Acta Oceanol. Sin., 2012, Vol. 31, No. 4, P. 154-160
DOI: 10.1007/s13131-012-0229-5
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Carbon, nitrogen and phosphorus stoichiometry in Japanese anchovy (*Engraulis japonicus*) from the Huanghai Sea, China

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Received 5 March 2011; accepted 21 September 2011

Abstract
Generally, nutrient cycle is closely related to the element distribution in biomass and the population dynamics in ecosystems. Carbon, nitrogen and phosphorus in Japanese anchovy (*Engraulis japonicus*) of different body lengths from the Huanghai Sea (Yellow Sea) were determined to better understand their variability and reasons during its life history. The mean content was 45.12%, 10.12% and 2.02% for C, N and P, respectively. Significant differences in C, N and P elemental composition were found among different sizes, which could be explained by varying proportions of storage compounds in whole body fish, and varying degrees of ossification. Considering abundant resources in Japanese anchovy, it was an important P-pool in the cycle of P. Moreover, the excreted N/P ratio was significantly different in fishes of different sizes, especially at high gross growth efficiency. In the past two decades, overexploitation tended to cause smaller body length in the community structure of anchovy, which presumably changed the nutrient cycle in food webs of the Huanghai Sea. Exptrapolation of the results indicates that Japanese anchovy may be important for conveying nutrient in the Huanghai Sea.

Key words: Japanese anchovy (*Engraulis japonicus*), nutrient elements, the Huanghai Sea

1 Introduction

Stoichiometric model is a very important tool in ecology, which is applied in many areas, such as food-web nutrient recycling (Sterner, 1990; Elser and Urabe, 1999), food-web dynamics (Sterner et al., 1997, 1998), and trophic interactions (Markow et al., 1999). It is ever hypothesized that body nutrient in consumer species was always homeostatic (Sterner, 1990; Schindler and Eby, 1997). However, body nutrient changes individually in some species (Elser et al., 1996). Furthermore, fish population structure always changed continuously for some reasons (e.g., fishing), so as to affect nutrient cycle, and the consequences of such changes are poorly known. Therefore, it was necessary to study the stoichiometry of key species in aquatic ecosystem all through lifetime.

Two major affecting factors for invertebrates' stoichiometry are growth rate and diet. For example, Hessen (1990) found that juveniles *Daphnia magna* had more body P than adults, probably due to the high growth rate experienced by small bodied organisms (Elser et al., 1996). Diet might also importantly affect the organism's body composition. For example, DeMott et al. (1998, 2004) discovered that P deficient diets can affect growth rate and body P content of daphniids. In vertebrates such as fish, body elemental changes may be particularly important due to bone formation during larval-juvenile developmental stage. More P was used for bone formation during vertebr-

Foundation item: The National Key Basic Research Program from the Ministry of Science and Technology of China under contract Nos 2006CB400601 and 2011CB409801; Science Fund for Creative Research Groups of the National Natural Science Foundation of China under contract No. 41021064.
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ates maturation, and thus P content in whole-body might increase and N/P ratio might decline with the increase of body size (Elser et al., 1996). Sterner and George (2000) also reported such relationship between size and change of body elemental composition in adult fishes. As proven by Pilati and Vanni (2007), body stoichiometry greatly changed individually in fishes, due to an inherent increase of relative P composition in bones.

Fish played an important role in nutrient cycle in aquatic food webs (Vanni, 2002), and excretion of young fishes was particularly important (Kraft, 1992). So changes in fish body nutrient content can influence nutrient excretion, and the population size might greatly affect the limited nutrient for primary producers. As found by Vanni et al. (2006), algae were P-limited in the spring and summer because larvae were very abundant, but tried to become N-limited in the autumn. Thus, it was quite necessary to study the change of body nutrient all through lifetime from young to adults for some key species in aquatic ecosystem, e.g., Japanese anchovy (*Engraulis japonicus*) from the Huanghai Sea (Yellow Sea).

As one of the small pelagic species, Japanese anchovy was considered as a key species in the Huanghai Sea ecosystem (Tang and Su, 2000; Tang et al., 2005). It was not only a small pelagic zooplankton feeder, but also prey for some important species of higher trophic level (Wei and Jiang, 1992; Aoki and Miyashit, 2000). Moreover, in the past two decades, its stock experienced three stages: under-exploitation, full development and over-exploitation (Xu and Jin, 2005). The dramatic variation in its anchovy abundance and the long-term high pressure of fishing might greatly affect the community structure of anchovy.

This study analyzed in detail nutrient content (C, N and P) in Japanese anchovy from the Huanghai Sea all through lifetime from young to adult. Firstly, it primarily aimed to better understand nutrient content (C, N and P) and nutrient ratio (C/N, C/P and N/P) of Japanese anchovy at different growth stages. More specifically, we will evaluate whether there are stoichiometric differences between anchovy and their food in the Huanghai Sea. Finally, it discussed the potential effect on nutrient recycling in food webs.

2 Materials and methods

2.1 *Composition of body nutrient*

All samples were collected from the Huanghai Sea in June 2001 (Fig. 1). Bottom trawl survey was conducted on Japanese anchovy. R/V *Beidou* used a net of circumference 836 mesh×12 cm, a 10 cm mesh size cod-end with a 2.4 cm mesh size liner. Captured fishes were measured and weighed on board. They were sorted into size class of 10 mm, generally sampled 1–3 replicates, and then always frozen at −20℃ until the transportation to land laboratory.

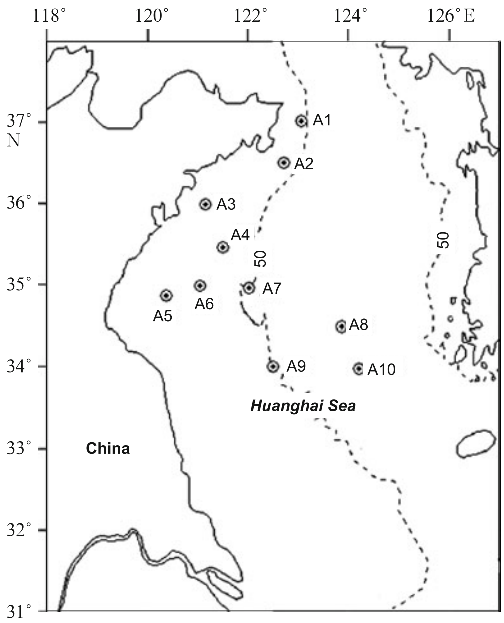

Fig.1. Study area: the Huanghai Sea.

In the laboratory, fish samples were unfrozen and rinsed with Milli-Q water. We used only one individual per sample and analyzed 1–3 replicates per sampling date. All samples were dissected through stainless steel scalpel, and gut content was removed. After freeze-dried, the samples were grinded to fine powder using a mortar and pestle.

In samples (about 2 mg), C and N contents were analyzed through Vario EL analyzer, and with acetanide as reference material. For total P analysis, about 1 000-mg samples were digested with 12 ml HNO_3 and 2 ml $HClO_4$ at 110℃, and then were analyzed by ICP-AES (PerkinElmer Plasma 2000). Reference material was the dried oyster tissue obtained from the National Institute of Standards Technology, and P had a recovery of more than 90%.

2.2 *Estimation model of recycled N/P ratio*

The N/P ratio recycled by Japanese anchovy was estimated through the following formula by the model proposed for fishes by Schindler and Eby (1997).

$$N/P_{\text{recycled}} = \frac{AE_N \times [N]_{\text{food}} - GCF_{\text{fish}} \times [N]_{\text{fish}}}{AE_P \times [P]_{\text{food}} - GCF_{\text{fish}} \times [P]_{\text{fish}}},$$

where AE is the assimilation efficiency for N and P, and GCE is the gross growth efficiency (growth/consumption). To be simplified, the assimilation efficiency was assumed as constantly 80% and 72% for N and P (Schindler and Eby, 1997).

2.3 *Statistical analysis*

Inter-group linkage cluster analysis of Pearson correlation was conducted through SPSS 10.0 software, which was an exploratory data analysis tool to solve classification problems. Based on elemental composition, Japanese anchovy was divided into different groups after the cluster analysis.

3 Results

3.1 *Body nutrient*

A total of 25 samples typical of Japanese anchovy were analyzed for C, N and P. Carbon content in whole body was the highest and lowest in a length of 140–150 mm and 30–40 mm at 51.25% and 36.65% respectively (mean 45.12%, Fig.2a); N content in whole body was the highest and lowest in a length of 130–140 mm and 140–150 mm at 11.4% and 7.77% (mean 10.2%, Fig. 2b); P content in whole body was the highest and lowest in a length of 50–60 mm and 140–150 mm at 3.11% and 1.22% respectively (mean 2.02%, Fig. 2c).

With the increase of fish length, C content increased, P content decreased, and N content decreased slowly. Body elemental ratio also varied all through lifetime, possibly driven by C and P. Because the change of N content was different with other nutrients, C/N ratio hardly changed at a length of being less than 100 mm, but increased rapidly at a length of being greater than 100 mm (Fig. 2d). At the increase of C content and the decrease of P content, C/P ratio increased quickly with length (Fig. 2e). Similarly, there was a significant relationship between N/P ratio and length (r^2=0.65, P <0.01) (Fig. 2f).

By cluster analysis, Japanese anchovy was divided into two groups (Fig. 3): being less than 100 mm length group with low C content, high P content and C/N ratio of 4.5, and being greater than 100 mm length group with high C content, low P content and C/N ratio of being greater than 4.5.

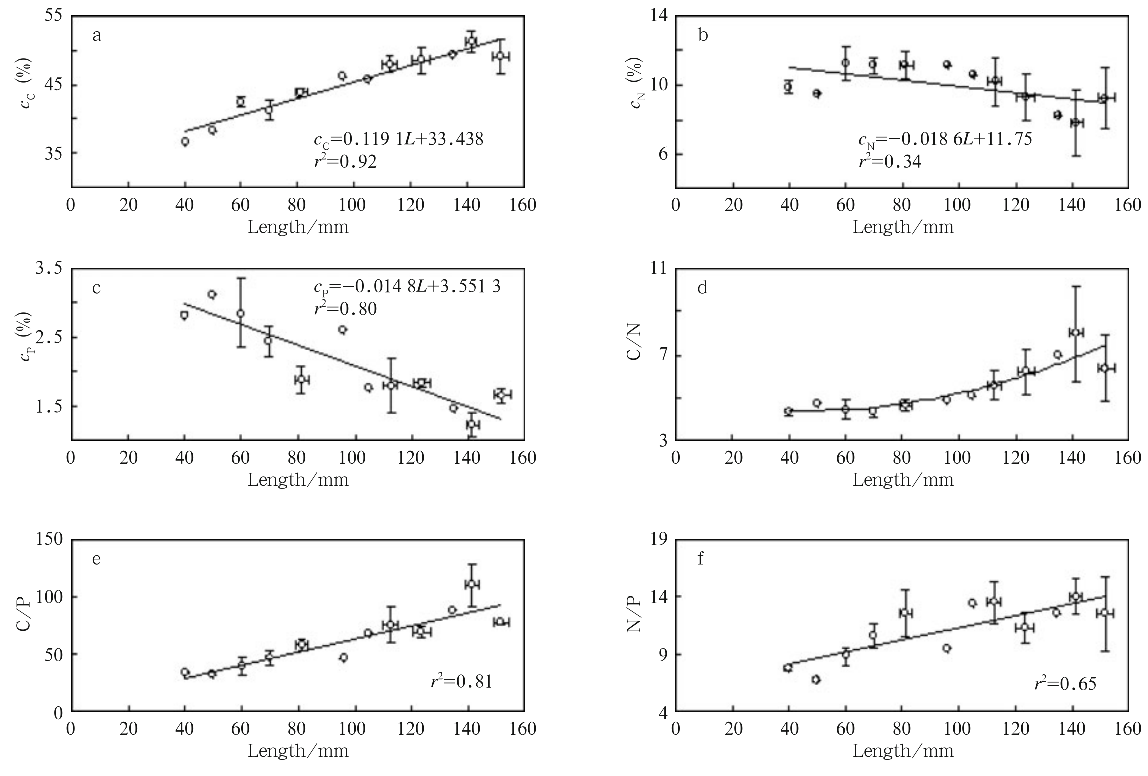

Fig.2. Ontogenetic change of carbon content (c_C) (a), nitrogen content (c_N) (b), phosphorus content (c_P) (c) (%), C/N (d), C/P (e) and N/P (f) ratios for Japanese anchovy (*Engraulis japonicus*).

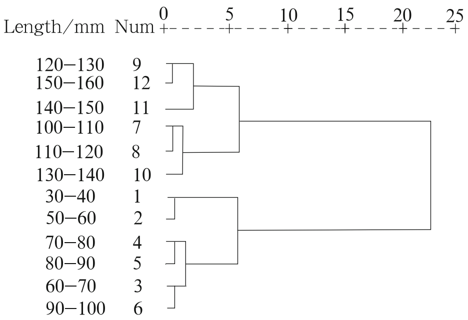

Fig.3. Cluster analysis chart of Japanese anchovy according to nutrient content at different life stages.

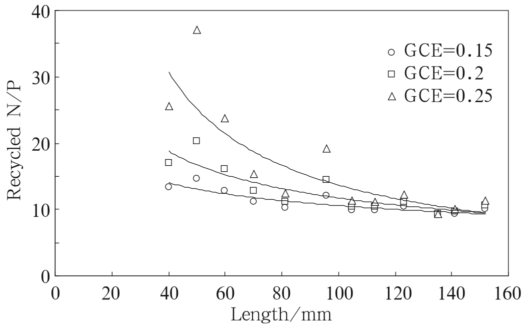

Fig.4. Calculated N/P ratio recycled in excreta from Japanese anchovy of different body lengths under different GCE.

3.2 *Nutrient recycle*

In this study, gut content was not separated from the fish because of lacking experience, however, because copepod (*Calanus sinicus*) was one of the most dominant food for Japanese anchovy (Meng, 2003), and had a mean N and P content of 10.3% and 1.3%, respectively (Uye and Matsuda, 1988; Cao et al., 2006), we used its data on Japanese anchovy and their food stoichiometry to explore potential imbalances in N and P recycling based on Schindler and Eby (1997) model (Fig. 4). The magnitude of imbalance greatly relied on the assumed value of GCE. With a length increase in Japanese anchovy, the recycled N/P ratio decreased just slightly at smaller GCE, but more significantly at higher GCE.

4 Discussion

4.1 *Fish C, N and P content*

There was yet not reported comparative data on Japanese anchovy. However, its C and N contents were similar and P content was different in other fishes (Table 1). Thus, P content seemingly changed more greatly than N content, mostly due to taxon (Sterner and George, 2000), and possibly due to the difference in feeding guild (Hendrixson et al., 2007). Phosphorus content was lower in planktivore fish (e.g., Japanese anchovy) than piscivore fishes (i.e., about 2% vs 3.5%).

For fishes, C and N were generally stored as lipids and protein in apparatus and muscle respectively, and nearly half of P was retained in bone and scales (DaCosta and Stern, 1956). Thus, the content and ratio of C, N and P indicated the relative composition of lipid, protein and bone in fishes (Czamanski et al., 2011). Lipid content was related to body length slightly (Hodder et al., 1973; Mcgurk et al., 1980), but positively during spawning period in the spring (Slotte, 1999). At spawning migration of Japanese anchovy, C content increased and lipid content increased with length (Zhang et al., 2004). With respects to nitrogen, lower N content in lipid-rich fish was consistent with the fact that lipids are usually N-poor compared with other biochemicals (Sterner and Elser, 2002). For example, glycerol and fatty acids do not contain N, hence the average C/N/P ratios of phospholipids is 39:0.8:1 (Sterner and Elser, 2002).

Table 1. Comparison of C, N and P content (dry mass, %) in whole fish tissues from some studies

	Location	Number of species	Species of fish	Number	c_C(%)	c_N(%)	c_P(%)
This study	China	1	*E. japonicus*	25	45.00(4.62)	10.21(1.12)	2.11(0.61)
Tanner et al. (2000)	Wisconsin	20	*P. promelas, C. inconstant, L. macrochirus* et al.	192	n.d.	11.26(0.07)	2.45(0.10)
Sterner and George (2000)	Ontario	4	*P. eos, P. neogaeus, M. promelas* et al.	242	46.00(3.8)	9.70(0.09)	1.49(0.33)
Dantas and Attayde (2007)	Brazil	6	*O. niloticus, C. monoculus, S. rhombeus* et al.	120	n.d.	10.40(0.13)	3.27(0.23)
	Sweden	2	*R. rutilus* and *P. fluviatilis*	50	n.d.	10.24(0.12)	2.53(0.28)

Notes: n.d. means not analyzed.

For P content, differences between young and adult could be explained by varying growth rates and ossification (Elser et al., 1996). As observed by Zhu and Iversen (1990), one-year anchovy of being less than 100 mm grew rapidly than two-year and three-year one of being greater than 100 mm. Thus, for fast-growing fishes, size increase might increase structural materials (including bone); and for slow-growing adult fishes, the pre-existing P in bone was diluted by other new substances (lipid). Phosphorus content increased with size in centrarchid (Davis and Boyd, 1978), but did not increase or increased just slightly with size in other species (Goodyear and Boyd, 1972; Sterner and George, 2000).

N/P ratio also changed all through lifetime in Japanese anchovy. N/P ratio increased opposite to P content, indicating that such trend was caused by P content. Thus, N/P ratio of new tissues added during the growth was greater than that of the whole lifetime, consistent with that in black bullheads *Ameiurus melas* (Hendrixson et al., 2007). Body C/P ratio increased with size for Japanese anchovy, because C content increased but P content decreased with size. As shown by a stoichiometry study of other cyprinid fishes (Sterner and George, 2000), C content and C/P ratio were positively related to fish size.

As a whole, biochemical compositions and growth rate of Japanese anchovy changed dramatically at being greater than 100 mm length (Zhang et al., 2004; Zhu and Iversen, 1990). Thus, this study divided Japanese anchovy into two groups according to elemental compositions (Fig. 3).

4.2 *Japanese anchovy's elemental stoichiometry and nutrient recycling*

In natural marine systems, the fact that marine invertebrates tend to be comparatively P-poor can be compared with marine fish, and that P assimilation efficiency tends to be lower than that of C and N, would result in an increased discrepancy between the elemental stoichiometry of digested food and the fish body (Czamanski et al., 2011). Consequently, this would be expected to result in variable nutrient recycled ratios (Sterner, 1990). For Japanese anchovy, the wide ontogenetic nutrients variation mean different recycled N/P ratio between small and large fishes, especially at higher GCE. As reported by Pilati and vanni (2007), N/P resupply ratios from larvae and juveniles of the gizzard shad, were extremely variable, in the range less than 10:1 to about 80:1. These authors suggested that ontogenic variations in N/P ratios were driven more by the change of food N/P ratio than that of body N/P. In our study, the nutrient of the copepod *Calanus sinicus* was used as the value of food in the simplified model but in practice, its diet changed at different life stages. The anchovy of being less than 80 mm fork length mainly ate copepodites (Meng, 2003), whose P content exceeded that in copepods at higher growth rate (Walve and Larsson, 1999). Thus, under actual conditions, the recycled N/P ratio might vary more significantly in fishes of different size.

In addition, due to long-term high pressure of fishing, the diet for Japanese anchovy changed dramatically in the Huanghai Sea since the mid-1980s, and more and more young fishes became fishing targets year by year (Fig. 5) (Jin et al., 2001). It might significantly change nutrient cycle in the food webs of the Huanghai Sea according to ecological stoichiometry (Elser et al., 1996). As observed by Vanni et al. (2006), population dynamics (age structure) greatly affected nutrient cycle in the Acton Lake. Such top-down effects have been also evidenced in marine reefs communities (Pinnegar et al., 2007).

Of further significance, Japanese anchovy is known to migrate from place to place, and be a key species in food webs. The fact that its fecal pellets would be P-rich in comparison to C or N, could be potentially enhance the export of P toward the deep sea through the sinking of P-rich fecal pellets. Robinson and Bailey (1981) reported that the sinking rates of fish fecal pellets were about 1 000 m/d. Moreover, a recent estimate of the biomass of Japanese anchovy in the Huanghai Sea is of the order of about 102 millions (Jin et al., 2001). If we apply the mean water content of 74% and the P content of 2% recorded in our study, we could compute yields to a phosphorus pool from this single fish specie of 1.7×10^8 mol. Potentially, it may reinforce the P-limition of marine productivity by

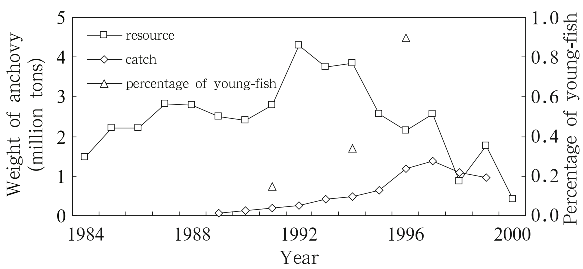

Fig.5. Variation of anchovy resources, catch and proportion of young-fish in the Huanghai Sea during 1984–2000 (Jin et al., 2001).

sequestering phosphorus in their tissues. For example, Hjerne and Hansson (2002) found that fish biomass increases during the summer months, and may explain up to one third of the summer decrease in total phosphorus in the upper 40 m of the water column in the Baltic Sea. Therefore, the implication of this change could be easily assessed only after intensive study.

All in all, this study initially highlights the need to improve our understanding of the role of Japanese anchovy in nutrient cycle in food webs in the Huanghai Sea.

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