bay reached its highest peak in the summer of 2010, when concentration of Chl *a* attained to 97.9 µg/L. The conditions on the 1st August were post rainfall, related to heavy rainfall on 29th July which flushed huge amounts of freshwater and nutrients into the estuary. On 29th July, heavy rainfall in which the total precipitation was recorded at 42 mm/day, occurred (Figure 3). During rainfall, there are significant relationships between the discharge and the nutrients found in the Umeda River. Two discharge peaks occurred during a flood event, followed by alteration of particulate and dissolved nutrient concentrations.

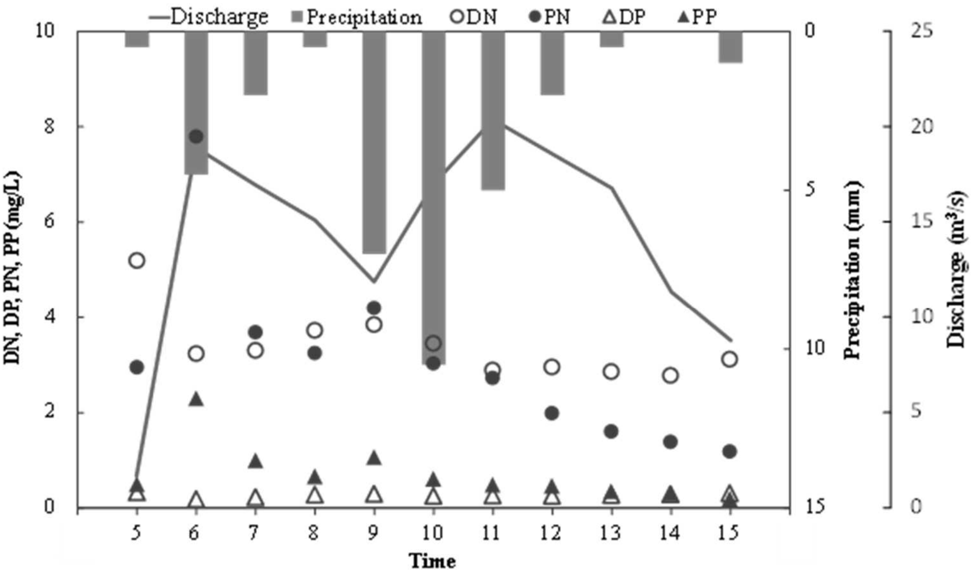

Figure 3 Precipitation, discharge and nutrients on 29th July during rainfall at Umeda River.

Generally, particulate nutrients were more dominant than dissolved nutrients as a result of particulate release from the soil. In particular for PN and PP the concentration decreased at the first peak, then was stable between the two peaks, and later leveled out after the second peak. On the other hand, the DN and DP concentrations showed a different trend, decreasing after a flood occurred and being relatively stable during the flood event. The decrease of DN and DP concentrations are assumed to be the effect of dilution by freshwater. During observation, the discharge-weighted averages of DN, DP, PN, and PP were 3.23 mg/L, 0.26 mg/L, 3.35 mg/L, and 0.79 mg/L, respectively. Eventually, these inputs were later to be the source of the change in water quality in Atsumi Bay on 1st August.

3.2 Water Quality Condition at Estuary Stations

During our study in the summer of 2010, we found that total nitrogen and phosphorus concentrations in Atsumi Bay exceeded the environmental standard in Japan. According to the Japanese Ministry of Environment, the environmental quality standard for total nitrogen and phosphorus of Atsumi Bay, which is class III for coastal waters, are 0.6 mg/L and 0.05 mg/L, respectively. We also found typical changes of eutrophic water in Atsumi Bay as an effect of riverine freshwater inputs. In relation with rainfall, an increase of water discharge after rainfall transported fresh water mixed with nutrients and suspended solid through the estuary and seaward. In addition, excessive nutrient inputs and a proper water temperature, around 22-30 °C, also stimulated algal blooming in the bay. Moreover, meteorological conditions became important factors on the water quality dynamics of the estuary.

The time series graphs in Figures 4 and 5 depict the water quality changes at four observation stations along Atsumi Bay during the study period. On several days, such as 12th July, 1st August and 28th September, a depression of salinity occurred at the surface layer due to mixing of freshwater and seawater. Salinity tended to be stable in the bottom layer at a higher level than in the surface layer, which indicates that river inputs dominantly forced surface layer circulation.

Significant water quality changes occurred on 1st August and 28th September (Figures 4 and 5). Both days went through a large rain event with total precipitation of 42 mm/day and 81 mm/day, respectively. The difference between both days was that on August 1st there were post-rainfall conditions, exactly three days after rainfall, while September 28th was during rainfall. Both days experienced a decline of salinity and an increase of particulate nitrogen as a result of river inputs. However, escalation of Chl *a* and depletion of dissolved nutrients only occurred on 1st August.

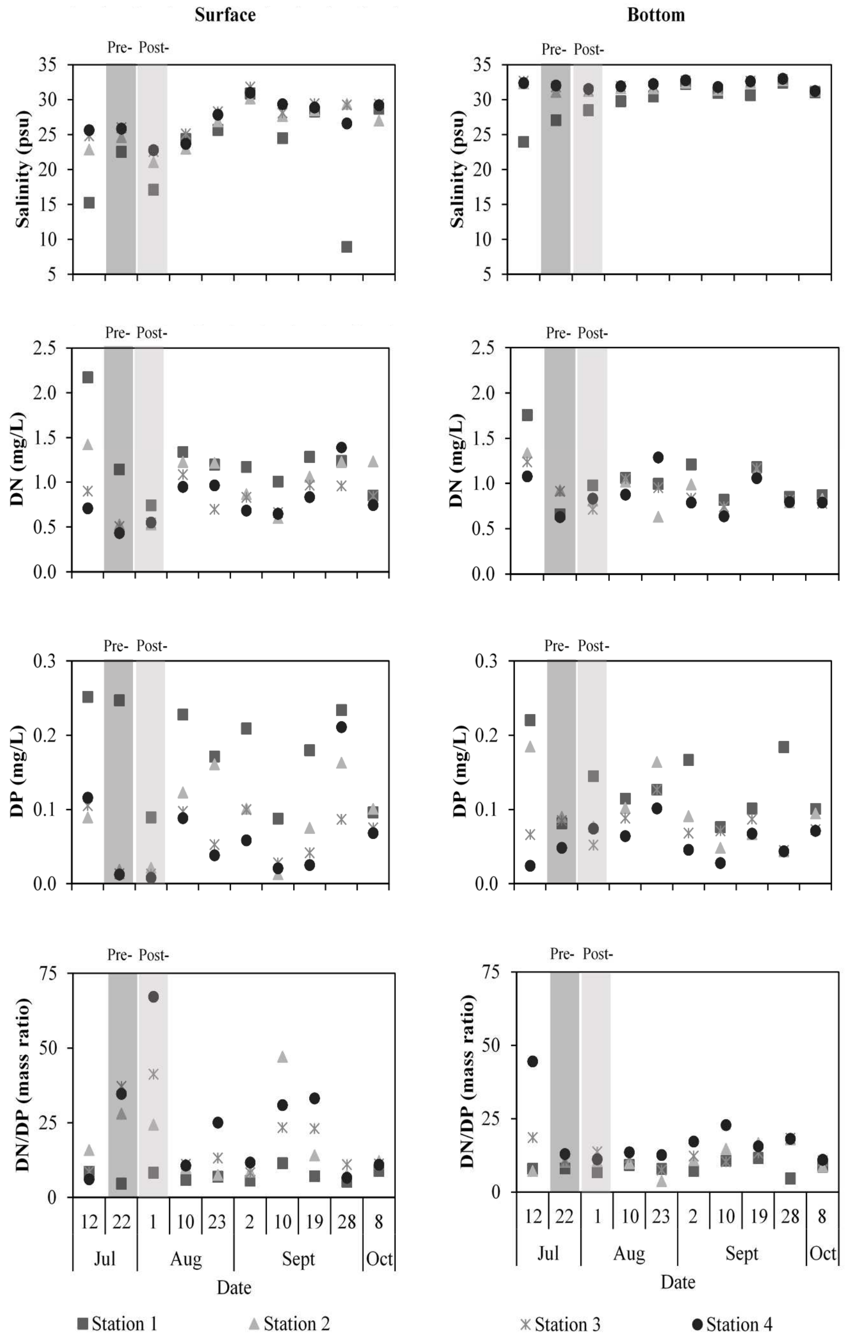

Figure 4 Salinity, DN, DP, and DN/DP ratio during the summer of 2010.

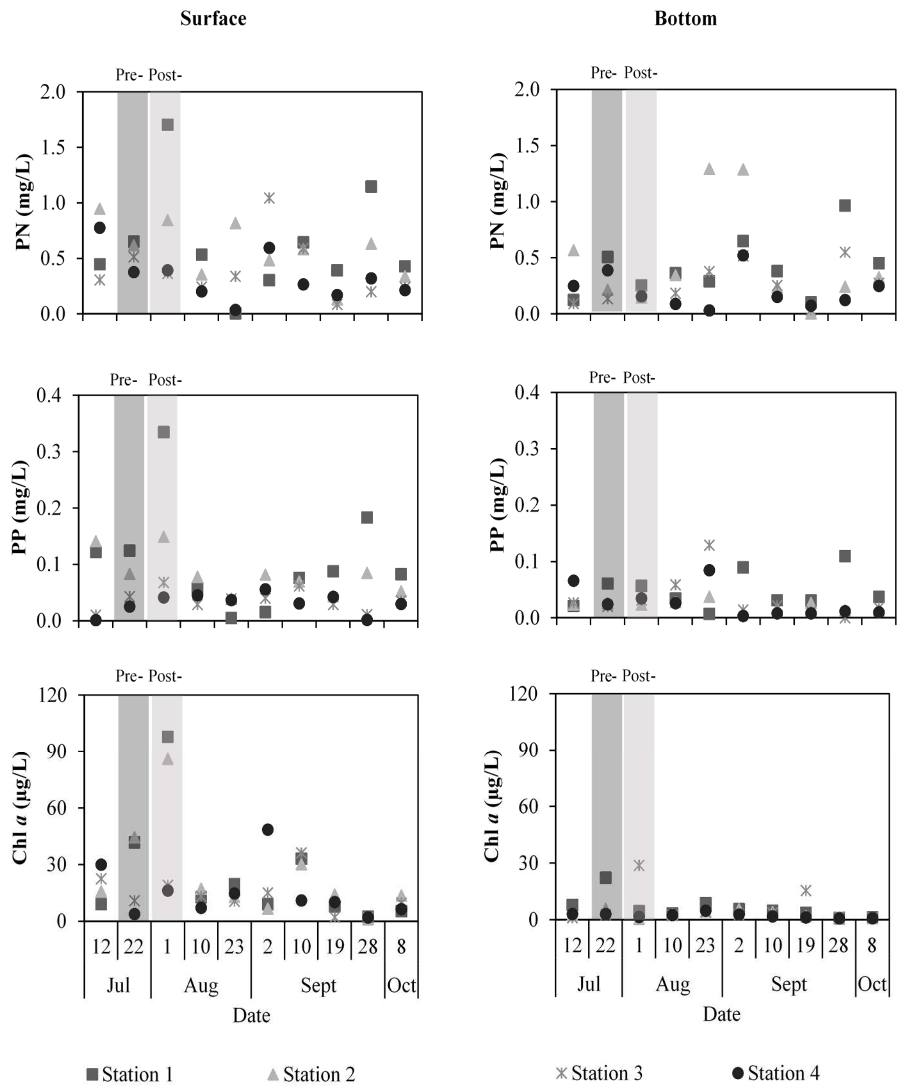

Figure 5 PN, PP, and Chl *a* during the summer of 2010.

The casualty came from the phytoplankton dynamic, which need to uptake dissolved nutrients for growing, as explained by Chen, *et al.* that assimilation or conversion of nutrients, especially dissolved nutrients, is needed by phytoplankton for growth [17]. The DN/DP ratios at all estuary stations also clearly showed limitation of N, except in the bottom layer of Station 1. Richards, *et al.* explained that the Redfield ratio of N and P is at a rate of 16:1

molar ratio or 7.2 mass ratio [18]. The ratio determines that limitation of N occurs when the Redfield ratio is below 7.2 mass ratio, while for the limitation of P it is vice versa.

3.3 Freshwater Influence on Salinity Alteration Pre and Post Rainfall

The high number of freshwater discharges during rainfall constitutes one of the important factors in controlling and changing estuarine salinity [19]. This input not only significantly influences the salinity of waterbodies but also affects the stratification and circulation of coastal waters [20]. Bárcena, *et al.* [21] in their study describe that huge freshwater inputs during rainfall are the main force of estuarine circulation and that at the same time the estuary is constantly being mixed. After heavy rainfall on 29th July, high freshwater inputs stratified salinity around Atsumi Bay. As explained before, the river inputs mostly influence surface circulation, which results in dominant alteration of the salinity gradient in the surface layer rather than in the bottom layer.

Figure 6 shows more details of the conditions on 22nd July (pre rainfall) and 1st August (post rainfall) of Atsumi Bay, as previously shown in Figures 4 and 5. Here, pre-rainfall surface salinity levels at Stations 1, 2, 3, and 4 were around 22.6 psu, 24.6 psu, 26.0 psu, and 25.9 psu, respectively. Post rainfall, the surface salinity gradually decreased to between 3.3-5.5 psu, where the salinity levels at Stations 1, 2, 3, and 4 were around 17.1 psu, 21.1 psu, 22.6 psu, and 22.8 psu, respectively. This suggests that high freshwater inputs during heavy rainfall influenced surface estuarine circulation and mixing, which resulted in a depletion of surface salinity.

Conversely, bottom salinity around the estuary in post-rainfall conditions tended to increase at the river mouth station and tended to be stable at the seaward stations. Post rainfall, the bottom salinity at Station 1 increased to 28.5 psu. On the other hand, the bottom salinity levels at Stations 2, 3, and 4 tended to be stable, with alterations around 0.1-0.5 psu. Eventually, the decline of salinity in the surface layer and the stable condition of the bottom layer indicates that the river inputs dominantly influenced the horizontal circulation of the estuary rather than the vertical circulation. Moreover, the observation data showed that the density difference of freshwater and seawater is an important factor in overlaying the high-salinity layer with a low-salinity layer. This is strengthened by Ji's statement that there is a tendency for low-density freshwater to flow over the surface estuarine layer when the surface layer circulation has a lower salinity than the bottom layer [22].

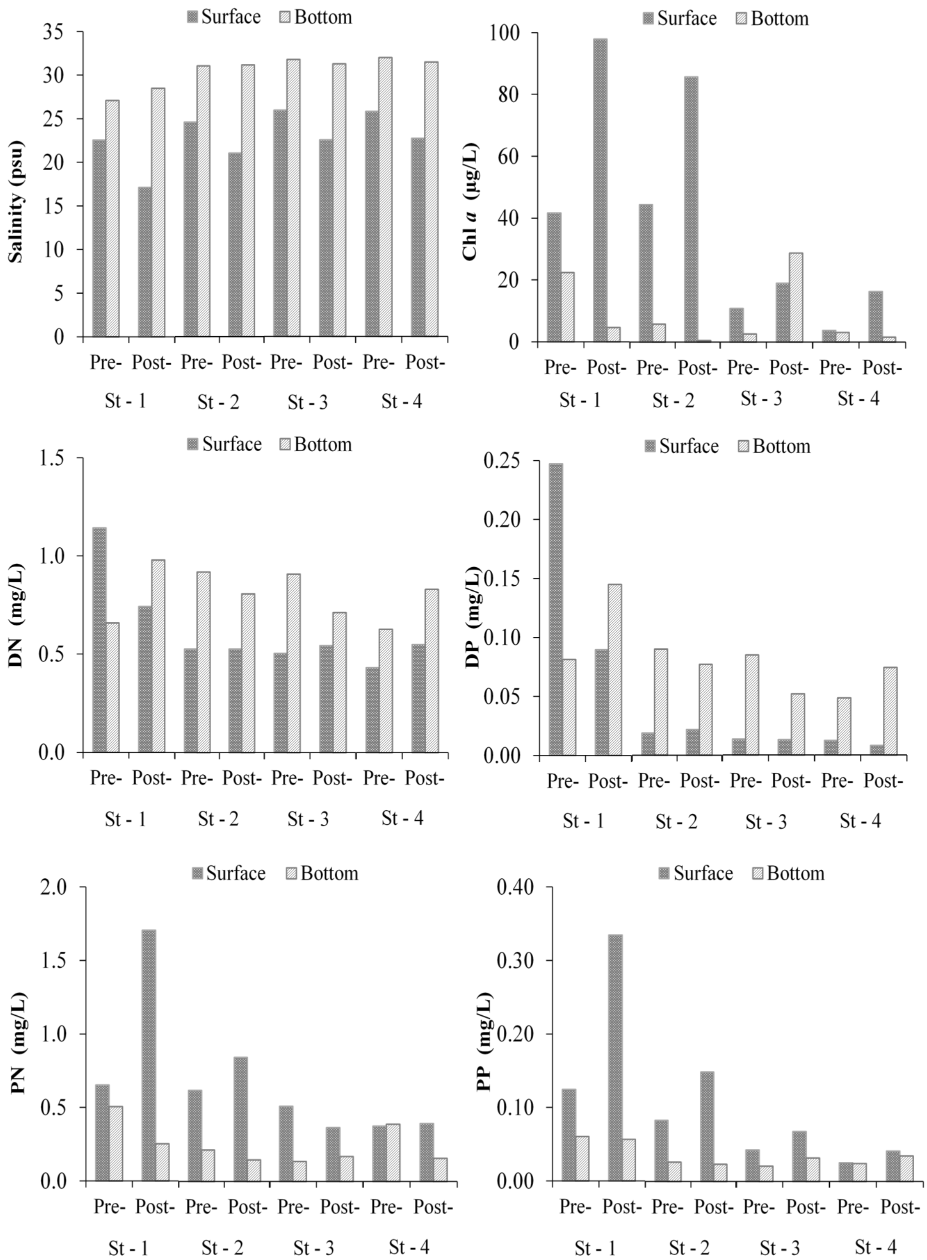

Figure 6 Estuarine salinity, Chl a, DN, DP, PN and PP between pre- and post-rainfall conditions.

Moreover, freshwater mostly influenced the area nearest to the river mouth, as shown by the data where salinity at stations close to the ocean tended to be higher than at other stations. In addition, the decrease of surface salinity by high freshwater inputs has a correlation with the decrease of DN and DP as an effect of dilution by freshwater in the surface layer. This correlation will be discussed in the next section.

3.4 Chl *a* and Nutrient Alterations Pre and Post Rainfall

Post-rainfall water quality changes significantly occurred in the surface layer rather than in the bottom layer due to dominant horizontal circulation from freshwater inputs into the estuary. In the surface layer, we found that Station 1, at the river mouth, experienced the highest rate of Chl *a* growth compared to the other estuarine stations. At Station 1, surface Chl *a* post rainfall was elevated two times higher than pre rainfall. The concentration was 41.7 µg/L pre rainfall, which was further elevated to 97.9 µg/L post rainfall (Figure 5). Other estuarine stations displayed a similar tendency. Pre rainfall, the Chl *a* concentrations at Stations 2, 3, and 4 were around 44.6 µg/L, 10.9 µg/L, and 3.8 µg/L, respectively, which later increased to 86.0 µg/L, 19.1 µg/L, and 16.3 µg/L post rainfall. This growth was also assisted by the influence of the summer water temperature, light intensity, and nutrient concentrations. Ji described that phytoplankton bloom may occur during favorable conditions with regard to nutrients, sunlight, and water temperature, until one or more of these factors are no longer available [23].

In situ observation showed that the average temperature at the estuarine station was around 29-32°. Based on Huertas, *et al.* [24] several marine phytoplankton are able to grow in warm water temperatures of around 22-35 °C. Regarding nutrient uptake, post-rainfall conditions showed an increase of Chl *a* and a decrease of DN and DP, which is assumed to have a positive correlation with nutrient uptake by phytoplankton besides dilution by freshwater. According to Figure 3, the input of dissolved nutrients from the river during rainfall represents the existence of huge levels of dissolved nutrients in the estuary. However, the post-rainfall data show a decline of surface DN and DP at estuarine Station 1 and a steady state of these concentrations at the other stations (Figure 6). Pre rainfall, DN concentrations at Stations 1, 2, 3, and 4 were 1.14 mg/L, 0.53 mg/L, 0.51 mg/L, and 0.43 mg/L, respectively. At the same event, DP concentrations were 0.25 mg/L at Station 1, 0.02 mg/L at Station 2, and 0.01 mg/L at the other two stations. Post rainfall, depletion of DN and DP occurred at Station 1, where the concentrations were 0.74 mg/L and 0.09 mg/L, respectively, or one third and two thirds lower than pre rainfall, respectively. Furthermore, both DN and DP at the other stations remained stable between pre and post rainfall.

The decline of DN and DP gives a negative correlation between river inputs and estuarine concentrations. The decrease of DN and DP is related to two conditions: dilution, which has a correlation with the decline of salinity as an effect of freshwater inputs, and phytoplankton uptake, which is correlated with the increase of Chl *a*, especially in the surface layer. Relation between salinity and DN and salinity and DP in Figure 7 shows good correlation, which is confirmed by the coefficients of correlation 0.86 and 0.98, respectively. These correlations indicate that freshwater inputs occupied the surface layer of the waterbodies, which resulted in a decline of salinity and assisted the dilution of DN and DP. In addition, the decrease of DN and DP also has a relationship with phytoplankton growth, which consumes dissolved nutrients in major amounts. Eventually, the post-rainfall DN and DP concentrations were what remained after dilution by freshwater and uptake by phytoplankton. The time between July 29th (during rainfall) and August 1st was an appropriate time for Chl *a* to grow by uptaking dissolved nutrients. In addition, Rasul, *et al.* in their study at the Toyo River transect of Atsumi Bay found similar conditions and corroborated that nutrient uptake by phytoplankton resulted in the decrease of surface dissolved nutrients [12].

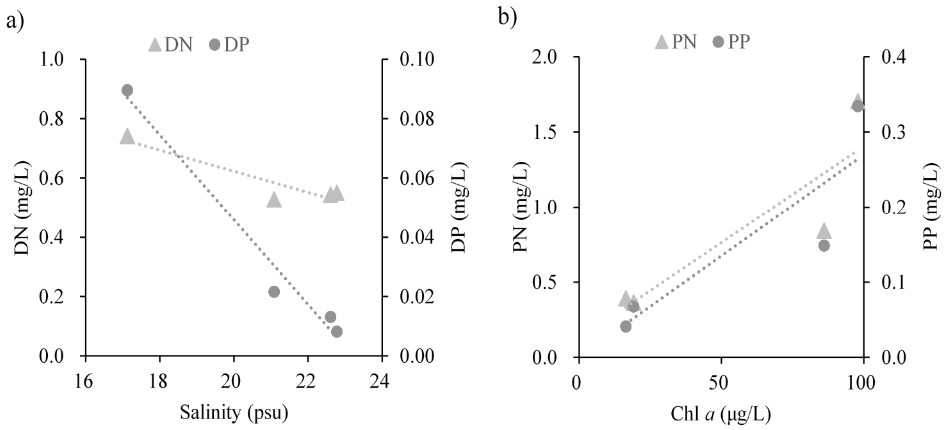

Figure 7 (a) Relation between surface salinity and dissolved nutrients (DN and DP); (b) relation between Chl *a* and particulate nutrients (PN and PP).

Apart from the decrease of dissolved nutrients, the escalation of Chl *a* post rainfall was followed by an increase of particulate nutrients PN and PP. Figure 6 shows an increase of particulate nutrients, which appeared significantly at the station closest to the river mouth. The highest concentrations of PN and PP were recorded at Station 1, where both concentrations were 2.6 times higher than pre rainfall. Similar results were found at Station 2, with increases of PN and PP of about 1.4 and 1.9 times, respectively, compared to pre rainfall. On the other hand, PN and PP at Station 3 showed a small decline; they are assumed to have sunk to the bottom layer. Moreover, PN and PP at Station 4, which lies in the open sea, were relatively more stable than at the other stations. The increase of PN and PP had a similar trend as Chl *a*, where a positive correlation was found between Chl *a* and PN, and Chl *a* and PP with the coefficients of determination being 0.78 and 0.77, respectively (Figure 7). These significant and positive values indicate that a concentration of Chl *a* was contained in the particulate bodies.

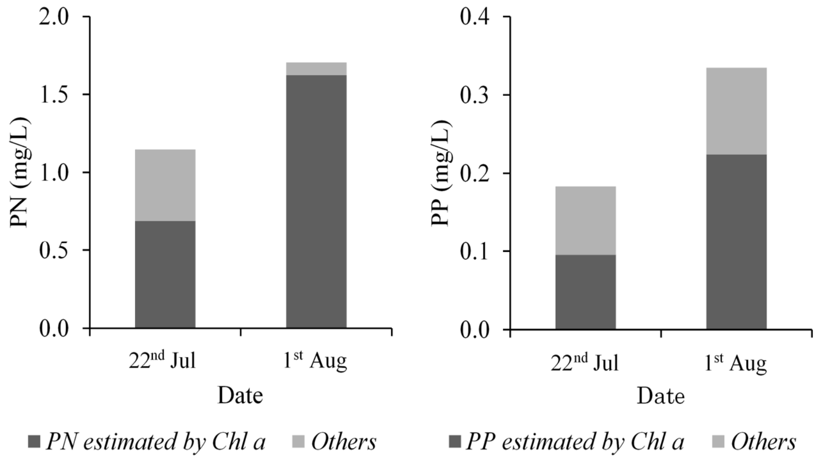

Figure 8 Phytoplankton biomass on PN and PP.

Based on this, we presume that PN and PP post rainfall were not only coming from river inputs but also from other sources, especially phytoplankton. In order to confirm this assumption, we used Chl *a*:N:P mass stoichiometry to verify the Chl *a* concentration in the particulate nutrient bodies. By referring to Rasul, *et al.* [4] estimation of Atsumi Bay's Chl *a*:N:P mass stoichiometry as 1:16.6:2.3, multiplication of N:Chl *a* and P:Chl *a* with Chl *a* concentration gave a correlation between PN, PP, and Chl *a*, as depicted in Figure 8. The figure shows the relation between PN, PP, and Chl *a* on July 22nd (pre rainfall) and August 1st (post rainfall), whether PN and PP were coming from phytoplankton growth in the estuary or from other sources. Pre rainfall, phytoplankton biomass on PN and PP showed lower values of Chl *a* influencing PN and PP, where about half of the PN and PP concentrations possibly came from the river and other sources. On the other hand, the data for the 1st of August show that high Chl *a* was not only related to particulate nutrient inputs from the river but also resulted from estuarine phytoplankton growth. The graph indicates that the phytoplankton biomasses on PN and PP as well as Chl *a* levels were higher post rainfall. This indicates that the source of PN and PP post rainfall dominantly came from phytoplankton growth compared to other sources.

4 Conclusion

In our study, freshwater inputs during rainfall changed post-rainfall salinity stratification in the Umeda River transect of Atsumi Bay. The increase of nutrients and the decline in salinity after rainfall proved that a large amount of freshwater, mixed with nutrients, affected water quality conditions in the estuary. The decrease of dissolved nutrients is an effect of freshwater dilution and phytoplankton uptake, which was verified by increased levels of Chl *a*, PN, PP, and decreased levels of DN and DP. Moreover, the influence of freshwater was dominant at river mouth Station 1 compared to the other stations. The larger distance between the station and the river mouth, the lower the influence, which shows the large differences in proportion between Chl *a*, dissolved nutrients and particulate nutrients at river mouth Station 1 and open sea Station 4. In addition, the surface layer was supplied to more than the bottom layer, because the inputs more influenced horizontal circulation than vertical circulation. This resulted in a small alteration of nutrient and Chl *a* concentrations in the bottom layer compared to the surface layer.

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