Spatial and seasonal variations in copepod communities related to turbidity maximum along the Chikugo estuarine gradient in the upper Ariake Bay, Japan

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Abstract

We investigated the spatial and seasonal variations in copepod assemblage, distribution and diversity in a wide spatial gradient (including the turbidity maximum) in the Chikugo estuary. Copepod samples were collected over a period of 12 months from April 2004 to March 2005. Hydrographical parameters such as temperature, salinity, turbidity (nephelometric turbidity unit, NTU), chlorophyll-*a* (chl-*a*), and phaeopigment (PhP) were measured. Two contrasting spatial zones were identified along the estuary: a zone of turbidity maximum exists in the low saline upper estuary, which was characterized by significantly higher turbidity and PhP concentrations than the downstream euryhaline zone. These two contrasting zones are characterized by contrasting copepod assemblages. A low saline upstream (true-estuarine) assemblage was dominated by an endemic calanoid coppeod, *Sinocalanus sinensis*, except during the summer when *Psedodiaptomus inopinus* dominated. In contrast, a euryhaline marine assemblage was observed in the downstream areas which was a multi-species assemblage dominated by common coastal marine species (*Acartia omorii*, *Oithona davisae*, *Paracalanus parvus*, *Pseudodiaptomus marinus*, etc.). There were two distinct groups of copepods based on the seasonal abundance: those showing a clear seasonal variability and those not. Most of the dominant copepod taxa did not show any well-defined seasonal pattern. Density, biomass, and diversity of copepods along the estuary are influenced by the hydrographic environments that are subject to significant spatio-temporal variations. Multivariate statistics showed that chl-*a* is the most significant predictor of overall copepod density, biomass and diversity as well as the density of dominant copepods and PhP is an additional predictor of copepod population dynamics in the ETM zones. This study indicates that the hydrographic and biological characteristics of ETM differ from that in the downstream marine areas and that spatial dynamics are more important than temporal dynamics in estuarine ecosystems.
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Keywords: spatial and seasonal variation; copepod community; estuarine turbidity maximum; Chikugo estuary; Ariake Bay

1. Introduction

Copepods are among the most important secondary producers in coastal and marine ecosystems, representing an important link between phytoplankton, microzooplankton and higher trophic levels such as fish. The variability observed in the distribution of copepod is due to a combination of both hydrographical variables such as temperature, salinity, turbidity, etc. and biotic parameters such as food habits and food availability (Roff et al., 1988; Christou, 1998; Escribano and Hidalgo, 2000; Beyst et al., 2001). However, copepod variability is difficult to relate to environmental factors because of complex multifactorial influences (Kiorboe and Nielsen, 1994). This is particularly true for estuarine environments because they are extremely dynamic and heterogenous ecosystems, subject to strong fluctuations of biotic and abiotic factors; therefore, estuarine ecosystems are of particular interest for studying copepod population dynamics.

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Spatio-temporal variations and habitat types are among the most important factors that influence observed patterns of species abundance, composition and size structure of estuarine planktons. Therefore, it is important that studies on estuarine copepods include a wide spatial and temporal scale. It has been shown that abundance and community structure of copepod display considerable variations on seasonal and spatial scales. Estuarine copepod communities are believed to be annually stable but show strong seasonal and spatial dynamics (Winkler et al., 2003) and, therefore, seasonal and spatial variations are generally more important than annual variations (David et al., 2005). Temperature, salinity, and food supply are among the most important factors that influence the observed spatial and seasonal patterns in demographic variations of copepods (Roddie et al., 1984; Hassel, 1986). Seasonal variations are mediated mainly by seasonal temperature variations (Durbin and Durbin, 1981) but salinity is also known to affect the distribution of copepods in estuaries (Lawrence et al., 2004). Interspecific differences in salinity tolerance often explain changes in the dominant species across the salinity gradient in estuary. Therefore, to achieve comprehensive understanding on the dynamics of estuarine copepods, studies should be based on multivariate considerations (Manning and Bucklin, 2005).

The estuarine turbidity maximum (ETM) is a ubiquitous feature in dynamic estuarine ecosystems with substantial impacts on copepod abundance and distribution. Previous studies have shown that planktonic predators encounter more prey under conditions of increased turbidity due to hydrodynamic accumulation of particles (POM) (Rothschild and Osborn, 1988; Gasparini et al., 1999; Roman et al., 2001; Winkler et al., 2003). Hydrodynamic conditions also result in high abundance and biomass of copepods in ETM (MacKenzie et al., 1994; MacKenzie and Kiørboe, 2000; Visser et al., 2001; David et al., 2005). ETM usually corresponds to areas just near the saltfront where freshwater mixes with seawater; therefore, ETM zones are resided by copepods with low salinity tolerance (Roman et al., 2001; Winkler et al., 2003). Consequently, ETM zones are characterized by low copepod diversity that is generally unique and different from that of upstream freshwater and downstream communities (Laprise and Dodson, 1994; Roman et al., 2001; Winkler et al., 2003).

The Chikugo estuary is comparable with the major estuarine systems of the world such as Chesapeake Bay, the St. Lawrence estuary, San Francisco Bay, the Gironde estuary and many others. The Chikugo estuary is characterized by sharp gradients in several environmental variables including salinity and turbidity and a zone of turbidity maximum in its upper part. Considerable amount of research has been done on copepod ecology in estuarine ecosystems in relation to turbidity maximum in Chesapeake Bay (Roman et al., 2001), the St. Lawrence estuary (Laprise and Dodson, 1994; Winkler et al., 2003), San Francisco Bay estuary (Kimmerer et al., 1998), the Gironde estuary (Irigoien and Castel, 1997; David et al., 2005) and in many other estuarine systems. However, the spatio-temporal variations in copepod distribution with respect to environmental factors in the Chikugo estuary have not

been sufficiently documented. Spatial variations in copepod distribution and feeding ecology have been previously described from samples collected in spring 2003 (Islam et al., 2004). In addition to the spatial variability, in this paper, we describe the seasonal patterns in copepod community structures and distribution from monthly samples over a period of one year from April 2004 to March 2005 along a wide spatial salinity gradient. The aim of this study was to characterize the spatial and seasonal variability in copepod abundance and distribution related to hydrographic environment along the Chikugo estuary in the upper Ariake Bay in Japan.

2. Materials and methods

The Ariake Bay (Fig. 1) is the largest tidal wetland in Japan. This is a rather shallow, semi-enclosed bay with the highest tidal range (~ 6.0 m) in Japan. The majority of the water supply comes from the East China Sea and the Pacific Ocean and a considerable mass of water drains into the bay from its large catchment through numerous channels. The Chikugo River is the largest river flowing into the bay at its western part; the river is approximately 200 m wide in the upper region and more than 250 m wide at the mouth during high tide. Therefore, a large estuarine system is formed at the
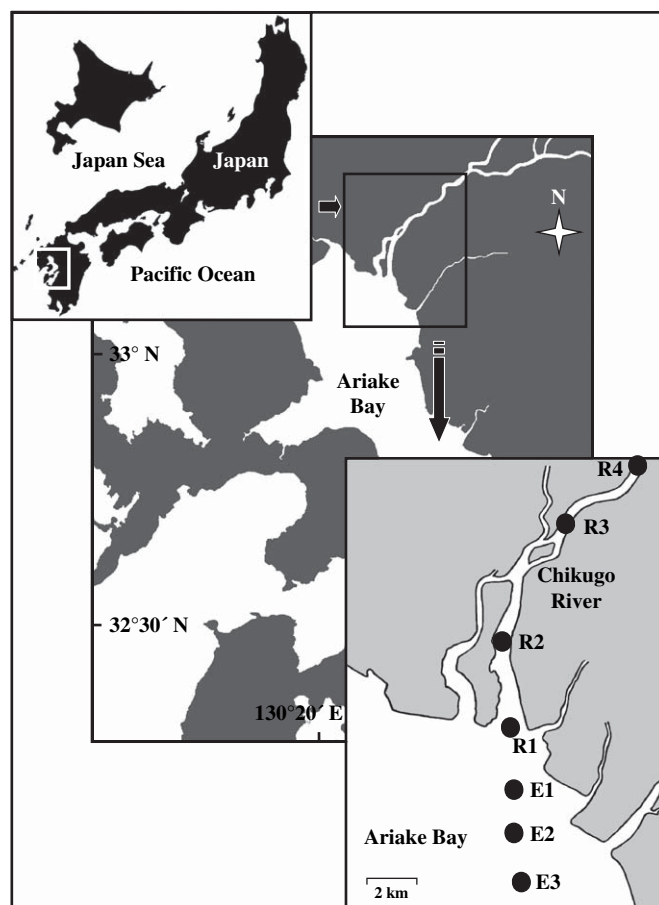

Fig. 1. Map of the Ariake Bay and the Chikugo River estuary showing the sampling stations.

mouth of the river. Considerable tidal influences of sea water reach to more than 15 km from the river mouth. Physically, the estuary is a well-mixed and dynamic estuary, characterized by a zone of nutrient-rich turbidity maximum in its upper region and an absence of vertical stratification. Being a dynamic and extremely heterogeneous ecotone, the abundance and distribution of planktonic copepods in the estuary are expected to be spatially and temporally variable.

Seven sampling stations were set up along the estuary (Fig. 1); the stations were lined along the tideway of the Chikugo River. Among them, four stations were along the river channel (R4, R3, R2 and R1) and the other three are outside the river mouth along the estuary (E1, E2 and E3). Station R1 is located at the river mouth, and R4 is the uppermost station, 16 km upstream from the mouth. Starting from the river mouth, the estuarine stations were situated on the tidal flat and E3 is the most distant station with the highest salinity.

Copepod samples were collected continuously over a period of 12 months from April 2004 to March 2005; samplings were conducted at the middle of each month and at the morning of each sampling day during high tide. Temperature ($^{\circ}$C), turbidity (nephelometric turbidity unit, NTU), salinity (practical salinity unit, PSU), chlorophyll-*a* (chl-*a*, μg l^{-1}) and phaeopigment (PhP, μg l^{-1}) were analyzed at each station. Temperature, turbidity, and salinity were recorded on the board by an Environmental Monitoring System (YSI 650 MDS, YSI Incorporated, USA). Details on the methods used for analyzing chlorophyll-*a* and phaeopigment concentrations have been described in our previous paper (Islam et al., 2004).

Copepod samples were collected by oblique tows of a plankton net (45 cm mouth diameter and 0.1 mm mesh size) equipped with a flow meter. The contents of the cod end were poured into a plastic container and immediately fixed in 10% seawater formalin and transported to the laboratory. Copepods were sorted from the suspended particles and detritus under a binocular stereomicroscope. The abundance of copepods was determined by identifying all copepods to the lowest possible taxonomic level and counting the total number; density was expressed as number m^{-3} of water. Copepod dry biomass of each major taxon was determined by drying samples at 45 $^{\circ}$C for 24 h in a thermostat oven and the dry weight was expressed as mg m^{-3}. Results of the copepod density and dry biomass for each month have been presented as a function of salinity. Copepod species diversity was assessed using the Shannon–Wiener index (H) of diversity, which was computed according to Berasategui et al. (2005).

Two-way analysis of variance (ANOVA) was used to examine the spatial and temporal differences, i.e., between the sampling stations and between months in all hydrographic variables, pigment concentrations, copepod density, biomass and diversity. Two-way ANOVA was used also to analyze the spatio-temporal variations in each dominant species. Copepod density data were natural log transformed to meet assumptions of normality (i.e., to minimize the influence of highly abundant species). Small deviations from normality or homogeneity after transformation were accepted because ANOVA is considered to be robust to such violations

(Underwood, 1997), particularly when there are many independent estimates of variance. Pearson's correlation coefficient was calculated to characterize the relationships between different hydrographic parameters, pigments, copepod density, biomass and diversity. To characterize the influence of the hydrographic variables and pigment concentrations on copepod density, biomass and diversity, multiple regressions were performed using the total copepod density as well as the density of each dominant species, biomass and diversity as dependent variables on the environmental variables. All analyses were performed using SPSS 12.0 for windows (SPSS Inc., 1989–2003).

3. Results

3.1. Hydrographic environment

Spatial and temporal variations in hydrographical parameters are given in Fig. 2. Temperature was not significantly different among stations ($P = 0.999$) but showed significant temporal variations ($P < 0.001$) with the mean ($\pm$SD) values ranging from 8.0 ± 0.9 $^{\circ}$C in January to 31.1 ± 0.3 $^{\circ}$C in August. The lowest mean salinity was 0.6 ± 0.7 recorded at R4 and increased significantly ($P < 0.001$) towards the sea with the highest value of 24.9 ± 5.2 at E3. The lowest salinity was recorded in May (3.3 ± 4.4) and the highest in June (20.5 ± 10.4) and showed significant ($P < 0.001$) temporal variation. The highest mean turbidity (258.4 ± 209.7 NTU) was recorded at R4 and decreased significantly ($P < 0.001$) toward the sea with the lowest mean value (28.8 ± 13.7 NTU) at E3. Turbidity showed significant monthly variations ($P < 0.05$), with the lowest value (44.1 ± 50.0 NTU) in June and the highest value (202.0 ± 156.7 NTU) in January. Turbidity had significant negative correlation (Table 1) with temperature ($P < 0.05$) and salinity ($P < 0.01$) and positive correlation with chl-*a* ($P < 0.05$) and PhP ($P < 0.01$).

The highest concentration of chl-*a* (11.4 ± 9.8 μg l^{-1}) was recorded at R4, decreased slightly toward the sea and the lowest concentration (7.0 ± 3.9 μg l^{-1}) was recorded at E3 (Fig. 2); however, spatial variations were not significant ($P = 0.293$). Temporal variations in chl-*a* concentration were significant ($P < 0.001$) with the lowest value (2.5 ± 0.6 μg l^{-1}) in May and the highest value (14.8 ± 3.4 μg l^{-1}) in August. Chl-*a* showed significant positive correlation with temperature ($P < 0.01$), turbidity ($P < 0.05$) and PhP ($P < 0.01$). In contrast to chl-*a*, PhP concentrations showed significant spatial variations ($P < 0.001$) with the highest concentration (16.1 ± 9.3 μg l^{-1}) at R4 and decreased sharply towards the sea, with the lowest value (2.9 ± 0.9 μg l^{-1}) at E3. Although temporal variations in PhP concentrations were significant ($P < 0.001$), there was no consistent seasonal trend; the lowest value (3.6 ± 1.3 μg l^{-1}) was found in May and the highest value (13.9 ± 5.1 μg l^{-1}) in February. PhP showed significant negative correlation with salinity and positive correlation with turbidity and chl-*a* (Table 1). The contrasting pattern between chl-*a* and phaeopigment was apparent when
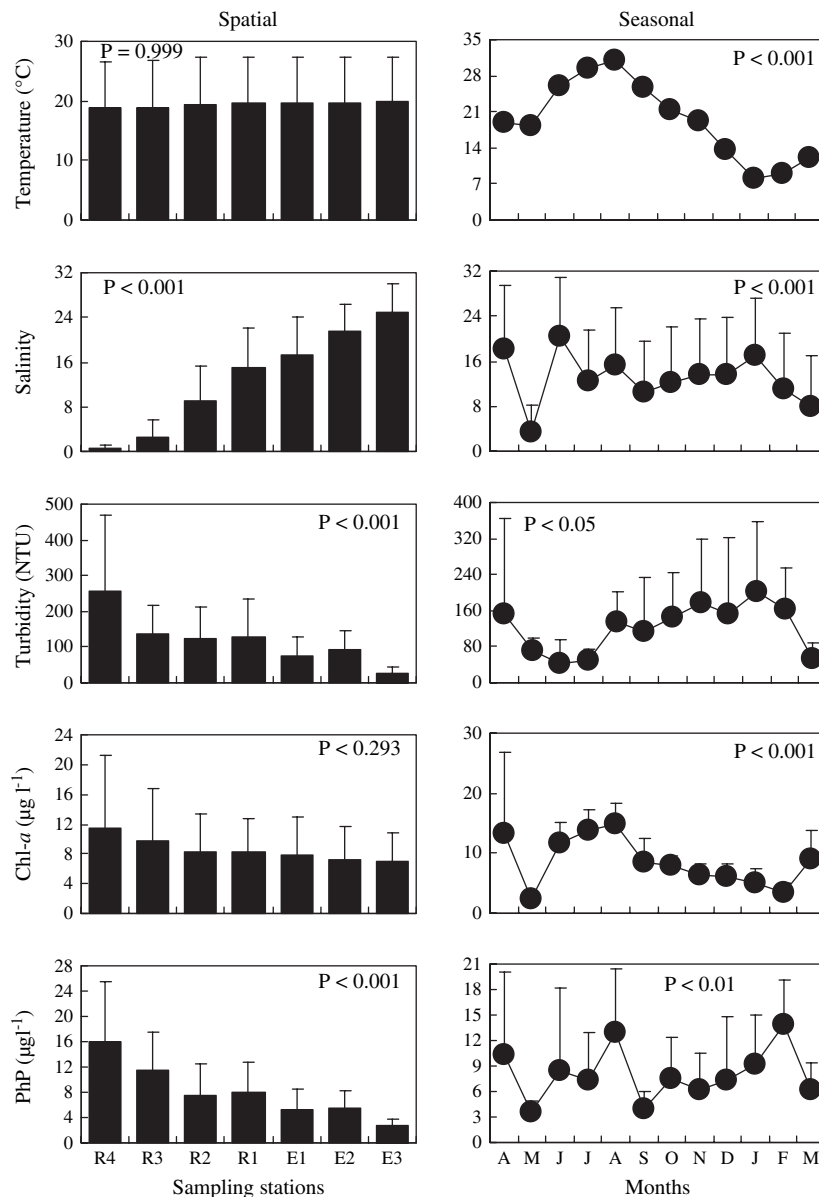
Fig. 2. Spatial and temporal variations in the hydrographical variables along the Chikugo estuary; values are mean ($\pm$SD) and *P* values indicate the results of ANOVA.

they were calculated as proportion of the total pigment. The ratio of chl-*a* to total pigment (TP) (chl-*a* + PhP) and that of PhP to TP were calculated as an index of chl-*a* and PhP production and their relative contribution to the total pigment over the spatial scale. Contrary to the chl-*a* concentrations which

showed no significant spatial variations, chl-*a*:TP ratio showed significant spatial variations ($P < 0.001$) with the lowest value at R4 and increased towards downstream and the highest value was observed at E3 (Fig. 3). Spatial variations of PhP:TP ratio also was significant ($P < 0.001$) and showed a completely

Table 1
Pearson's correlation coefficient between different hydrographical variables, pigments and copepod density, diversity and biomass collected over the spatio-temporal scales from the Chikugo estuary (*$P < 0.05$; **$P < 0.01$)

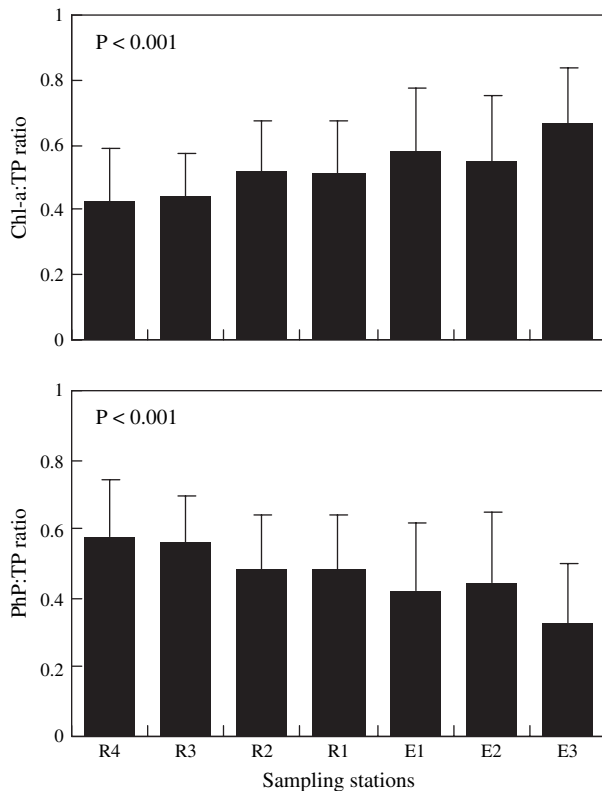|	Temperature	Salinity	Turbidity	Chl-*a*	PhP	Density	Biomass
Salinity	0.12	—					
Turbidity	−0.22*	−0.42**	—				
Chl-*a*	0.50**	−0.08	0.25*	—			
PhP	−0.07	−0.49**	0.68**	0.43**	—		
Density	0.43**	0.08	0.13	0.60**	0.22*	—	
Biomass	0.26*	−0.19	0.39**	0.69**	0.45**	0.82**	—
H	0.45**	0.27*	−0.20	0.04	−0.20	−0.14	−0.16

Fig. 3. Spatial variations in PhP:TP ratio along the Chikugo estuary (values are mean ± SD); TP is total pigment (chlorophyll-*a* + phaeopigment); *P* value indicates the results of ANOVA.

contrasting scenario, i.e., the highest value was recorded at R4 and decreased downstream and the lowest value was recorded at E3.

3.2. Copepod community structure, density, biomass and diversity

A total of 25 copepod taxa were recorded; among them, there were 21 consistently identified species and the others were either not identified to the species level or unidentified (Table 2). Overall, copepod species, in order of abundance (those contributing more than 1%) were *Oithona davisae* (31.3%), *Sinocalanus sinensis* (25.5%), *Paracalanus parvus* (12.8%), *Cyclopoida* spp. (6.6%), *Acartia omorii* (6.0%), *Pseudodiaptomus inopinus* (5.9%), *Pseudodiaptomus marinus* (2.7), Copepodite (2.4%), *Acartia erythraea* (1.9%), and *Acartia pacifica* (1.5%) and the other 15 taxa contributed a total of 3.4% of the total biomass. *S. sinensis* was the overwhelmingly dominant species based on overall biomass contribution; this species alone contributed as high as 44.6% of the total copepod biomass. *S. sinensis* was followed by *P. inopinus* (12.1%), *P. parvus* (10.7%), *O. davisae* (8.6%), *A. omorii* (7.3%), *Cyclopoida* spp. (5.0%), *P. marinus* (3.1), *A. erythraea* (2.4%), *A. pacifica* (1.8%), *Centropages abdominalis* (1.7%), and Copepodite (1.3%) and the other taxa contributed a total of only 1.4% of the overall copepod biomass.

Two clearly different copepod assemblages were identified along the estuary; one, the upstream low-saline assemblage in

Table 2

Contribution of each species to the overall density and biomass of copepods collected over a period of twelve months along the Chikugo estuary; species are arranged in order of abundance

| Density (number $\times 10^3$ m^{-3})			Biomass (mg m^{-3})		
%	Total	Species	%	Total	Species
31.28	415.33	*Oithona davisae*	44.60	1825.98	*Sinocalanus sinensis*
25.52	338.96	*Sinocalanus sinensis*	12.12	496.23	*Pseudodiaptomus inopinus*
12.81	170.06	*Paracalanus parvus*	10.66	436.30	*Paracalanus parvus*
6.61	87.78	*Cyclopoida* spp.	8.62	353.03	*Oithona davisae*
5.99	79.61	*Acartia omorii*	7.26	297.18	*Acartia omorii*
5.90	78.35	*Pseudodiaptomus inopinus*	5.03	205.81	*Cyclopoida* spp.
2.66	35.27	*Pseudodiaptomus marinus*	3.09	126.42	*Pseudodiaptomus marinus*
2.43	32.24	Copepodite	2.43	99.54	*Acartia erythraea*
1.87	24.88	*Acartia erythraea*	1.77	72.29	*Acartia pacifica*
1.51	20.08	*Acartia pacifica*	1.70	69.63	*Centropages abdominalis*
0.75	9.92	Nauplii	1.34	54.72	Copepodite
0.74	9.79	*Euterpina acutifrons*	0.49	20.05	*Sinocalanus tenellus*
0.38	5.11	*Centropages abdominalis*	0.25	10.06	*Hemicyclops japonicus*
0.34	4.54	*Hemicyclops japonicus*	0.15	6.31	Nauplii
0.34	4.50	Unidentified	0.12	4.89	*Euterpina acutifrons*
0.31	4.14	*Paracalanus crassirostris*	0.11	4.34	Unidentified
0.27	3.64	*Sinocalanus tenellus*	0.09	3.75	*Calanus sinicus*
0.07	0.91	*Centropages tenuiremis*	0.07	2.73	*Tortanus derjugini*
0.06	0.78	*Calanus sinicus*	0.04	1.69	*Microsetella* sp.
0.05	0.69	*Tortanus derjugini*	0.04	1.43	*Centropages tenuiremis*
0.04	0.54	*Microsetella* sp.	0.03	1.11	*Labidocera rotunda*
0.03	0.44	*Labidocera rotunda*	0.01	0.21	*Temora turbinata*
0.02	0.21	*Temora turbinata*	0.00	0.17	*Eurytemora pacifica*
0.01	0.17	*Eurytemora pacifica*			
0.00	0.04	*Centropages gracilis*			
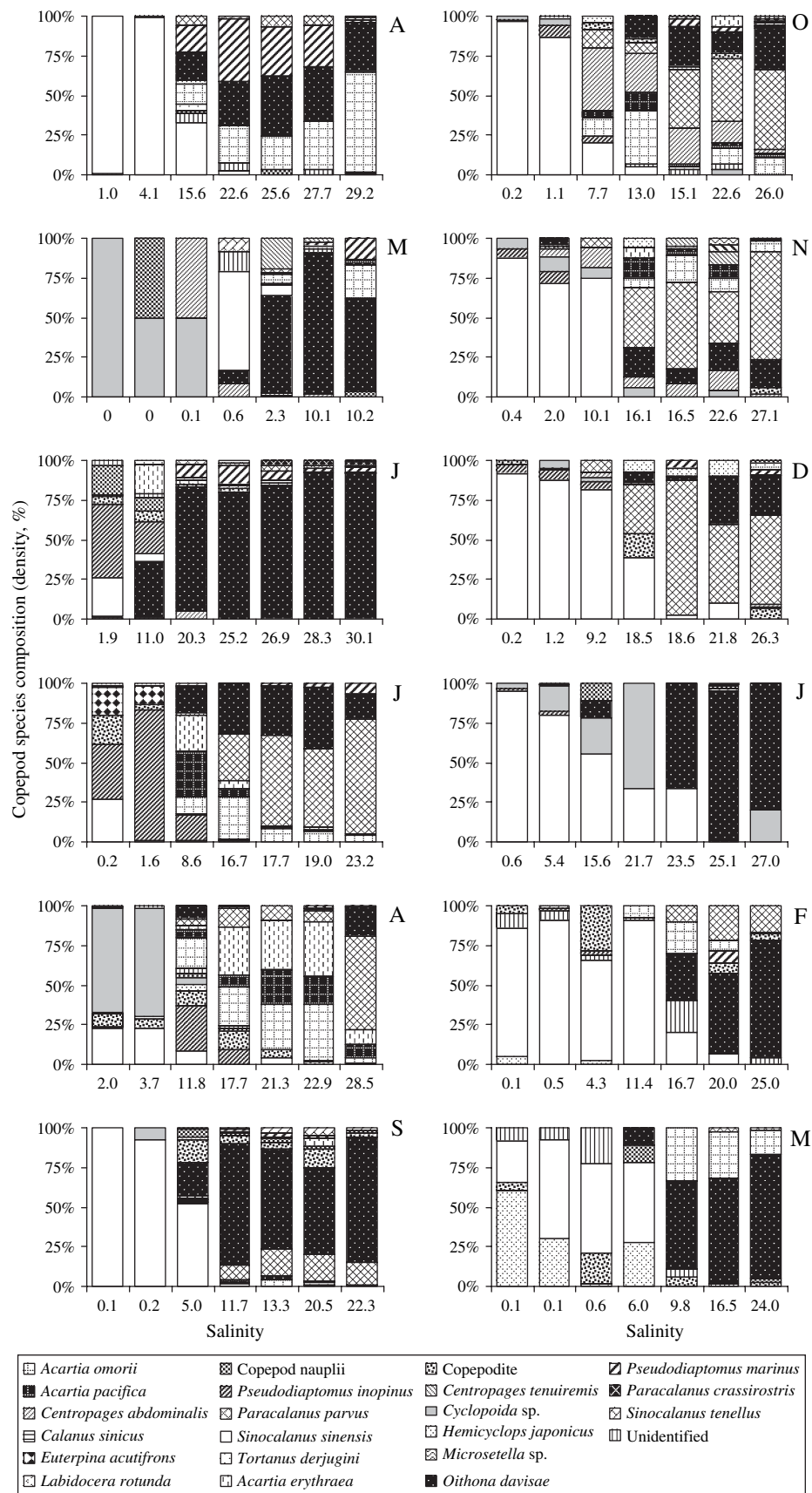
Fig. 4. Spatial and temporal variations in the numerical composition of copepod from April (A) 2004 to March (M) 2005 along the spatial gradient in Chikugo estuary. Note that the scale of the *x*-axis which shows the salinities are different.

Table 3
Two-way analysis of variance (ANOVA) summary results of the spatial (seven sampling stations) and temporal (12 months) variations in the density of the dominant copepod species along the Chikugo estuary

Copepod species	Sources of variation	df	F	P
Cyclopoida sp.	Temporal	11	5.21	0.000
	Spatial	6	7.02	0.000
Sinocalanus sinensis	Temporal	11	1.90	0.055
	Spatial	6	21.76	0.000
Nauplii	Temporal	11	2.42	0.013
	Spatial	6	0.82	0.556
Acartia omorii	Temporal	11	5.08	0.000
	Spatial	6	17.49	0.000
Hemicyclops japonicus	Temporal	11	2.61	0.008
	Spatial	6	2.30	0.045
Oithona davisae	Temporal	11	5.61	0.000
	Spatial	6	23.61	0.000
Pseudodiaptomus inopinus	Temporal	11	3.34	0.001
	Spatial	6	10.00	0.000
Pseudodiaptomus marinus	Temporal	11	4.89	0.000
	Spatial	6	8.87	0.000
Paracalanus parvus	Temporal	11	5.97	0.000
	Spatial	6	20.30	0.000
Copepodite	Temporal	11	3.98	0.000
	Spatial	6	0.73	0.626
Acartia erythraea	Temporal	11	8.51	0.000
	Spatial	6	2.98	0.012
Acartia pacifica	Temporal	11	9.00	0.000
	Spatial	6	6.67	0.000

Table 4
Relationships (Pearson's correlation coefficient) between the monthly patterns of temperature and chl-a and the monthly abundance of dominant species of copepods ($**P < 0.01$; $*P < 0.05$)

Species	Temperature	Chl-a
S. sinensis	−0.01	0.39
O. davisae	0.44	0.28
A. omorii	0.38	0.68*
Cyclopoida spp.	0.46	0.48
Nauplii	0.41	0.16
C. abdominalis	0.17	0.01
P. parvus	0.47	0.41
P. inopinus	0.62*	0.60*
P. marinus	0.64*	0.72**
A. erythraea	0.69*	0.66*
A. pacifica	0.74**	0.66*
H. japonicus	0.76**	0.68*
Copepodite	0.74**	0.54

station R4−R2 (extended down to R1 during winter) and, the other, the high salinity assemblage in station R1−E3. The low salinity assemblage was dominated by only a few species, mainly *S. sinensis* during almost the whole survey period (Fig. 4). Exception occurs during May−August when *Cyclopoida* spp. and *P. inopinus* dominated. In contrast, the high salinity downstream assemblage was dominated by a number of species which were seasonally variable. Major species of this assemblage were *O. davisae*, *P. parvus*, *A. omorii*, *P. marinus* and some other common coastal copepods. *O. davisae* dominated overwhelmingly during May, June, September and January and *P. parvus* in July and from October to December. *A. omorii* dominated during February−March; in April, *A. omorii*, *O. davisae* and *P. marinus* co-occurred and contributed almost equally.

Spatio-temporal variations of most of the dominant copepods were significant (Table 3). There were three groups based on spatial distribution: (1) those showing significantly (Table 3) higher density in the upper estuary (*S. sinensis*, *Cyclopoida*, *P. inopinus* and *H. japonicus*), (2) those showing no significant spatial variation and (3) those showing significantly higher density in the lower estuary (*O. davisae*, *A. omorii*, *A. erythraea*, *A. pacifica*, *P. parvus*, *P. marinus*) (Fig. 5). Monthly variations in the abundance of dominant species showed that most of the dominant species were generally highly abundant during June−August (Fig. 6), corresponding with the periods of high temperature. Thus, the monthly abundance of *A. erythraea*, *A. pacifica*, Copepodite, *P. inopinus*, *P. marinus*, and *H. japonicus* correlated significantly with the monthly patterns of temperature and chl-a (Table 4). In contrast, *S. sinensis*,

O. davisae, *P. parvus*, *A. omorii*, copepod nauplii and *Cyclopoida* spp. did not show consistent monthly patterns and were not correlated with monthly temperature variations although temporal variations in the density of these copepods were significant. The dominant species in the two contrasting copepod communities showed a completely contrasting spatial distribution pattern in their density. Copepods of the low saline upstream assemblage (*S. sinensis* and *P. inopinus*) showed a decrease in abundance towards downstream while those of the high saline marine assemblage (*O. davisae*, *P. parvus*, *Acartia* spp. and *P. marinus*) showed an increase in abundance toward the sea (Fig. 7).

Copepod density showed significant spatial variations ($P < 0.05$) with the highest mean density of $(32.6 \pm 39.7) \times 10^3$ m^{-3} at E3 and the lowest mean density of $(7.9 \pm 10.3) \times 10^3$ m^{-3} at R2. There were three major spatial zones based on copepod density; a high density zone in the lower estuary (E2−E3), a medium density zone in the upper estuary (R4−R3) and a low density zone in between (R2−E1) (Fig. 8). In contrast to copepod density, the highest standing biomass of 121.6 ± 217.6 mg m^{-3} was recorded at the uppermost station and the lowest value of 18.5 ± 20.0 mg m^{-3} was recorded at R1 and varied significantly between stations ($P < 0.05$). Similar to copepod density, there were three major spatial zones based on copepod standing biomass. However, in contrast to the density patterns, the zone of high biomass occurred in the upper estuary (R4−R3), the zone of medium biomass in the lower estuary (E2−E3) and the zone of low biomass in between (R2−E1). Copepod density and standing biomass showed significant ($P < 0.005$ and $P < 0.05$ respectively) temporal variations; higher density and biomass were observed during April−September (except for May). There were two distinct regions based on all the indexes of diversity used. The upper estuary (R4−R3) had significantly lower diversity than the lower estuary (R2−E3) and this pattern was consistent with the Shannon−Weiner index ($P < 0.001$), species richness ($P < 0.05$) and evenness ($P < 0.01$). The highest values of all the indexes were observed at R2 and the lowest value at R4.
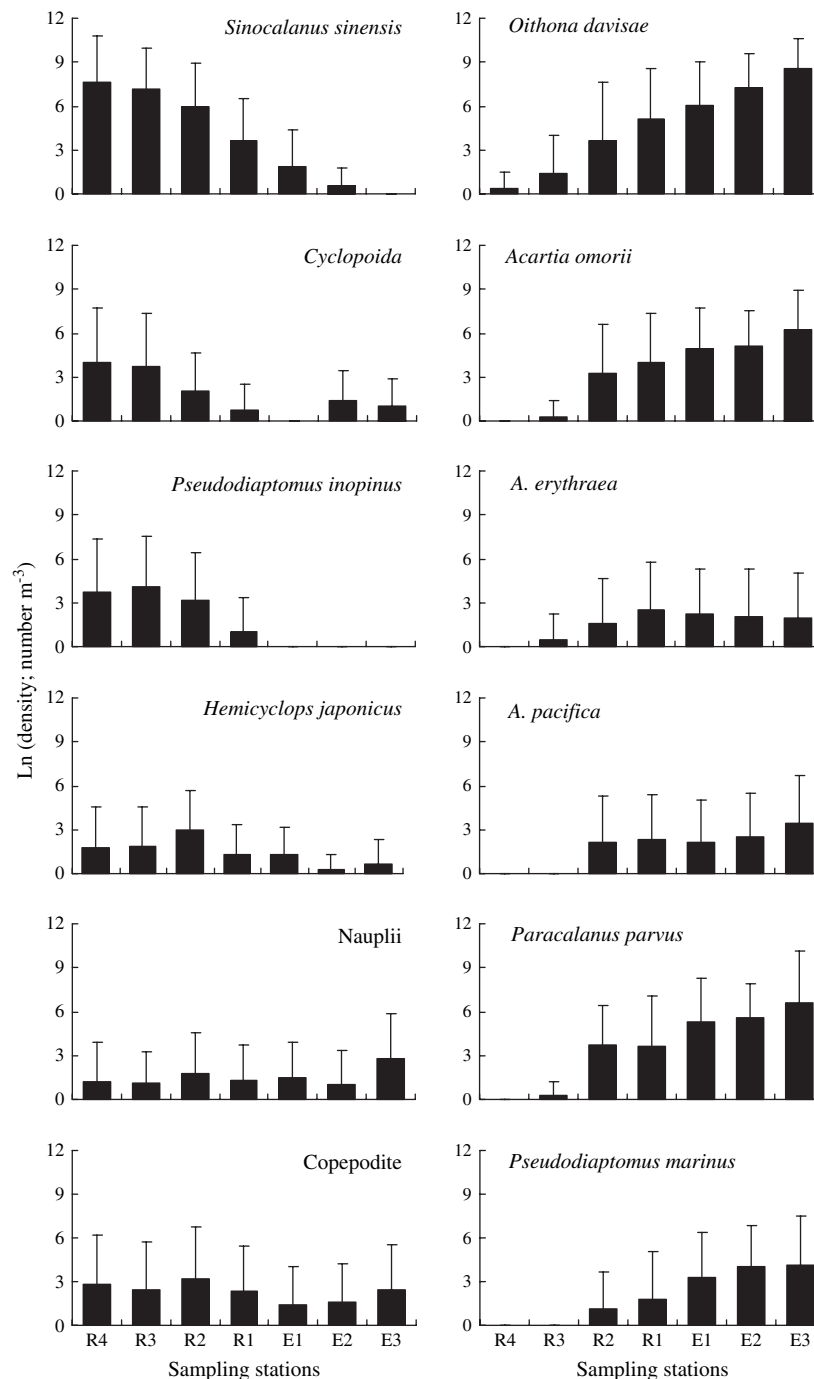
Fig. 5. Spatial variation in the density of each of the dominant copepod species. Mean (±SD) values are natural log transformed density (number m^{-3}) data generated from 12 months samples collected from April 2004 to March 2005 along the spatial gradient in the Chikugo River estuary.

3.3. Role of hydrographic variables in copepod abundance and diversity

The influence of the hydrographic environment on copepod density, biomass and diversity were characterized using multiple regression analysis. Temperature, salinity, turbidity, chl-a, and PhP were used as independent variables in the multiple regression model to characterize their influence on the dependent variables of copepod density, biomass and diversity.

Significant multiple regression models were produced for copepod density ($F = 8.27$; adjusted $R^2 = 0.38$; $P < 0.0001$), biomass ($F = 13.08$; adjusted $R^2 = 0.51$; $P < 0.0001$) and diversity ($F = 4.64$; adjusted $R^2 = 0.235$; $P < 0.0001$) (Table 5). The model showed that chl-a was the common predictor for all of the dependent variables (P values were <0.05, <0.001, and <0.05 for density, biomass and diversity respectively). For biomass and diversity, two additional variables such as turbidity for biomass ($P < 0.05$) and salinity for diversity
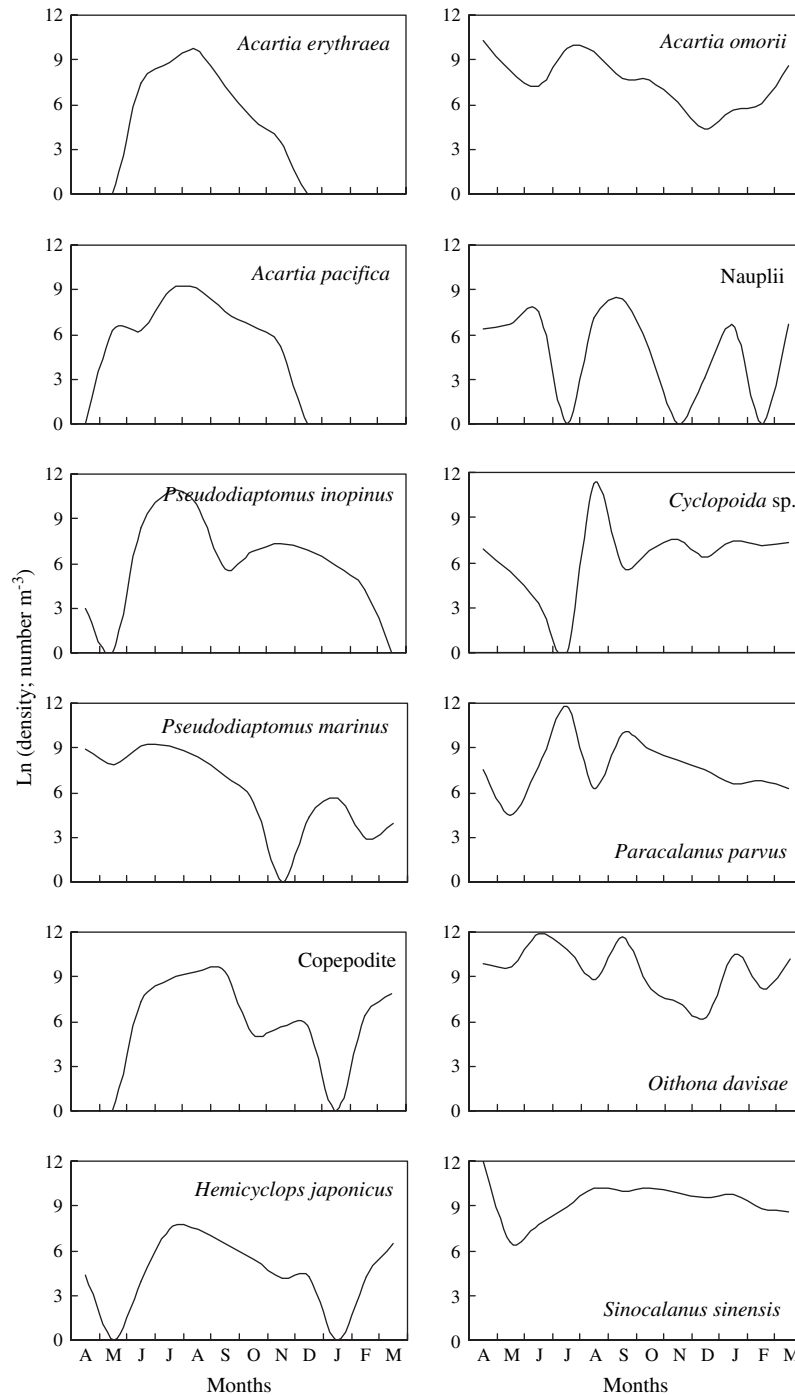
Fig. 6. Temporal variation in the density of each of the dominant copepods. Values are natural log transformed density (number m^{-3}) collected from April 2004 to March 2005 along the spatial gradient in the Chikugo River estuary.

($P < 0.05$) were found as significant predictors. The environmental parameters and copepod density, biomass and diversity were calculated separately for four main seasons, i.e., winter (December–February), spring (March–May), summer (June–August) and autumn (September–November) and the Pearson's correlation analysis was performed to examine the relationships among the variables. Only temperature, chl-*a* and copepod density showed significant correlations (Fig. 9). When multiple regressions were done using each

dominant species as the dependent variable, only the two most dominant species such as *S. sinensis* ($F = 4.95$; adjusted $R^2 = 0.74$; $P < 0.05$) and *O. davisae* ($F = 6.54$; adjusted $R^2 = 0.801$; $P < 0.05$) produced significant models. Again, chl-*a* was the significant common predictor variable for both *S. sinensis* ($P < 0.05$) and *O. davisae* ($P < 0.05$) and one additional variable such as PhP ($P < 0.05$) was significant predictor for *S. sinensis*. Multiple regression models with the other species were not significant, suggesting that either the
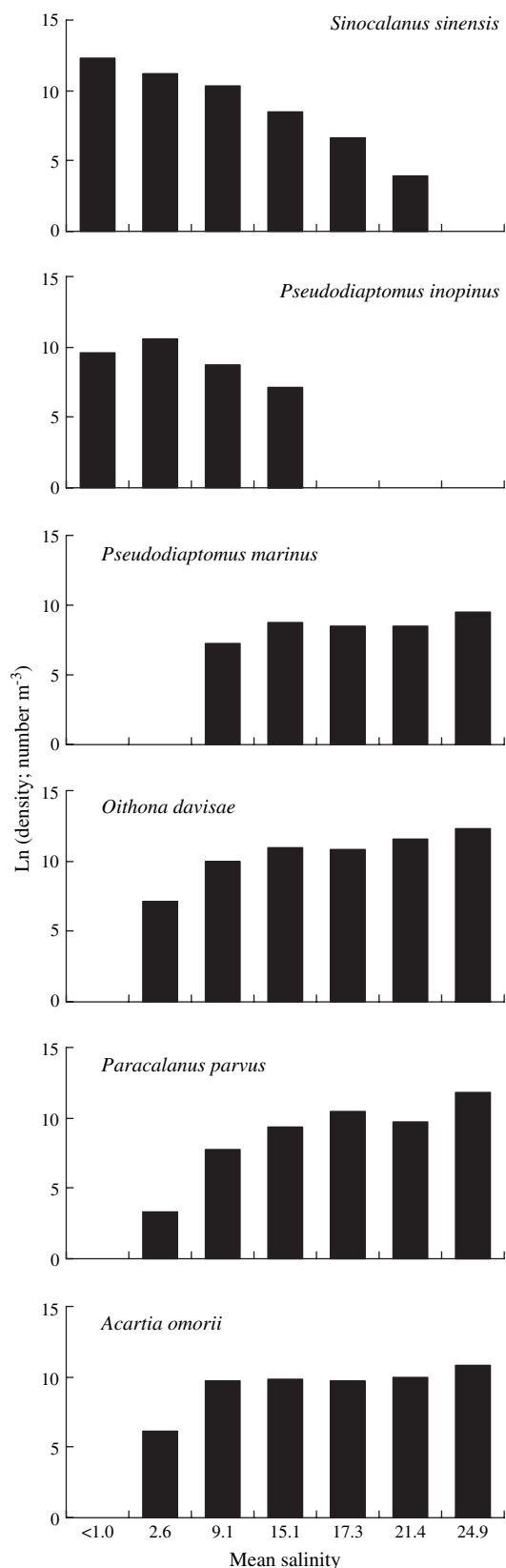
Fig. 7. Variations in the density of dominant copepods with salinity; the oligo-haline copepods *S. sinensis* and *P. inopinus* showed a seaward decrease in abundance while the euryhaline copepods *P. inopinus*, *O. davisae*, *P. parvus* and *A. omorii* showed seaward increase in abundance. Values are natural log transformed density (number m^{-3}).

Table 5
Summary results for multiple regression on the effects of environmental variables on density, biomass and diversity of all copepods and on the density of the two most dominant species (*S. sinensis* and *O. davisae*); the R^2 values are adjusted R^2

Dependent variable	Design summary (ANOVA)	Independent variable	Beta value	P
Density	$F = 8.27$; $R^2 = 0.38$; $P < 0.0001$	Chl-*a*	0.325	0.030
Biomass	$F = 13.08$; $R^2 = 0.51$; $P < 0.0001$	Chl-*a*	0.549	0.000
		NTU	0.427	0.046
Diversity	$F = 4.64$; $R^2 = 0.24$; $P < 0.0001$	Chl-*a*	0.331	0.046
		Salinity	0.293	0.026
S. sinensis	$F = 4.95$; $R^2 = 0.74$; $P < 0.050$	Chl-*a*	3.48	0.021
		PhP	−6.36	0.025
O. davisae	$F = 6.54$; $R^2 = 0.80$; $P < 0.050$	Chl-*a*	−2.53	0.035

species are not very strongly influenced by the environmental factors or their relationships with the environmental parameters measured are not apparent.

4. Discussion

Turbidity had negative correlation with temperature and salinity, indicating that higher turbidity occurs in low salinity areas during winter. Chl-*a* had significant positive relation with temperature but were independent of salinity. In contrast, PhP appeared independent of temperature but correlated negatively with salinity and positively with turbidity. This makes sense because these relations imply that higher PhP is associated with lower salinity and higher turbidity, indicating that phaeopigment is associated with the turbidity maximum. This also implies a significant partitioning between chl-*a* and PhP, as chl-*a* did not show significant spatial variations while PhP did. Based on strong correlation between salinity and chl-*a*, Winkler et al. (2003) reported that the physical processes are important in controlling the distribution of phytoplankton and therefore, chl-*a*. However, we have found no correlation between salinity and chl-*a*, rather have found correlation between salinity and PhP, which indicates that the physical processes are associated with PhP but did not influence the spatial distribution of chl-*a*. Although it is generally argued that little phytoplanktonic primary production is possible in ETM areas due to higher turbidity and resultant low light availability, in our study, chl-*a* concentration in the ETM were similar to that found in the low turbidity zones of the lower estuary. Vincent et al. (1996) reported that photosynthetic rates per unit chl-*a* remain high across the ETM zone and low light penetration is offset by a shallow mean mixing depth and these processes might be responsible in maintaining a spatially stable primary production along the estuarine gradient.

We have observed a longitudinal succession of two well-defined copepod assemblages: one is the oligohaline (0.5–2.5) assemblage, associated with the ETM in the upper estuary and the other is a euryhaline (9.0–25.0) assemblage seaward of the ETM in the lower estuary. *S. sinensis* is the dominant species in the low saline areas of the estuary and was
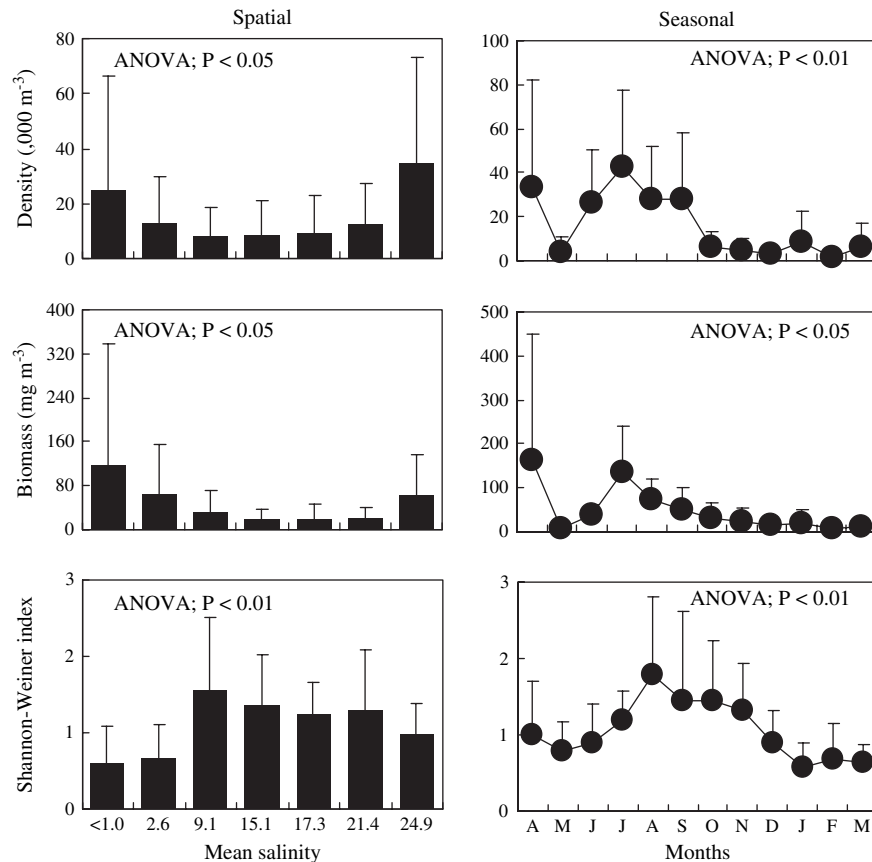
Fig. 8. Spatial and temporal variations in the total copepod density, biomass and diversity; values are mean ($\pm$SD) and P values indicate the results of ANOVA.

associated with the turbidity and PhP maximum, particularly during autumn to spring. In summer, this species occurred in a very low density even in the low salinity areas of the upper estuary and was replaced to a great extent by other species such as *P. inopinus* and *Cyclopoida* spp. *S. sinensis* occurs only in Chikugo estuary in Japan and the mechanism of its association with the ETM and the survival strategy is poorly known. Its seasonal variability is significantly and inversely correlated with salinity, probably resulting from its seasonal cycle and ecological requirements; its maxima occur in winter and early spring which corresponds to periods of high river flow and therefore higher turbidities and lower salinities. Being highly abundant in winter, the abundance of *S. sinensis* appears to be independent of the seasonal patterns of temperature and chl-*a* as revealed by the absence of significant correlation. *S. sinensis* is also present in summer but in lower densities, and seems then to be outcompeted by other species such as *P. inopinus* and *Cyclopoida* spp. and associated with higher temperature. Summer temperatures and elevated salinity appear to provide *P. inopinus* a competitive advantage allowing them to extend their distribution upstream. A similar scenario has been reported in the Gironde estuary turbidity maximum (David et al., 2005) with *Eurytemora affinis* which is a typical brackishwater calanoid copepod species in this estuary. David et al. reported that the maximum abundance of this species occurs during winter to early spring and is correlated negatively

with salinity; in summer, this species is replaced by euryhaline species (*Acartia* spp.) whose abundance correlated positively with salinity.

In contrast to the low saline upstream community where a few species dominated, a relatively more diverse and multi-species assemblage was formed in the high salinity areas of the lower estuary where a number of species dominated. *O. davisae*, *P. parvus*, *A. omorii* were the three most dominant species in this community. The copepod assemblage in this area was characterized by commonly occurring coastal copepods; similar and related species have been reported in many coastal estuaries in the world (Plourde et al., 2002; Satapoomin et al., 2004; David et al., 2005). An exactly similar pattern of longitudinal zooplankton assemblage was reported along the St. Lawrence ETM (Bousfield et al., 1975; Laprise and Dodson, 1994; Winkler et al., 2003). These authors have reported a longitudinal succession three well-defined assemblages of zooplankton along the estuary. A tidal freshwater assemblage dominated by *Bosmina longirostris* and *Gammarus* sp. reaches highest abundances at the upstream limit of ETM. Further downstream, this freshwater community grades into a true estuarine assemblage composed of *Eurytemora affinis*, *Neomysis americana* and *Mysis stenolepis* occurring in salinities of 0.5—5 which correspond to the ETM and large zooplankton of this assemblage was reported as the most abundant of the estuary. At higher salinities, the estuarine community is replaced by
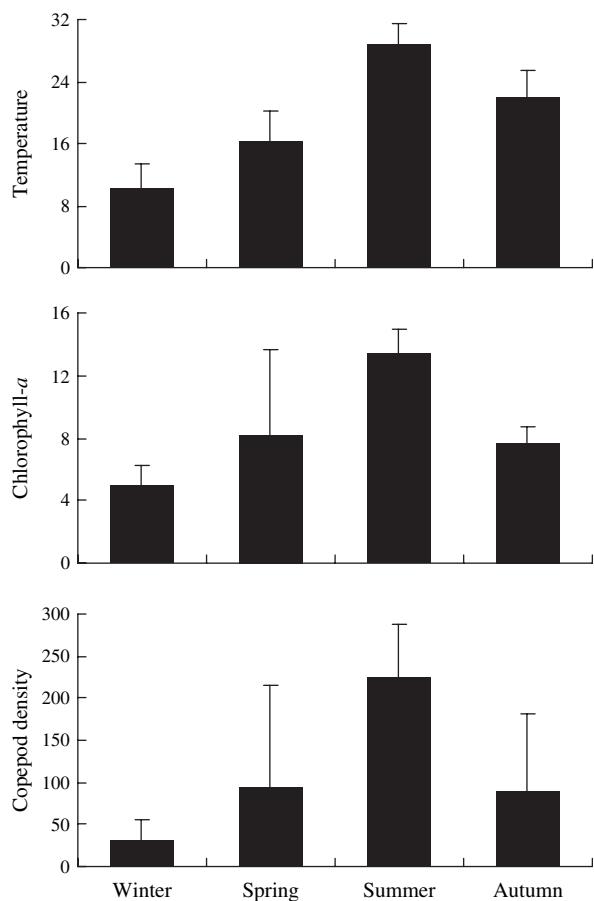
Fig. 9. Patterns of interrelationships among temperature (°C), chl-*a* (μg l^{-1}) and copepod density (number m^{-3}) during four main seasons. Values are mean and error bars are standard deviations of three samples during each season. Pearson's correlation coefficient showed that seasonal patterns in temperature, chl-*a* and density were significantly related to each other (values or *r* were 0.917 ($P < 0.05$), 0.921 ($P < 0.05$) and 0.997 ($P < 0.01$) for temperature vs chl-*a*, temperature vs density and chl-*a* vs density respectively.

associated with high summer temperature (*P. inopinus* in the upper and *A. erythraea*, *A. pacifica*, *P. marinus*, *H. japonicus* in the lower estuary). The highest total copepod density occurred at E3, the most downstream station; in contrast, the highest standing biomass occurred at R4, the most upstream station, indicating that *S. sinensis* accounts for most of the secondary production by copepods in the estuary. As a single species, *S. sinensis*, which is associated with the ETM zone of the estuary contributed as high as 44.6% of the overall copepod biomass production and, in association with *P. inopinus*, which is also associated with ETM, contributed 56.7% of the total copepod standing biomass in the estuary. This implies that the ETM zone accounts for majority of the copepod biomass production and is consistent with other well mixed estuarine systems that are characterized by an ETM zone (Laprise and Dodson, 1994; Winkler et al., 2003). Both density and biomass showed higher values during the summer period and had significant correlation with temperature, chl-*a* and PhP; this implies that the seasonal cycle in pigment production, which is influenced by seasonal temperature patterns, plays an important role in copepod abundance and biomass production in the estuary (Kiorboe and Nielsen, 1994).

Abundance of *S. sinensis* and *P. inopinus*, which are members of the low saline upstream assemblage, decreased sharply toward the sea in contrast to the abundance of the dominant species of the low saline marine community which showed sharp upstream decrease. This contrasting spatial abundance pattern explains the habitat partitioning between these two communities. This implies that the main habitats of the copepods of the upstream assemblage are associated with the low salinity waters flowing and carrying them downstream while habitats of the euryhaline marine species are formed in more saline deep waters flowing and carrying them upstream, suggesting that animals may be confined in waters that transport them away from the centers of their respective populations. We, therefore, speculate that the original habitat of the oligohaline community is located towards more upstream and that of the euryhaline community towards more downstream because the highest density of the two communities occurred at the two ends of the gradient. This pattern is in strong agreement with that reported by Bousfield et al. (1975) and Dodson et al. (1989). Bousfield et al. (1975) suggested that the copepods that are transported from their original habitats are mostly moribund, non-reproductive animals, suggesting that the transported habitats may constitute an extreme environment for these species. Consequently, lowest copepod abundances are obtainable in areas between the original low saline upstream estuarine assemblage and euryhaline downstream marine assemblage. In contrast, the edge effects, caused by mixing individuals from the two assemblages, result in increasing the species richness. Therefore, species richness may give erroneous impressions on the evolution of copepod species diversity along the spatial gradient in an estuary (Laprise and Dodson, 1994).

Chl-*a* was found as the common and the most important predictor variable for total copepod density, biomass and

a euryhaline marine assemblage composed of *Calanus* spp. and other commonly occurring coastal marine zooplanktons (Bousfield et al., 1975; Laprise and Dodson, 1994).

Similar to *S. sinensis* in the upper estuary, the dominant species in the lower estuary (*O. davisae*, *P. parvus*, *A. omorii*, *Cyclopoida* spp.) did not show significant relation with monthly temperature cycle. In contrast, most of the medium abundant species (*A. erythraea*, *A. pacifica*, *P. marinus*, *H. japonicus*) showed generally high abundance during the summer months and had highly significant correlation with temperature and chl-*a*, indicating that production of these species depends on seasonal cycle of temperature and is, therefore, associated with active primary production by phytoplankton. Therefore, there were two major categories of copepod assemblages based on seasonal abundance patterns: the first category comprised of highly abundant and dominant species which are independent of seasonal temperature cycle (*S. sinensis* in the upper and *O. davisae*, *P. parvus* and *A. omorii* in the lower estuary) and the second category includes the medium abundant species that are dependent on seasonal temperature cycle and are

diversity as well as the density of dominant species. Salinity, by virtue of its spatial differences, was a significant predictor of the diversity estimates; this was expected because higher diversity was recorded in the higher salinity regions. Chl-*a* was found as a significant predictor for *O. davisae*, indicating that the abundance of *O. davisae* is dependent on primary production by phytoplankton and is presumably independent of PhP. In contrast, PhP was an additional variable for *S. sinensis*, which indicates that the abundance of *S. sinensis* is controlled to a significant extent by the supply of phaeopigment sources. Therefore, chl-*a* is the most important variable that can describe the observed variability in copepod density, biomass and diversity in the estuary as a whole, and PhP is the additional variable for copepods in the upper estuary alone. Similar scenarios are reported in other comparable ecosystems such as in the Gironde estuary (Irigoien and Castel, 1997; David et al., 2005), the St. Lawrence River estuary (Winkler et al., 2003), and in Chesapeake Bay turbidity maximum (Roman et al., 2001). High turbidities alter the nutritional environment and thus affect feeding, survival and reproduction of the copepod species residing in the ETM areas. As a consequence, the copepod populations of ETM zones are mainly localized as a result of hydrodynamic processes. In contrast, *O. davisae* and *Acartia* spp. that are dominant copepod species seaward of the maximum turbidity area were distributed preferentially downstream, illustrating their greater tolerance to higher salinity. Similar distribution patterns were reported by David et al. (2005) in the Gironde estuary. They reported that a calanoid copepod *Eurytemora affinis* was restrictedly distributed in the turbidity maximum area while *Acartia* spp. were distributed toward the downstream areas. However, copepod species assemblages are not simply the result of species sorting independently according to their salinity tolerance and, as in some other studies (Laprise and Dodson, 1994), our results support the hypothesis that environmental variability is a major factor influencing distribution and species diversity of copepod in estuaries.

Acknowledgements

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