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Regional Characteristics of Lower Trophic Level Food Web Structure in the Seto Inland Sea

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Abstract—To elucidate food web structure is important for understanding the links associated with bioaccumulation of chemical pollutants such as POPs in wild animals. The Seto Inland Sea (SIS) of Japan is divided into subsystems with high environmental heterogeneity that may vary in their respective food chain lengths, which may account for variance in bioaccumulation of pollutants. The number of trophic interactions can vary at the basal food web level due to effects of the microbial food chain. In this study, we investigated regional variability in the number of trophic links at the lower trophic level in the SIS by estimating trophic level of copepods, which represents the top trophic consumers within the lower trophic level using stable isotope analysis. The trophic level of copepods was *ca*. 2 in the most regions but was as high as *ca*. 3 in Bisan-seto. There was a very strong positive relationship between the relative amount of ciliates in the plankton community and trophic level of copepods. These results suggested that the microbial food chain dominated in the Bisan-seto and that the risk for higher bioaccumulation in fishes is higher in the Bisan-seto region than in other regions. Additionally, very low nitrogen stable isotope values of copepods in the Kii-channel suggested that the Kii-channel ecosystem was largely affected by imported organisms and chemicals from the Pacific Ocean.

Keywords: microbial food chain, copepod, stable isotope, food web structure, Seto Inland Sea

INTRODUCTION

Food webs process material and energy dynamics through biological trophic interactions. Therefore, it is a necessary to elucidate the basic functioning of food webs for understanding material and energy flow through the ecosystem (Begon *et al*., 1999), and in the case of pollutants, the bioaccumulation processes in wild animals (Kelly *et al*., 2007). It is well known that the structure of lower trophic levels in aquatic food webs, which consists largely of phytoplankton, bacteria and zooplankton, can vary substantially with the dominance of the microbial food

Table 1. Characteristics of each region of the Seto Inland Sea.

Region	Area (km^2)	Mean depth (m)	Volume ($\times 10^8$ m^3)
Bungo Channel	2575	72.5	1865
Suo Nada	3100	23.7	736
Iyo Nada	3974	46.1	2136
Aki Nada	1909	26.8	522
Hiuchi Nada	2250	14.7	380
Bisan Seto	916	13.9	127
Harima Nada	3426	25.6	887
Osaka Bay	1529	27.5	418
Kii Channel	1554	56.0	870

chain (Azam *et al.*, 1983). In classical grazing food chains, the number of trophic links from primary production to large zooplankton such as copepods, which are very important food for many marine fishes, is often 1.0 in which zooplankton directly feed on primary production. However, the number of trophic links can be elongated by the added contribution of microbial food chains because primary production is mainly consumed by protozoans, and then the protozoan is consumed by copepods when the microbial food chain dominates.

The Seto Inland Sea is a semi-enclosed coastal sea surrounded by Honshu, Shikoku and Kyushu Islands and it is a representative coastal sea in Japan (Takeoka, 2002). The sea is divided by islands and peninsulas into wide basins ("nada") and these basins are connected by narrow channels called "seto". This complicated geometry results in wide variations in the marine environment and may result in a variable food web structure in each region (Table 1). In the Seto Inland Sea, it has been shown that large differences in the transfer rates of organic matter from primary production to large zooplankton among each region exist (7–37%; Hashimoto *et al.*, 1997), suggesting regional variation in the number of trophic interactions from primary production to large zooplankton. However, it is unknown as to whether the grazing or microbial food chain dominates in each region of the SIS.

In this study, using stable isotope analysis we examined the lower trophic level food web structure by evaluating the trophic level (TL) of copepods from each region of the SIS. Additionally, we discussed the possible effects of differences in lower trophic level food web structure on the source of organic matter and POPs, and bioaccumulation of POPs in each regional sub-ecosystem.

MATERIALS AND METHODS

During late spring (early May to early June 2009) and summer (middle July to middle August 2009), copepods and primary producers (particulate organic matter (POM, predominantly phytoplankton) and littoral benthic microalgae) were sampled for stable isotope analysis from nine regions of the Seto Inland Sea

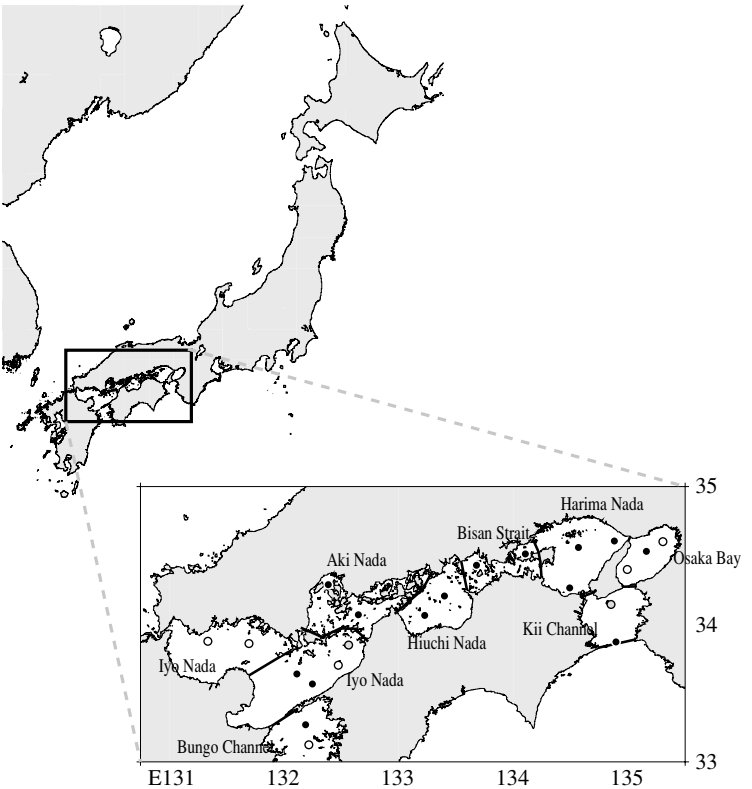

Fig. 1. The map of the Seto Inland Sea, Japan and sampling locations are shown as circles (black: collected in both period, grey: collected in late spring period, white: collected in summer period).

(Fig. 1). For collection of POM samples, we collected surface water (depth 1 to 3 m) from offshore waters and filtered the water through 100 μm mesh to remove large zooplankton. POM samples were collected by filtering the pre-filtered surface water using Whatman GF/F grass-fiber filters (pre-combusted at 550°C for 3 h). In addition, we preserved 2 L of the water sample in 2% formalin to examine components smaller than micro-plankton. Micro-plankton components were examined using a microscope and we calculated the proportion of ciliate to all plankton cells. Copepods were collected horizontally from surface water with a 200 μm mesh plankton net, and copepods were subsequently picked under a stereomicroscope. The collected copepods were rinsed with milli-Q water to remove organic residue and salt. For collection of littoral benthic microalgae, we took some stones at the subtidal shore and then brushed off epilithic microalgae. All of the samples for stable isotope analysis were preserved at –20°C until analysis.

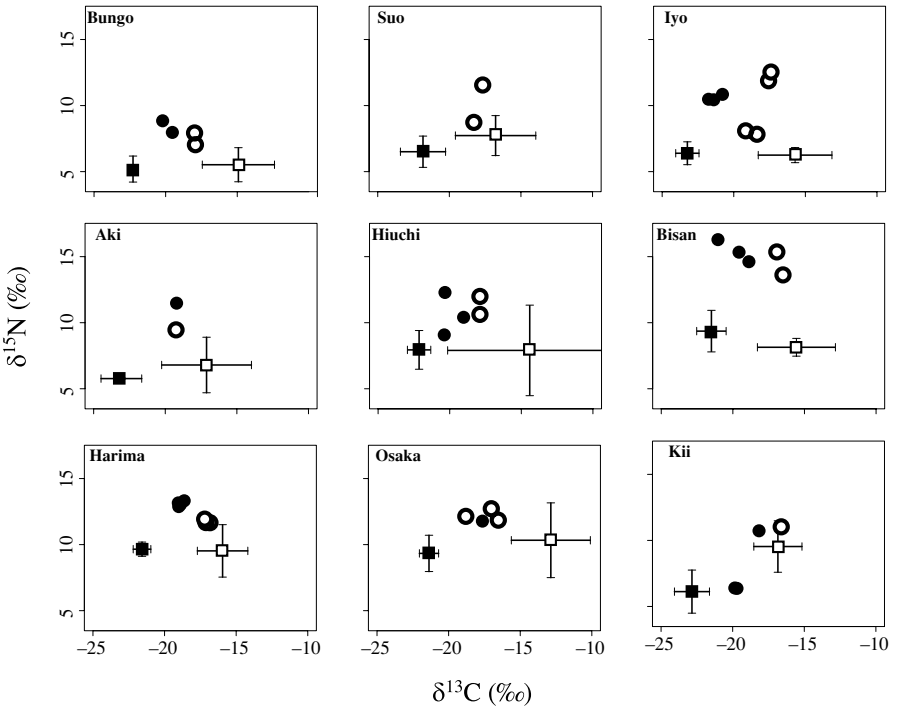

Fig. 2. Carbon and nitrogen stable isotope ratio distribution in each sub-region in the SIS. Circles show copepod values (closed: late spring period, open: summer period). Primary producers are shown as regional mean value with standard deviations (closed square: POM, opened square: littoral benthic microalgae).

STABLE ISOTOPE ANALYSIS

POM and littoral benthic microalgae samples were dried at 60°C and treated by vapor exposing with full strength HCl or directly with 0.5N HCl for 24 h to remove inorganic carbon prior to isotope analysis. Copepods were ground into a fine powder after being dried at 60°C and treated with 2:1 chloroholm; methanol solution for 24h to remove lipids (Bligh and Dyer, 1959). Carbon and nitrogen stable isotope ratios were measured with a continuous flow isotope ratio mass spectrometer (ANCA-SL; PDZ Europa Ltd.). Isotopic notations of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) were expressed as per mil (‰) deviation from a standard (atmospheric N_2 gas for nitrogen and PeeDee belemnite carbonate for carbon) as defined by the following equation:

$$\delta^{15}N \text{ or } \delta^{13}C = (R_{sample} - R_{standard})/R_{standard} \times 1000(‰),$$

where R denotes $^{15}N/^{14}N$ or $^{13}C/^{12}C$, respectively. Analytical errors of reproducibility were usually ±0.3‰ for both $\delta^{15}N$ and $\delta^{13}C$, but were ±0.6~0.8‰

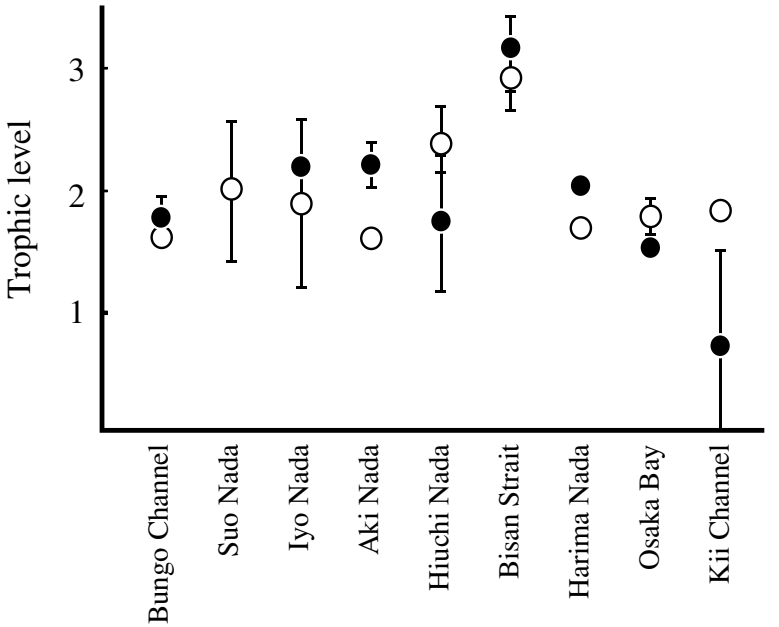

Fig. 3. Trophic level of copepods in each sub-region in the SIS (closed circle: late spring period, opened: summer period). Error bars show the standard deviation.

for $\delta^{15}N$ of some POM samples with low nitrogen content. Some samples of copepods and POM were not of sufficient mass to analyze for stable isotope ratios and were excluded from analysis.

Trophic level (TL) of copepods were estimated using the following 2-source isotope mixing models (Post, 2002) with a trophic enrichment factor of 0.8‰ for $\delta^{13}C$ (DeNiro and Epstein, 1978; Vander Zanden and Rasmussen, 2001) and 3.4‰ for $\delta^{15}N$ (Minagawa and Wada, 1984; Vander Zanden and Rasmussen, 1999):

$$TL = 1 + [\delta^{15}N_c - \{\alpha\delta^{15}N_p + (1 - \alpha)\delta^{15}N_l\}]/3.4 \quad (1)$$

$$\alpha = \{\delta^{13}C_c - \delta^{13}C_l - 0.8(TL - 1)\}/(\delta^{13}C_p - \delta^{13}C_l), \quad (2)$$

where the subscripts c, p and l refer to the stable isotope ratio of copepods, POM and littoral benthic microalgae, respectively; α denotes the contribution of POM to copepod biomass.

RESULTS AND DISCUSSION

Based on the distribution of stable isotopes in each region (Fig. 2), the trophic level of copepods were estimated using the stable isotope mixing model

Table 2. Community structure of small plankton. Cell proportions are shown.

	Bungo Channel	Suo Nada	Iyo Nada	Aki Nada	Hiuchi Nada	Bisan Strait	Harima Nada	Osaka Bay	Kii Channel
late Spring									
Diatom	0.62	0.88	*	0.89	0.64	0.73	0.02	0.92	0.44
Raphidophyceae	0.01	0.00	*	0.00	0.04	0.03	0.00	0.00	0.01
Silicoflagellates	0.00	0.01	*	0.00	0.02	0.00	0.00	0.00	0.00
Dinoflagellate	0.33	0.04	*	0.08	0.21	0.11	0.83	0.08	0.48
Ciliate	0.03	0.04	*	0.01	0.07	0.12	0.06	0.00	0.03
Crustacean (nauplii)	0.01	0.02	*	0.02	0.02	0.00	0.06	0.00	0.04
Fish egg	0.00	0.00	*	0.00	0.00	0.00	0.02	0.00	0.00
mid Summer									
Diatom	0.78	0.94	0.76	0.87	0.27	0.62	0.21	0.47	0.02
Raphidophyceae	0.00	0.00	0.02	0.00	0.00	0.03	0.14	0.00	0.00
Silicoflagellates	0.04	0.02	0.01	0.02	0.00	0.01	0.00	0.00	0.00
Dinoflagellate	0.13	0.04	0.16	0.11	0.44	0.20	0.46	0.33	0.91
Ciliate	0.05	0.00	0.03	0.00	0.05	0.14	0.10	0.08	0.01
Crustacean (nauplii)	0.00	0.00	0.02	0.00	0.05	0.00	0.08	0.11	0.05
Fish egg	0.00	0.00	0.00	0.00	0.20	0.00	0.00	0.01	0.01

*We can not evaluate the cell proportion because density of planktons were few.

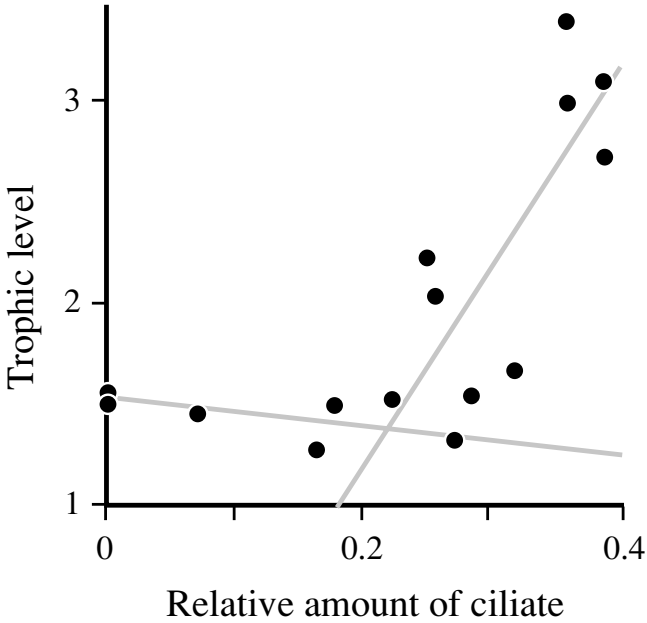

Fig. 4. Relationship between the relative amount of ciliate in the plankton community (shown as angular transformed value) and the trophic level of copepods. Lines show regression lines of piecewise regression analysis.

(Fig. 3). In the Seto Inland Sea, all nine subsystems did not consist of a common lower trophic level food web structure with the dominance of a classical grazing food chain.

Copepods collected from the Kii-channel in late spring showed a markedly different trophic level of below 1.0, because the copepods had comparatively depleted δ^{15}N values to that of primary producers in the Kii-channel (Fig. 2). This suggests that the copepods originated from another place where primary producers had very low δ^{15}N values, for example Kuroshio-current in the Pacific Ocean (POM from the Kuroshio shows 2.4‰ δ^{15}N in mean, Minagawa *et al*., 2001). The Kuroshio Current flows into the Kii-channel from May to June (Komai *et al*., 2008), when copepods in the Kii-channel showed very low nitrogen stable isotope values. Thus, the Kii-channel ecosystem may be largely affected by the inflow of plankton or material from the Pacific Ocean by means of the Kuroshio Current. This means that transportation of organisms and possibly some materials from the Pacific Ocean with the Kuroshio Current may largely affect the coastal ecosystems and chemical exposure levels for coastal organisms.

In almost of all subsystems, copepods were close to TL 2 (Fig. 3), suggesting a more grazing food chain dominated-system. However, in the Bisan-seto subsystem, copepods were usually at TL *ca*. 3 (Fig. 3). Ciliates, which play as

important intermediates between primary production and upper-trophic levels in the microbial food chain (Azam *et al.*, 1983), were more abundant in the Bisan-seto than in other sub-regions (Table 2). There was a significant positive relationship between abundance of ciliates and the copepod trophic level (piecewise regression, $n = 15$, $P < 0.01$; Fig. 4), supporting that where ciliates were abundant, a more microbial food chain-dominated lower food web existed in the Bisan-seto sub-system. In the Bisan-seto, the microbial food chain seemed to play an important role in the transportation of organic matter and consequently raise the trophic level of copepods to 3. This suggests that the bioaccumulation of chemical contaminants in copepods and higher consumers such as fish can became more elevated in the Bisan-seto.

Our results suggests that the food web structure of lower trophic levels can vary across relatively small spatial scales depending on regional geological and biological environmental characteristics, and we determine the number of feeding steps from primary producers up to copepods and fishes. To fully understand ecological risk of chemical pollutants, further investigation about the mechanisms driving the lower trophic level food web structure are warranted because the characteristics of the lower trophic level food web may be largely associated with the risk of pollutant exposure for fishes, especially juveniles of fishes that are most vulnerable life stage to chemical toxicity.

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