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Dissolved Free Amino Acids in Coastal Seawater Using a Modified Fluorometric Method

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The method of Parsons *et al*. (1984) for measuring dissolved free amino acids (DFAA) in coastal seawater was modified. We found considerable interference in DFAA determination from ammonia dissolved in coastal seawater, although the interference of urea could be ignored. For DFAA analysis for coastal seawater samples, ammonia determinations for the same sample are needed to correct DFAA values. For coastal surface seawater samples from all over the Seto Inland Sea, Japan, values of DFAA ranged from undetectable to 1.87 μg-at N/l when corrected for ammonia, while uncorrected values ranged from undetectable to 2.61 μg-at N/l. DFAA, urea, nitrate + nitrite, ammonia and DON concentrations in surface seawater collected in the Seto Inland Sea were analyzed simultaneously. DFAA at four seasons constituted from 1.4 to 10.1% of DON, with a mean value of 6.5%. The concentration of urea was similar to that of DFAA and often higher than that of ammonium, although generally lower than that of nitrate.

Keywords:
· DFAA,
· fluorometric method,
· the Seto Inland Sea,
· DON,
· urea.

1. Introduction

Although dissolved free amino acids (DFAA) are present in trace amounts in natural water, there has been great interest in studying DFAA in oceanic and coastal waters because they are a good nitrogen source for marine microalgae and bacteria. The role of DFAA in the growth of marine microalgae has been reviewed (Flynn and Butler, 1986).

The fluorometric method for measuring DFAA described by Parsons *et al*. (1984) is very useful for routine analysis because the method is fast, simple and there is no need for expensive equipment, such as HPLC. However, the amino acid composition cannot be determined by this method. The sensitivity of the fluorometric determination of amino acids is much better than that of the older colorimetric ninhydrin method. The fluorescent reagent, o-phthalaldehyde in the presence of 2-mercaptoethanol, reacts with primary amines and the fluorescent products are measured using a fluorometer with a 1-cm cell. However, there is considerable interference in the fluorometric determination of DFAA, because ammonia and urea in seawater react positively with the fluorometric reagent, o-phthalaldehyde. The measurement of DFAA should be corrected when analyzing coastal waters which contain high concentrations of ammonia and urea. In this paper, we examine the magnitude of the interference of ammonia and urea dissolved in natural seawater when measuring DFAA. Furthermore, we examine DFAA, urea, nitrate + nitrite, ammonia and dissolved organic nitrogen (DON) concentrations in surface seawater collected in the semi-enclosed Seto Inland Sea, Japan.These compounds have not previously been determined simultaneously.

2. Materials and Methods

2.1 Samples

Surface seawater samples were collected with a clean bucket at the 39 stations in the Seto Inland Sea, Japan (Fig. 1) during four cruises (October 1993, January, April and July 1994) on board the *T.R.V. Toyoshio-Maru* of Hiroshima University. The seawater samples for the analysis of DFAA, ammonia, urea and total dissolved nitrogen were immediately filtered through a pre-combusted Whatman GF/F filter (450°C, 2 h) and preserved until analysis at –20°C.

2.2 Chemical analyses

DFAA were determined by the fluorometric method described by Parsons *et al*. (1984). This method is a modification of the techniques outlined by Benson and Hare (1975), Josefsson *et al*. (1977), Dawson and Pritchard (1978) and Lindroth and Mopper (1979). A HITACHI 204 fluorescence spectrophotometer, equipped with a 1 cm cell was used. The optimum excitation wavelength was 342 nm, and optimal emission wavelength was 452 nm. Borate-buffered solution, including o-phthalaldehyde (0.048%) and 2-mercaptoethanol, was mixed with the sample solution (1:1) and allowed to stand for 2 min. at room temperature before measurement. The standard curve for amino acids was determined using glycine and concentrations of total amino

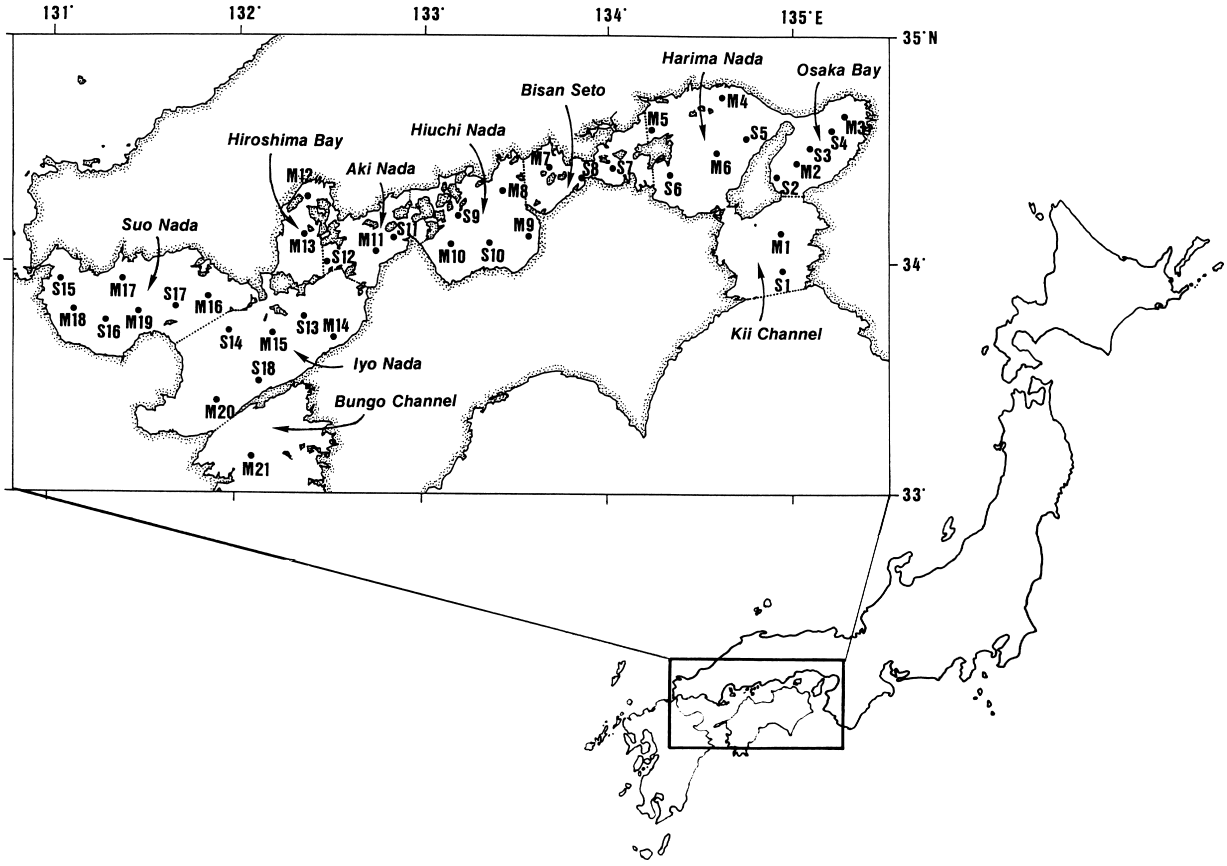

Fig. 1. Location of sampling stations in the Seto Inland Sea, Japan.

acids were calculated as a glycine equivalent. The reason why glycine was used is that glycine is the most predominant amino acid in DFAA and relative fluorescent intensities of other dominant amino acids are similar to that of glycine (Parsons *et al*., 1984).

Ammonia and nitrate + nitrite were measured with a Technicon Autoanalyzer II. Urea was measured by the method of Matsunaga and Nishimura (1972), which is an improvement of the diacetyl monoxime method of Newell *et al*. (1967). The DON value was calculated from the difference of total dissolved nitrogen (TDN) and dissolved inorganic nitrogen (DIN; nitrate + nitrite + ammonia). TDN was measured by the high-temperature catalytic oxidation (HTCO) method (Maita and Yanada, 1990) using a Sumigraph N-200 (Sumitomo Chemical Industries Co.). Duplicate or triplicate analyses on all determinations of DFAA, ammonia, urea and TDN gave results with a coefficient of variation less than 14%.

3. Results and Discussion

3.1 Interference in DFAA determination

Standard solutions of glycine, ammonia and urea were measured by the method used for DFAA determination. At the wavelength used to determine DFAA fluorescence, fluorescence increased with increasing concentrations of ammonia (Fig. 2). The relative fluorescence intensities per μmol-nitrogen of DFAA and ammonia were 34.6 and 2.55, respectively. The fluorescence of urea was much lower than similar concentrations of DFAA and ammonia. Moreover, the relative fluorescence intensities per μmol-nitrogen of DFAA were higher by one order of magnitude than that of ammonia. Therefore, considering the concentrations of these compounds, there was considerable interference by ammonia for DFAA measurements, while the interference of urea was almost negligible. The fluorescence due to various ammonia concentrations in a glycine solution increased when ammonia concentration was higher than 3 μg-at N/l (Fig. 3).

For seawater samples collected from all over the Seto Inland Sea, concentrations of DFAA, ammonia, and urea varied from undetectable to 2.61, undetectable to 24.2, and undetectable to 4.22 μg-at N/l, respectively (Tables 1 to 4). Although DFAA concentrations were high at Stn. 10 in October and Stn. 1 in April, no pattern of DFAA concentrations was apparent through the entire Seto Inland Sea. Although the concentrations of ammonia were relatively low, or lower than the detection limit, except for a few stations in April 1994, the ammonia concentrations of all

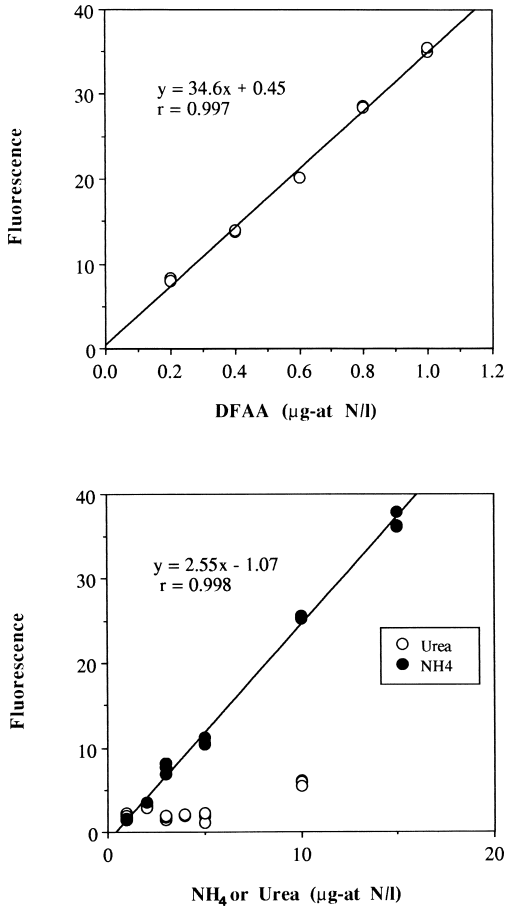

Fig. 2. Relationship between relative fluorescence intensities and concentrations of DFAA (glycine), ammonia and urea.

samples were higher than 1.1 μg-at N/l in October 1993. Since urea concentrations were usually lower than 4.22 μg-at N/l, the interference of the urea for DFAA determinations can be ignored. However, ammonia concentrations were often sufficiently high to enhance the fluorescence intensities in DFAA determinations.

Using the equations for relationships between relative fluorescence intensities and concentrations of the standard solutions of glycine and ammonia shown in Fig. 2, the interference of ammonia in DFAA determination was esti-

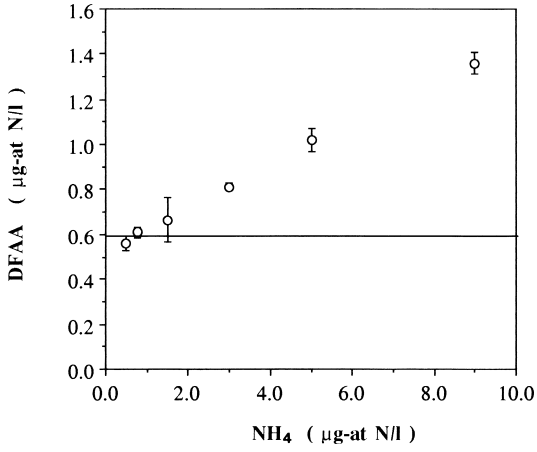

Fig. 3. Increase in DFAA concentrations in a 0.6 μg-at N/l glycine solution due to the addition of ammonia. The line shows the concentration of the glycine solution with no ammonia. Bars indicate the range of duplicates.

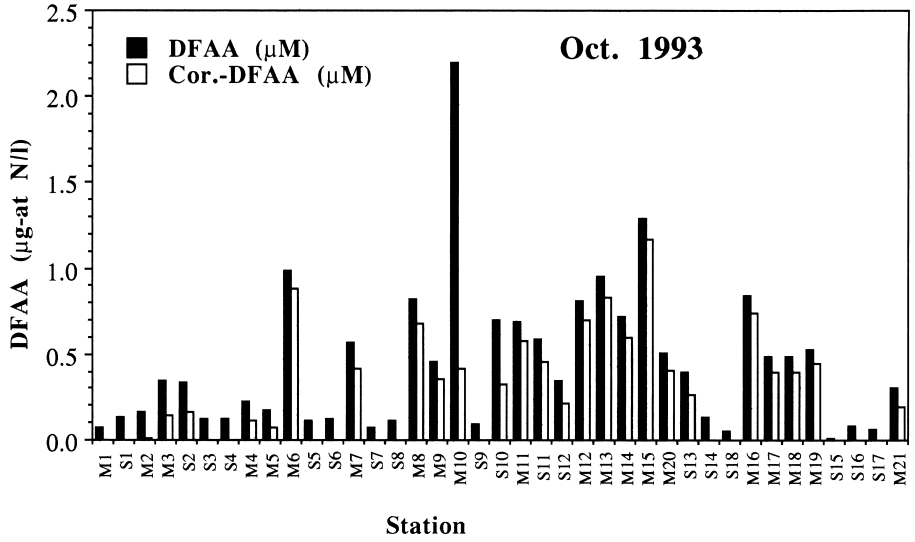

Fig. 4. DFAA concentrations before (black bar) and after (white bar) correction for ammonia in samples from stations in the Seto Inland Sea during October 1993.

Table 1. Results of DFAA (before ammonium correction), cor.-DFAA (after ammonium correction), nitrate + nitrite, ammonia, urea and DON concentrations for seawater samples collected in the Seto Inland Sea.

	Stn.	DFAA	cor.-DFAA	$NO_2 + NO_3$	NH_4	Urea	DON
		(μg·at N/l)					
Kii Channel	M1	0.07	0.00	2.55	2.11	0.25	2.06
	S1	0.13	0.00	1.91	1.74	0.47	n.d.
Osaka Bay	M2	0.16	0.01	3.79	2.06	0.25	0.87
	M3	0.35	0.14	21.9	2.91	0.58	n.d.
	S2	0.34	0.16	2.63	2.49	0.21	4.46
	S3	0.12	0.00	6.08	1.84	0.15	0.85
	S4	0.12	0.00	5.35	1.98	0.00	2.90
Harima Nada	M4	0.22	0.11	1.14	1.47	0.39	3.79
	M5	0.17	0.07	1.99	1.41	0.17	3.65
	M6	0.99	0.88	2.64	1.54	2.49	5.79
	S5	0.11	0.00	4.79	1.99	0.13	2.96
	S6	0.12	0.00	3.48	1.87	0.37	2.77
Bisan Strait	M7	0.57	0.42	10.4	2.07	0.57	4.50
	S7	0.07	0.00	15.8	1.75	0.48	n.d.
	S8	0.11	0.00	20.7	1.93	0.17	n.d.
Hiuchi Nada	M8	0.82	0.68	0.27	1.87	0.39	4.42
	M9	0.46	0.36	u.d.	1.36	0.06	4.06
	M10	2.20	0.42	1.00	24.2	0.56	10.6
	S9	0.09	0.00	3.74	2.24	0.09	3.44
	S10	0.70	0.33	0.04	5.04	0.04	5.96
Aki Nada	M11	0.69	0.58	5.04	1.48	0.10	1.81
	S11	0.59	0.46	5.27	1.70	0.11	1.96
	S12	0.35	0.21	2.78	1.84	0.00	6.26
Hiroshima Bay	M12	0.81	0.70	0.04	1.43	0.00	3.31
	M13	0.96	0.83	0.15	1.73	1.18	5.40
Iyo Nada	M14	0.72	0.60	3.12	1.63	0.60	n.d.
	M15	1.29	1.17	2.88	1.66	1.55	1.39
	M20	0.51	0.41	3.37	1.34	0.10	0.32
	S13	0.40	0.26	4.19	1.87	0.92	0.43
	S14	0.13	0.00	3.02	1.74	0.13	0.76
	S18	0.05	0.00	2.94	2.00	0.02	2.37
Suo Nada	M16	0.84	0.74	u.d.	1.41	1.15	3.47
	M17	0.49	0.40	0.19	1.28	0.17	2.91
	M18	0.49	0.40	0.75	1.19	0.17	2.61
	M19	0.53	0.45	0.04	1.14	0.02	4.99
	S15	0.01	0.00	0.01	1.84	u.d.	1.56
	S16	0.08	0.00	0.05	1.77	u.d.	1.75
	S17	0.06	0.00	0.12	1.80	u.d.	n.d.
Bungo Channel	M21	0.30	0.19	3.93	1.46	0.27	n.d.

u.d.: undetectable; n.d.: no data.

mated for each seawater sample and the concentrations of DFAA were corrected. Namely, the portions of fluorescence due to ammonia dissolved in individual samples were subtracted from the fluorescence values of seawater samples for DFAA determinations. Before the ammonium correction, DFAA concentrations ranged from undetectable to 2.61 μg-at N/l, while after ammonium correction, DFAA decreased and ranged from 0 to 1.87 μg-at N/l. Although the ten, seven and three DFAA values were corrected in all the samples collected in January 1993, April and July 1994, respectively, all values were corrected in October 1993, because ammonia concentrations were high. Particularly in the case of the samples which contained ammonia higher than 2.0 μg-at N/l, DFAA values were corrected and decreased by 20 to 100% compared to the values before correction. Considering one result as an example, Fig. 4 shows DFAA

Table 2. As Table 1 but in the January cruise.

	Stn.	DFAA	cor.-DFAA	$NO_2 + NO_3$	NH_4	Urea	DON
		(μg·at N/l)					
Kii Channel	M1	0.13	0.12	12.8	0.74	0.08	0.73
	S1	0.31	0.28	12.8	1.03	0.73	2.56
Osaka Bay	M2	0.33	0.28	13.3	1.21	0.11	0.59
	M3	1.82	0.92	25.5	12.8	0.15	2.81
	S2	0.38	0.30	13.7	1.72	0.59	n.d.
	S3	0.12	0.10	11.3	0.89	0.49	0.83
	S4	0.26	0.18	13.3	1.69	u.d.	1.54
Harima Nada	M4	0.46	0.46	4.05	0.42	0.99	7.64
	M5	0.10	0.10	2.58	0.16	u.d.	8.52
	M6	0.15	0.15	8.43	0.32	0.27	0.94
	S5	0.46	0.46	9.09	0.51	0.89	1.96
	S6	0.20	0.20	6.45	0.65	0.41	3.88
Bisan Strait	M7	0.06	0.06	1.78	0.31	0.04	6.02
	S7	0.24	0.22	6.02	0.85	0.51	5.28
	S8	0.14	0.11	5.24	1.05	0.33	3.96
Hiuchi Nada	M8	0.01	0.01	3.78	0.36	0.12	5.40
	M9	0.23	0.23	2.79	0.25	0.58	6.79
	M10	0.03	0.03	5.07	0.15	0.30	5.47
	S9	0.09	0.09	4.85	0.07	0.41	4.89
	S10	0.22	0.19	3.82	1.01	0.32	5.86
Aki Nada	M11	0.24	0.24	4.59	0.28	0.65	4.53
	S11	0.15	0.15	4.51	0.13	0.44	2.68
	S12	0.16	0.16	4.58	0.27	0.24	3.35
Hiroshima Bay	M12	0.34	0.34	8.05	2.56	0.54	1.36
	M13	0.37	0.37	4.73	0.29	0.97	6.84
Iyo Nada	M14	0.19	0.19	3.62	0.12	0.44	5.48
	M20	0.14	0.14	5.99	u.d.	0.42	3.96
	S18	0.29	0.29	5.72	0.16	0.59	2.39
Suo Nada	M16	0.24	0.24	2.65	0.11	0.03	6.46
	M17	0.51	0.51	0.15	0.17	0.27	8.46
	M18	0.16	0.16	0.09	0.21	u.d.	6.87
	M19	0.47	0.47	0.21	0.09	0.49	7.90
	S15	0.18	0.18	0.13	0.22	0.19	6.97
	S16	0.24	0.24	0.07	0.21	0.05	7.48
	S17	0.15	0.15	0.10	0.14	u.d.	7.62
Bungo Channel	M21	0.26	0.26	5.62	0.04	0.13	4.00

u.d.: undetectable; n.d.: no data.

concentrations before and after correction for ammonia in samples during October 1993.

Although the interference of ammonium in DFAA determinations can be ignored in the open ocean, it cannot be ignored in coastal waters, where ammonia concentrations are often higher than a few μg-at N/l, as in the Seto Inland Sea. Our study clearly showed that there was considerable interference in DFAA determination by ammonia dissolved in coastal seawater and thus DFAA should be corrected for ammonia. Furthermore, the interference of urea in DFAA determination can be ignored when urea concentrations are less than 10 μg-at N/l.

Mopper and Zika (1987) reported unexpectedly high concentrations of DFAA in oceanic rainwaters, averaging about 6.5 μg-at N/l and ranging from 1.1 to 15.2 μg-at N/l. Similar high concentrations of ammonia in rainwater were also reported by Keene *et al*. (1986) and Tamaki *et al*. (1991). The method of Parsons *et al*. (1984) cannot be used without the modification described here because of the high ammonia concentrations in rainwater.

3.2 DFAA and other nitrogen compounds in coastal seawater

In this study, DFAA, urea, nitrate + nitrite, ammonia and DON concentrations in surface seawater collected at

Table 3. As Table 1 but in the April cruise.

	Stn.	DFAA	cor.-DFAA	$NO_2 + NO_3$	NH_4	Urea	DON
		(μg·at N/l)					
Kii Channel	M1	2.61	1.53	2.22	15.3	1.21	6.27
	S1	0.49	0.49	2.21	0.23	2.19	5.01
Osaka Bay	M2	0.39	0.39	0.73	0.17	1.13	1.87
	M3	0.54	0.54	5.89	0.26	1.77	3.22
	S2	0.55	0.44	2.86	2.04	1.90	7.76
	S3	0.56	0.47	2.88	1.84	2.22	4.37
	S4	0.31	0.31	2.34	0.32	1.13	3.55
Harima Nada	M4	0.76	0.34	8.65	6.24	1.82	4.64
	M5	0.23	0.23	0.31	u.d.	2.11	2.74
	M6	0.46	0.46	0.79	0.17	2.48	2.78
	S5	0.49	0.47	1.81	0.82	1.66	5.23
	S6	0.55	0.55	0.17	0.43	2.48	5.47
Bisan Strait	M7	0.38	0.37	1.15	0.79	1.82	3.58
	S7	0.55	0.55	0.43	0.31	2.61	7.53
	S8	0.68	0.67	0.46	0.76	2.11	6.23
Hiuchi Nada	M8	0.32	0.32	0.10	u.d.	1.42	4.32
	M9	0.26	0.26	0.03	u.d.	1.06	4.96
	M10	0.13	0.13	0.03	u.d.	0.71	8.38
	S9	0.67	0.67	0.03	u.d.	2.02	6.04
	S10	0.52	0.52	u.d.	u.d.	1.17	5.25
Aki Nada	M11	0.31	0.31	0.13	u.d.	0.87	4.84
	S11	0.27	0.27	0.08	u.d.	1.06	9.15
	S12	0.63	0.63	0.16	0.08	1.35	5.69
Hiroshima Bay	M12	0.54	0.54	0.17	u.d.	1.06	4.53
	M13	0.09	0.09	0.03	u.d.	0.85	4.39
Iyo Nada	M14	0.28	0.28	0.16	u.d.	1.35	3.85
	M15	0.39	0.39	0.30	u.d.	0.14	4.40
	M20	0.39	0.39	0.10	u.d.	0.01	8.44
	S13	1.87	1.87	0.18	u.d.	4.22	6.85
	S14	0.28	0.28	u.d.	u.d.	0.35	4.84
	S18	0.21	0.21	0.31	u.d.	0.12	7.68
Suo Nada	M16	0.73	0.73	0.08	u.d.	0.37	5.72
	M17	0.58	0.58	0.25	u.d.	0.00	6.51
	M18	0.58	0.58	0.11	u.d.	0.25	5.82
	M19	0.36	0.36	0.03	u.d.	0.00	4.67
	S15	1.02	1.02	0.02	u.d.	0.90	8.52
	S16	0.48	0.48	0.01	u.d.	0.27	3.31
	S17	0.21	0.21	u.d.	u.d.	u.d.	9.37
Bungo Channel	M21	0.55	0.55	1.86	0.16	0.18	5.15

u.d.: undetectable.

Seto Inland Sea were determined simultaneously. Tables 1 to 4 shows these values, although DFAA values range from undetectable to 1.87 μg-at N/l after ammonium correction, as described previously. The concentrations of nitrate + nitrite, ammonia, urea and DON varied from undetectable to 23.5 μg-at N/l, undetectable to 24.2 μg-at N/l, undetectable to 4.22 μg-at N/l and 0.32 to 25.7 μg-at N/l, respectively (Tables 1 to 4). Direct analyses showed that DFAA in coastal waters ranged from 0.04 to 2.2 μg-at N/l (Sharp, 1983). Our measurement values in the present study seem to fall within the range commonly encountered in coastal waters. The mean concentrations of DFAA in surface seawaters in each cruise were 0.31 μg-at N/l, 0.24 μg-at N/l, 0.50 μg-at N/l and 0.17 μg-at N/l in October, January, April and June, respectively. DFAA in each cruise constituted 10.1, 5.2, 9.2, and 1.4% of DON in October, January, April and June, respectively. The average value (6.5%) was similar to that reported in oceanic waters (7%; Sharp, 1982).

Table 4. As Table 1 but in the June cruise.

	Stn.	DFAA	cor.-DFAA	$NO_2 + NO_3$	NH_4	Urea	DON
		(μg·at N/l)					
Kii Channel	M1	0.37	0.37	0.15	u.d.	0.03	8.67
	S1	u.d.	0.00	0.10	0.06	u.d.	11.8
Osaka Bay	M2	0.06	0.06	4.79	0.07	0.46	8.15
	M3	1.00	0.91	0.19	1.86	u.d.	14.4
	S2	u.d.	0.00	4.39	0.27	u.d.	13.8
	S3	0.07	0.07	5.65	0.49	u.d.	9.98
	S4	0.06	0.05	0.12	0.69	u.d.	15.5
Harima Nada	M4	0.05	0.05	0.11	0.06	u.d.	25.7
	M5	0.04	0.04	0.18	0.22	u.d.	8.42
	M6	0.17	0.17	0.18	0.11	u.d.	10.1
	S5	0.31	0.31	3.35	0.55	0.52	12.0
	S6	0.06	0.06	0.12	0.23	0.69	13.1
Bisan Strait	M7	0.17	0.11	2.38	1.46	0.05	9.95
	S7	0.13	0.13	3.27	0.37	0.57	12.5
	S8	0.22	0.22	3.29	0.61	0.78	11.3
Hiuchi Nada	M8	u.d.	0.00	0.23	0.08	u.d.	9.13
	M9	0.21	0.21	0.06	0.05	0.47	10.8
	M10	u.d.	0.00	0.11	0.02	u.d.	10.6
	S9	u.d.	0.00	0.18	0.07	0.17	14.8
	S10	0.13	0.13	0.14	0.26	0.68	14.6
Aki Nada	M11	0.09	0.09	0.25	0.09	u.d.	9.10
	S11	0.06	0.06	0.28	0.26	0.05	12.3
	S12	0.38	0.38	0.11	0.08	1.56	16.6
Hiroshima Bay	M12	0.98	0.98	0.94	0.16	0.55	14.1
	M13	0.34	0.34	0.11	0.15	0.27	10.3
Iyo Nada	M14	0.26	0.26	0.36	0.08	0.17	10.2
	M15	0.11	0.11	0.13	0.14	1.11	10.4
	M20	0.09	0.09	0.29	0.11	0.72	10.4
	S13	0.17	0.17	1.27	0.12	2.61	12.7
	S14	0.07	0.07	0.27	0.09	0.53	12.3
Suo Nada	M16	0.16	0.16	0.11	0.49	0.53	11.2
	M17	0.10	0.10	0.10	0.04	1.26	12.4
	M18	0.13	0.13	0.06	0.07	1.42	13.7
	M19	0.17	0.17	0.21	0.09	0.50	11.6
	S15	0.13	0.13	0.75	0.15	0.88	13.4
	S16	0.09	0.09	0.18	0.07	0.53	13.2
	S17	0.06	0.06	0.10	0.04	0.86	14.4
Bungo Channel	M21	0.08	0.08	1.33	0.09	0.57	11.4

u.d.: undetectable.

Nitrate + nitrite, ammonium, urea and DFAA have not previously been determined simultaneously. In lower forms of dissolved nitrogen (nitrate + nitrite, ammonium, urea and DFAA), the seasonal trend of these compounds is high in winter and low in summer. Ammonia and nitrate + nitrite were high in January due to vertical mixing in the water column. Furthermore, in October 1993, ammonia and nitrate + nitrite were also high over the entire Seto Inland Sea, which was due to heavy rainfall during June to September in 1993 (Yamamoto *et al*., 1997). The concentration of urea was similar to that of DFAA and often higher than that of ammonium, although generally lower than that of nitrate. However, urea variation was not parallel to DFAA variation (Tables 1 to 4 and Fig. 5). It is interesting to note that both DFAA and urea were high in April (Fig. 5). Chl.*a* concentration in seawater was not high in April compared to other seasons (Tada *et al*., 1998). However, it is believed that the sample collection was done after the phytoplankton bloom, because nutrient concentrations in April had already decreased compared to those in January. It is well known that

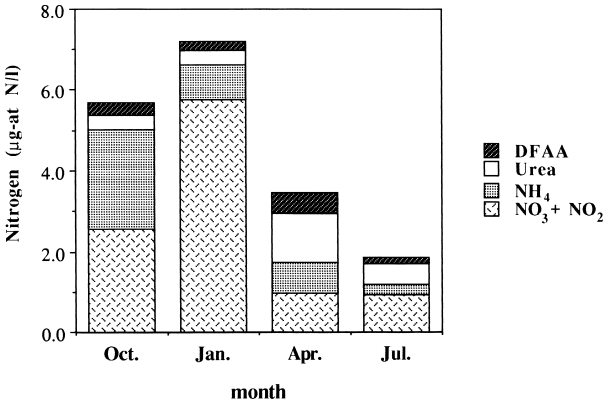

Fig. 5. Average concentrations of DFAA, Urea, Ammonia and Nitrate + Nitrite at each cruise in the Seto Inland Sea.

free amino acids are released into seawater by biological processes, such as phytoplankton excretion (e.g.; Hammer and Brockman, 1983; Carlucci *et al*., 1984) and sloppy feeding of grazers (Lampert, 1978). Our data suggest that DFAA was actively released from phytoplankton or the effect of sloppy feeding by grazers could not be ignored during the phytoplankton bloom.

Figure 5 also indicates that neither DFAA nor urea have been loaded in large quantities from river water, or removed rapidly by microorganisms, because the concentrations of these compounds were low, even in October. DFAA concentration was relatively constant in all seasons. DFAA constitutes 3.3% to 14.5% of lower forms of dissolved nitrogen, with a mean value of 8.1%. Flynn and Butler (1986) suggested that the levels of DFAA which we can measure in the marine environment are for the most part residual levels and that amino acids present at very low concentrations are the result of microbial activity. With the advent of DFAA analysis by HPLC, it has been concluded that DFAA are utilized by bacteria rather than by algae, and that the bacterial incorporation of amino acids leads to a regeneration of ammonium, which is subsequently used by the phytoplankton (e.g.; Williams, 1970). On the other hand, the importance of DFAA as a nitrogen source for marine invertebrates has been pointed out (e.g.; Stephen and Schinske, 1961; Nell *et al*., 1983; Manahan *et al*., 1983). Manahan (1989) reported that *Crassostrea gigas* larvae could account for 100% of their oxidative need by the uptake of amino acids from seawater at concentrations on the order of 10 μM. According to the report of Manahan (1989), the concentration level of DFAA which we measured in this study would satisfy the nitrogen requirements of *Crassostrea gigas*.

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