Seasonal variations of the ichthyoplankton assemblage in the Yangtze Estuary and its relationship with environmental factors

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Seasonal variations of the ichthyoplankton, as well as the relationship between the assemblage and the environmental factors, were analyzed based on four seasonal surveys during 2012. And historical data was also collected to be compared with the results of other years in order to indicate the seasonal and inter-annual variation of the ichthyoplankton assemblage in the Yangtze Estuary and its adjacent waters. A total of 3,688 individuals, belonging to 5 orders, 9 families and 15 species, were collected. No samples were collected in the winter cruise. Then, all samples were separated into four ecotypes in 2012, which is the same as other years. The *Engraulis japonicus* was the most abundant species of all teleost fishes. The *E. japonicus* was captured in every season, contributed most to total ichthyoplankton abundance and also much bigger than the abundance of other years. This result may be caused by the periodic fluctuations of *E. japonicus* or spawning ground was moving to the offshores because of the environmental reflection. The diversity indexes of the assemblage were significantly different among seasons, with the number of the species and the abundant peaked in the spring, while richness index, evenness index and diversity index was peaked in the autumn. The Species richness of the ichthyoplankton varied from 0.74-1.62, the Pielou evenness index varied from 0.10-0.49 and the Shannon-Wiener index varied from 0.19-1.04. The results of CCA analysis showed that the major factors affecting the ichthyoplankton assemblage in different seasons were not the same. Chla factor was the key factor affecting the ichthyoplankton in 2012. These seasonal and inter-annual variations likely resulted from both migrations associated with fish spawning and the environment. Compared with previous literatures, the relationship between the assemblage structure of ichthyoplankton and environmental variables have undergone a decline change.

Title

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Abstract

Seasonal variations of the ichthyoplankton, as well as the relationship between the assemblage and the environmental factors, were analyzed based on four seasonal surveys during 2012. And historical data was also collected to be compared with the results of other years in order to indicate the seasonal and inter-annual variation of the ichthyoplankton assemblage in the Yangtze Estuary and its adjacent waters. A total of 3,688 individuals, belonging to 5 orders, 9 families and 15 species, were collected. No samples were collected in the winter cruise. Then, all samples were separated into four ecotypes in 2012, which is the same as other years. The *Engraulis japonicus* was the most abundant species of all teleost fishes. The *E. japonicus* was captured in every season, contributed most to total ichthyoplankton abundance and also much bigger than the abundance of other years. This result may be caused by the periodic fluctuations of *E. japonicus* or spawning ground was moving to the offshores because of the environmental reflection. The diversity indexes of the assemblage were significantly different among seasons, with the number of the species and the abundant peaked in the spring, while richness index, evenness index and diversity index was peaked in the autumn. The Species richness of the ichthyoplankton varied from 0.74-1.62, the Pielou evenness index varied from 0.10-0.49 and the Shannon-Wiener index varied from 0.19-1.04. The results of CCA analysis showed that the major factors affecting the ichthyoplankton assemblage in different seasons were not the same. Chla factor was the key factor affecting the ichthyoplankton in 2012. These seasonal and inter-annual variations likely resulted from both migrations associated with fish spawning and the environment. Compared with previous literatures, the relationship between the assemblage structure of ichthyoplankton and environmental variables have undergone a decline change.

Introduction

As the transition region of seawater and fresh water, the Yangtze Estuary has an advantaged geographical location and distinct ecological environment. Profited from freshwater runoff of Yangtze River, as well as Taiwan Warm Current, East China Sea Coastal Current and Yellow Sea Coast Current, the Yangtze River have become an excellent spawning and nursing ground of diversiform economic fish species and a crucial fishery ground in China (Luo and Shen, 1994). However, the Yangtze River basin, especially the estuary area, is featured by the high industrialization and urbanization (Chai et al, 2009), which means the estuary are exposed to the anthropogenic inputs from the populated areas in upstream and industries located near the estuary. The construction and operation of Three Gorges Reservoir have resulted in the short-term and long-term impacts not only on the ecosystem in Yangtze Estuary, but also the distribution and community structure of marine organism (Xian et al, 1994). Due to the heavy intensity of trawl operations and environmental pollution, the structure of marine fishery resources was prominently characterized by the recession of economic species, and the variation in fishing species (Shan and Jin, 2011). Thus it's necessary to reveal the relationship between the ichthyoplanton assemblage and the environmental variation.

The spatial and temporal variation of ichthyoplankton assemblages has always been widely studied in the field of marine ecology(such as Yang et al, 1990; Zhu et al, 2002; Zhong et al, 2007; Zhang et al, 2015;2016.). With the increasing insight of the status of Yangtze Estuary, many domestic scholars have studied seasonal variation of species composition and biodiversity, as well as the characteristics of ichthyoplankton assemblage structure and its relationship with environmental factors such as depth, dissolved oxygen, temperature and salinity in this region. Liu et al (2008) reported a total of 11540 ichthyoplankton individuals were taxonomically identified, belonging to 11 orders, 18 families and 32 species in spring of 1999 and 2001 in Yangtze Estuary and salinity, depth, dissolved oxygen, and total suspended particulate matter were the major factors affecting the ichthyoplankton assemblages in the study areas. Wei et al (2012) reported that a total of 93 ichthyoplankton samples were collected at 15 stations in

Hangzhou Bay In summer from 2004 to 2010. As results, 233 eggs and 29 825 larvae were obtained. And the correlation was significant between ichthyoplankton logarithm density and factors of hydrological conditions. Based on the investigation data of ichthyoplankton and environmental conditions from four cruises in 2012, this paper aimed to show the characters of ichthyoplankton assemblage in Yangtze Estuary by gathering the data of species composition and biodiversity and to reveal the relationship between the spatial-temporal distribution patterns in the ichthyoplankton assemblage and environmental factors, which could provide scientific basis for the management and sustainable utilization of fishery resources in the Yangtze Estuary.

Materials and Methods

Data collection

A total of 40 stations located at the Yangtze Estuary and its adjacent waters (30°45′-32°00′N, 121°00′-123°20′E) (Fig.1) and the samples were collected by using the trawl, guiding with " Specification of Oceanographic Investigation" (GB12763-2007) during February, May, August and November in 2012. This gear has a horizontal opening of 0.8m and a vertical opening of 2.8m (mesh size of 0.5mm). The trawl was monitored horizontally with the vessel speed of approximately 2 knots, lasting 10-min in each station. Samples from each trawl were immediately preserved in 5% formalin buffer for later sorting. Real-time data on the environmental parameters of water column including temperature (T), salinity (S), total nitrogen (TN), total phosphorus (TP), pH, suspended matter (SPM), depth (D), dissolved oxygen (DO), chemical oxygen demand (COD), and chlorophyll a (Chla) was measured. Collections of all data were under the guidance of "Specification of Oceanographic Investigation" (GB12763-2007).

Species identification

At the laboratory, fish eggs and larvae were counted and sorted to the lowest possible taxonomic level at each station according to the morphological characteristics under the guidance of literatures (Zhang et al, 1985; Cheng and Zheng, 1987; Wu et al, 2012;), and classified into different ecotype by different ecological habit based on the description in the book and literature (Yang et al, 1990; Luo and Shen, 1994). Numerous fish eggs and larvae lacking clear morphological features could not be identified by morphological approach, so the molecular identification was applied to prevent misidentification.

Data analyses

Abundance of ichthyoplankton was standardized and expressed as the total individual of fish eggs and larvae per 10 min-trawling (ind/trawl). The dominant species were determined using the Index of Relative Importance (IRI) developed by Zhu et al (2002):

$$IRI = \mathrm{N} * 100\% * \mathrm{F} * 100\%$$

N ∗ 100% and F ∗ 100% are the relative abundance and frequency of occurrence, respectively. The IRI of the dominant species should be greater than 100.

The Margalef's richness (D), Shannon-Wiener index (H′, $\log_e$), Pielou's evenness (J′) were calculated in each station. Related equations were as follows (Ludwig and Reynolds, 1988; Qian and Ma, 1994):

$$D = (S-1)/\ln N$$

$$H^{'} = -\sum_{i=1}^{S} P_i \cdot \ln P_i$$

$$J^{'} = H^{'}/\ln S$$

Where “S” is the number of species, “N” is total individuals and “Pi“is the proportion of ith species individuals to the total individuals.

The homoscedasticity was measured with the method Levene's test, after that, one-way ANOVA was performed to assess the difference in abundance, biomass, species richness and biodiversity index among four cruises. When significant difference was detected, the Duncan's test was applied for the multiple comparisons. Canonical correspondence analysis (CCA) was applied to analyze the correlation between environmental factors and the distribution pattern of ichthyoplankton assemblages. To eliminate the effect of few dominant species and plenty of zeros in species data and highly variable value in environment data, all data matrix were transformed by log(x+1).

All the maps were drawn with Surfer 8.0 and statistical analyses were performed with PRIMER 5.0, SPSS 16.0 and CANOCO 4.5.

Results

Species composition

A total of 3688 individuals, including 689 fish eggs and 2999 larvae from 4 cruises, were sorted. All samples belong to 7 orders, 12 families and 15 species including one unidentified specie (Table 1). Abundance and biomass of Engraulidae, including were both dominant in 2012.

According to the habitats and distribution characteristics of ichthyoplankton, 4 ecotypes were included in this study (Table. 1):

Fresh water species, including *P. engraulis*, which finished the whole life history in fresh water. This species has distributed in fresh waters or oligo-salt waters adjacent to the inner sider of the estuary and had the fewest individuals, accounting for 1.97% of the whole abundance in four seasons.

Brackish water species, which used the estuary as habits but finished the early development stages in the waters close to estuary, including catadromous species and anadromous species. This species contained *C. nasus, C. mystus, C. spinosus*, *H. sajori*, and one species belonging to Takifugu, accounting for 11.30% of the total abundance.

Coastal species, most of which always gathered in costal shallow water for reproduction and development in spring and summer and migrated to abyssal region in winter. Four species were included: *A. commersoni*, *L. polyactis, A. bleekeri* and *M. monodactylus,* accounting for 4.55% of the total abundance.

Marine species, which will migrate to profundal zone (> 30 m) for ingestion as they hit adulthood and returned to estuary or coastal water for spawning and breeding. This species contents *E. japonicus, S. japonicus, T. japonicus, S. acua* and *L. litulon,* which were contributed the most to the total abundance accounting for 82.30%.

The largest number of species were collected in spring, including 3 brackish water species, 4 coastal species and 3 marine species, followed by autumn with 9 species were collected, including 4 brackish water species, 2 coastal species and 3 marine species, The minimum number of species were caught in the summer, including 1 fresh water species and 1 marine species, 3 brackish water species and 3 coastal species. In the summer, ichthyoplankton assemblages was dominant by brackish water species and coastal species, while coastal species and brackish water species was dominant in the spring and autumn respectively.

E. japonicus, C. mystus, A. commersoni, A. bleekeri and *C. spinosus* were widespread, which were captured in all four seasons. On the contrary, 7 species (54.55% of the total species) were captured only in single season, such as *S. japonicus, L. polyactis* and *M. monodactylus* were only collected in the spring; *P. engraulis* was only collected in the summer and *S. acua, H. sajori, T. japonicus* were only captured in the autumn. The distribution pattern of ichthyoplankton assemblages occurred seasonal variation on account of the driven of ecological habit and different species composition in different seasons.

Index of relative importance (IRI) was used to discuss the dominant species. Species with the index greater than 1000 and varied from 100 to 1000 were considered as dominant species and common species respectively, which combined together as the important species. A clear variation occurred in the composition of the dominant species in every season (Table 2). As is shown in Table 2, *E.japonicus* and *C. nasus* as the important species contributed the most (98.63%) to the total abundance, followed by *E. japonicus* which occupied 93.80% in the spring. *E. japonicus* occupied the most proportion of abundance in the spring and autumn. The characteristics and composition of dominant species and the variation in the degree of dominance showed distinct difference during three investigations, which indicated the seasonal variation in ichthyoplankton assemblage structure.

Spatial and temporal variation

The spatial distribution of ichthyoplankton abundance in the Yangtze Estuary in 2012 showed significant seasonal variation (Fig 2), with the highest abundant in the spring and the lowest in the autumn.

A total of 2604 individuals were captured in the spring, including 317 fish eggs and 2287 larvae. Larvae were widespread apart from the river channel and the north part in investigation areas. The most widely distributed was *E. japonicus*, followed by *A. bleekeri, C. mystus, C. spinosus, L. polyactis.* In total, 366 individuals were recorded in the summer, including 120 fish eggs and 246 larvae, which was primarily distribute in the river channel and the south and east of study areas. *E. japonicus* had the largest number of larvae, followed by *P. engraulis* and *C. mystus*, which shared the similar distribution range. Only 76 larvae was collected in the autumn lacking fish eggs, which mainly distributed in the river channel and the south of study areas, with the *C. nasus* was the majority, followed by *E. japonicus, A. bleekeri, A.commersoni* and *H. sajori.*

Biodiversity

The statistical result of Levene's test (df1=2, df2=9, sig=0.165>0.05) indicated that the difference of homoscedasticity of the index is not significant. After this, we used One-way ANOVA test for further analysis. The One-way ANOVA test revealed that significant difference occurred among three diversity indexes during four seasons (df of inter-season=2, df of intra-season=9, F=4.601, P=0.0095<0.01).

According to the consequence, autumn presented the highest diversity indexes and the lowest was in the spring (Table 3). Furthermore, according to the result of multiple comparisons, all the diversity indexes had significant difference between spring and autumn (P<0.01). Furthermore, *H′* and *J′* between spring and summer as well *J′* between summer and winter also occurred significant difference. But no significant difference was detected for *D* between spring and summer (F=3.24, P=0.10＞0.05) as well as for *D* (F=4.30, P=0.08＞0.05) and *H′* (F=2.96, P=0.07＞0.05) between summer and autumn.

CCA analysis

The relationships between the environmental factors and species were clarified in the CCA ordination diagram using the data of 15 species and the set of 10 environmental factors. The first axes (eigenvalues=0.497) and the second axes (eigenvalues=0.290) of CCA plot explained 14.4% of "species data" variation and 65.6% of variation in "species-environment relation". The species-environment correlations coefficients of this two axes were 0.832 and 0.621 respectively. Monte-Carlo test (Table 4) indicated that Chla was the key environmental factor affecting ichthyoplankton assemblages ($P<0.05$). As is shown in the plot, the first axes was strongly correlated with Chla, SPM, TP, COD, DO, and the remaining environmental factors displayed a higher correlation with axes 2 than axes 1. SPM, Chla, TP and temperature exerted positive effect on the first axes, in addition, depth showed positive correlation and TN showed negative correlation with the second axes respectively. The CCA ordination plot of sampling stations (Fig 3) revealed that stations in the spring were located in the area where was significantly connected with dissolved oxygen and had higher level of dissolved oxygen, TN, pH and salinity values. The location of sampling stations in the summer were relatively scattered and mainly characterized by higher level of TN, TP, Chla, SPM, temperature and dissolved oxygen values. As for investigating areas in the autumn, sampling stations was mainly distributed in the areas with higher pH and salinity values.

As shown in the CCA ordination plot of ichthyoplankton species (Fig 4), the correlation between environmental factors and the distribution of different species was inconsistent. *E. japonicus* showed a strong relationship with dissolved oxygen and less affected by the remaining factors and *S. japonicus* was mainly affected by TP, which indicated that the distribution pattern of different species belonging to the same ecotype may affected by different environmental factors. *L. polyactis* and *A.commersoni* were also revealed significantly positive correlation with dissolved oxygen, which mainly distributed in the region of higher dissolved oxygen content. *C. spinosus* showed a distinct distribution pattern positively associated with the higher value of Chla, SPM and temperature. The distribution pattern of *C. nasus* was positively and mainly correlated with deeper and higher concentration of nutrients areas.

Species such as *H. sajori* (*hesa*), *A. bleekeri* (*Albl*), *M. monodactylus* (*Mimo*) and *S. acua* (*syca*) had positive correlation with pH and salinity, but *A. bleekeri* had lower demand for pH and salinity than the other three species.

Discussion

The ichthyoplankton assemblages in estuaries are complex both in species composition and distribution. Studies show that the organization of ichthyoplankton in estuarine systems is influenced by the interactive effects of a multitude of biotic and abiotic processes. Biological factors include the location, timing and manners of spawning, larval life history, larval behavior, rates of predation, and feeding (Leis 1991; Azeiteiro 2006). Physical factors include salinity (Whitfield 1999), temperature (Blaxter 1992), turbidity (Islam et al. 2006), dissolved oxygen (Rakocinski et al. 1996), depth (Wantiez et al. 1996), river flow (Faria et al. 2006), sediment characteristics and hydrographic events such as currents, winds, eddies, upwelling and stratification of the water column (Gray 1993). The present study was based on surveys at four seasons in 2012. Our aims are to provide detailed characterizations of the ichthyoplankton assemblage in 2012, and evaluate the influence of environmental factors on the spatial distribution and intra-annual variations of ichthyoplankton assemblages associated with the Yangtze Estuary.

Species composition and seasonal variation

In the last decades, many scholars have reported the community structure and biodiversity of ichthyoplankton assemblages and its relationship with environmental factors. The study of Yang et al (1990), was carried out from 1985 to 1986 with 10 cruises in Yangtze Estuary, have collected 94 species. Another study based on the four cruises in 2007 collected 45 species (Liu and Xian, 2009), which shared the same investigation area with this study. Zhang et al (2015; 2016) studied the ichthyoplankton assemblages in springs (1999-2007) and autumns (1998-2009). In spring forty-two ichthyoplankton belonging to 23 families were collected. Engraulidae was the most abundant family, including six species and comprising 67.91% of the total catch (Zhang et al, 2015), while in autumn a total of 969 ichthyoplankton constituting 33 species from 19 families and 10 orders were collected during the seven sampling autumns in the Yangtze Estuary, including 226 fish eggs and 743 larvae and juveniles (Zhang et al, 2016). Species composition of ichthyoplankton assemblages in Spring showed a descending trend with 20 species in 1999 (Zhu et al, 2002) , 31 in 2001(Zhang et al, 2015), 12 in 2004 (Zhang et al, 2015) , 17 in 2007 (Zhang et al, 2015) and only 10 in 2012 (the present study). The comparison of these studies showed that species composition of ichthyoplankton assemblages has been suffering the recession (Fig 5). Composition and numbers of fresh waters species in this study was less than investigation results in 2007 (Liu and Xian, 2009; Zhang et al, 2015), only *P. engrauli* was collected. This phenomenon indicated that the composition and numbers of fresh water species have been experiencing a significantly decline due to the impact of anthropogenic activity and natural environment. Zhong et al (2007) have presented that salinity conditions in Yangtze Estuary and its adjacent waters was significantly influenced by the surface runoff which maybe result in the decline of fresh water species.

The abundance of ichthyoplankton assemblage in this study was highly concentrate on the dominant species- *E. japonicus,* which was the common characteristics of ichthyoplankton assemblages (Harrison and Whitfield, 1990; Whitfield, 1999). *E. japonicus* resources have declined dramatically than before based on the comparison in May of 1999, 2001, 2004 and 2007 (Zhang et al, 2015) , and May and June of 2008(Shan and Jin, 2011). Nevertheless, this study showed that the quantity of *E. japonicus* resources in every season of 2012 had a rising trend, which was not consistent with the previous results. Watanabe (2007) repotted that *E. japonicus* resources had relatively steady fluctuations in quantity as a result of climate and environment changes which was mainly caused by water circulation and fluctuation of temperature. Although Watanabe did not point out the duration of the fluctuation, this conclusion was also supported by the investigation results in this study.

Based on the results of this study and the comparison with the results with other scholars, the community structure of ichthyoplankton assemblages in the Yangtze Estuary has suffered great changed in a short time. On the one hand, this phenomenon was related to the alternate usage of coastal water in different month by dominant species for breeding and feeding (Shan and Jin, 2011), on the other hand, the sensitivity of the different species with different disruptive factors such as fishing and environmental changes was also different due to the difference of ecological niche and habits among species.

Biodiversity and its spatial - temporal variation

In this study, the biodiversity indexes in each season showed significant difference, but all the indexes were relatively low (Table 3). The investigation in 2007 (Zhang et al, 2015;2016) collected 52 fish eggs and 638 larvae in the spring, 3973 fish eggs and 1342 larvae in the summer and 6 fish eggs and 450 larvae in the autumn, which were significantly greater than results in this study in terms of quantity and species composition. So, significant differences existed in the spatial and temporal variation of ichthyoplankton assemblages and biodiversity in the Yangtze Estuary from 2007 to 2012. Furthermore, the composition of dominant species in different seasons presented clear difference between 2007 and 2012. In 2007, *A. bleekeri, C. mystus,* and *E. japonicus* was as dominant species in the spring, and *E. japonicus*, *C. mystus,* and *S. elongata* as dominant species in the summer and *H. prognathous, E.japonicus* and *C. stigmatias* as dominant species in the autumn. As for 2012: *E. japonicus* was dominant species in the spring, *E. japonicus* and *C. nasus* as dominant species in the summer and *A. bleekeri* as dominant species in the autumn.

Most of the marine fish's spawning season was in the spring and summer, which makes the abundance of ichthyoplankton assemblages reaching the maximum in this period (Young and Potter, 2003; Sabatés et al, 2007). In this study, the abundance and the number of species reached the highest in the spring which was consistent with above conclusion. However, due to the highly concentration of the *E. japonicus* in the spring, accounting for 93.80% of the total abundance, the diversity indexes in the spring was less than summer and autumn.

The seasonal variation of biodiversity in estuary mainly depended on the selection of reproductive areas by grown fish (Hernández et al, 2003) and the influence of seasonal variation of water environment in spawning area on the spawning behavior (Lam, 1983). With the increasing intensity of fishing, a variety of fish reached the sexual maturity earlier which led to the spawning period ahead of time than before. That will further caused the peak abundance value of ichthyoplankton occurred earlier than before, which maybe one of the reasons of seasonal variation in ichthyoplankton abundance in the Yangtze Estuary. The environment in Yangtze Estuary was complicated and changeable along with the drastic fluctuation (Luo and Shen, 1994), which makes the fish were incapable of adapting to the environmental changes in time, hence the assemblage biodiversity of ichthyoplankton was relatively low.

Relationship between the distribution of ichthyoplankton assemblages and environmental factors

Distribution pattern of ichthyoplankton assemblages in estuary was mainly affected by both abiotic factors and environmental factors (Zhu et al, 2002). In general, salinity was the major factor which determined the structural changes of plankton community in estuaries (Wooldridge, 1999). Due to the specific geographical conditions and the inflow of fresh water in the estuary, salinity showed clear gradient along with the direction of the runoff. Ichthyoplankton assemblages appeared different ecotype according to the variation of salinity content. CCA ordination results indicated that the key factor affecting the assemblage structure of ichthyoplankton was not salinity but Chla, which differed with some researches from Kushlan (1976), Thiel et al (1995) and Fraser (1997). The salinity condition in Yangtze Estuary was significantly influenced by the surface runoff which maybe results in the decline of fresh water species (Zhong et al, 2007). In the present work, only one fresh water specie was collected and other species are not sensitive to the variation of salinity. This may be the reason that salinity is not the key factor. Due to the fluctuation of water environment and species composition in different season, the influence mechanism of environmental factors was not consistent among different seasons and years. Harris et al (1999) presented that DO was the leading indicator accounting for the variation of the community structure and abundance of ichthyoplankton assemblages, which was also correspond with other scholars (Castillo-Rivera et al, 2002) as well. In this study, DO was also confirmed as the dominating factor affecting the assemblage structure in the Yangtze Estuary, and temperature, nutrients content, COD, pH also made great contributions to this affection. The CCA ordination only explained 14.4% and 65.6% of the variation in species and environment, respectively, thus, more biotic factors and environmental factors need to be collected in later investigations to understand the environmental-biological relationships.

Conclusion

During the four surveys in 2012, 3688 individuals of 15 species were collected. We found significant seasonal differences occurred in the species number and abundance of ichthyoplankton assemblages in the Yangtze Estuary with low biodiversity. Chla is the key environmental factors affecting the assemblage structure of ichthyoplankton in 2012, which was different with precious research.

With the rapid development of industrialization, urbanization and marine fishery, the ichthyoplankton resources is declining significantly, which would strengthen the trend of the vulnerabilization and simplification of fishery resources in the Yangtze Estuary. Protection on the fishery resources and the continuous tracking and monitoring is necessary and imperative in the Yangtze Estuary.

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Table 1(on next page)

Presence (+) of species in ichthyoplankton samples in the present study

Table 1 Presence (+) of species in ichthyoplankton samples in the present study

Species	**Code**	**Ecotype**	**Month**			
			Feb	**May.**	**Aug.**	**Nov.**
Engraulidae						
Engraulis japonicus	*Enja*	marine		+	+	+
Anchoviella commersoni	*Anco*	coastal		+	+	+
Coilia nasus	*Cona*	brackish water			+	+
Coilia mystus	*Comy*	brackish water		+	+	+
Cyprinidae						
Pseudolaubuca engraulis	*Psen*	fresh water			+	
Sciaenidae						
Larimichthys polyactis	*Lapo*	coastal		+		
Scombridae						
Scomber japonicus	*Scja*	marine		+		
Trichiuridae						
Trichiurus japonicus	*Trja*	marine				+
Atherinidae						
Allanetta bleekeri	*Albl*	coastal		+	+	+
Scorpaenidae						
Minous monodactylus	*Mimo*	coastal		+	+	
Triglidae						
Chelidonichthys spinosus	*Trfa*	brackish water		+	+	+
Hemiramphidae						
Hemiramphus sajori	*Hesa*	brackish water				+
Syngnathidae						
Syngnathus acua	*Syac*	marine				+
Lophiidae						
Lophius litulon	*Loli*	marine		+		
Tetraodontidae						
Takifugu sp.	*Tasp*	brackish water		+		

Table 2(on next page)

Composition of dominant ichthyoplankton species in different seasons

Table 2 Composition of dominant ichthyoplankton species in different seasons

Dominant species	**Spring**		**Summer**		**Autumn**	
	IRI	**Percentage of quantity（%）**	**IRI**	**Percentage of quantity（%）**	**IRI**	**Percentage of quantity（%）**
Engraulis japonicus	5120.99	90.2	1532.11	74.61	19.74	3.95
Coilia mystus	158.99	3.6	40.37	10.01	3.29	1.32
Allanetta bleekeri	11.27	1.13	1.15	0.46	52.63	5.26
Chelidonichthys spinosus	4.22	0.85	1.15	0.46	3.29	1.32
Anchoviella commersoni	2.11	0.42	1.15	0.46	26.32	5.26
Larimichthys polyactis	1.52	0.61				
Scomber japonicus	0.12	0.025				
Minous monodactylus	0.12	0.025				
Lophius litulon	0.12	0.025				
Takifugu sp.	0.12	0.025				
Coilia nasus			2.30	2.02	19.74	3.95
Pseudolaubuca engraulis			300.25	12.00		
Hemirhamphus sajori					39.47	5.26
Syngnathus acua					13.16	2.63
Trichiurus japonicus					3.29	1.32

Table 3(on next page)

Diversity index of ichthyoplankton in different seasons

P<0.01, Numbers with different superscript are significantly different with each other.

Table 3 Diversity index of ichthyoplankton in different seasons

	Spring	Summer	Autumn	Winter
Species richness (*D*)	$0.79±0.05^{A}$	$0.74±0.08^{A}$	$1.62±0.11^{B}$	$0.00±0.00^{C}$
Pielou evenness index (*J'*)	$0.10±0.06^{A}$	$0.38±0.08^{B}$	$0.49±0.05^{B}$	$0.00±0.00^{C}$
Shannon-Wiener index (*H'*)	$0.19±0.05^{A}$	$0.61±0.09^{B}$	$1.04±0.10^{C}$	$0.00±0.00^{D}$

Note: $P<0.01$, Numbers with different superscript are significantly different with each other.

Table 4(on next page)

Conditional effects and correlations of environmental variables with the CCA

1 Table 4 Conditional effects and correlations of environmental variables with the CCA axes

Environmental factors	Lambda A	P	Axis 1	Axis 2
Chla	0.23	0.046	0.7541	0.1330
pH	0.16	0.148	-0.0108	0.0124
DO	0.13	0.334	-0.0649	-0.0482
D	0.1	0.414	0.0208	0.1146
TP	0.11	0.396	0.4464	0.2961
COD	0.09	0.610	0.1636	-0.0699
SPM	0.08	0.514	0.6435	0.1287
TN	0.14	0.298	0.0251	-0.1837
T	0.08	0.64	0.1195	0.0236
S	0.08	0.682	-0.0912	0.1521

2

Figure 1

Location of survey stations of ichthyoplankton in Yangtze estuary

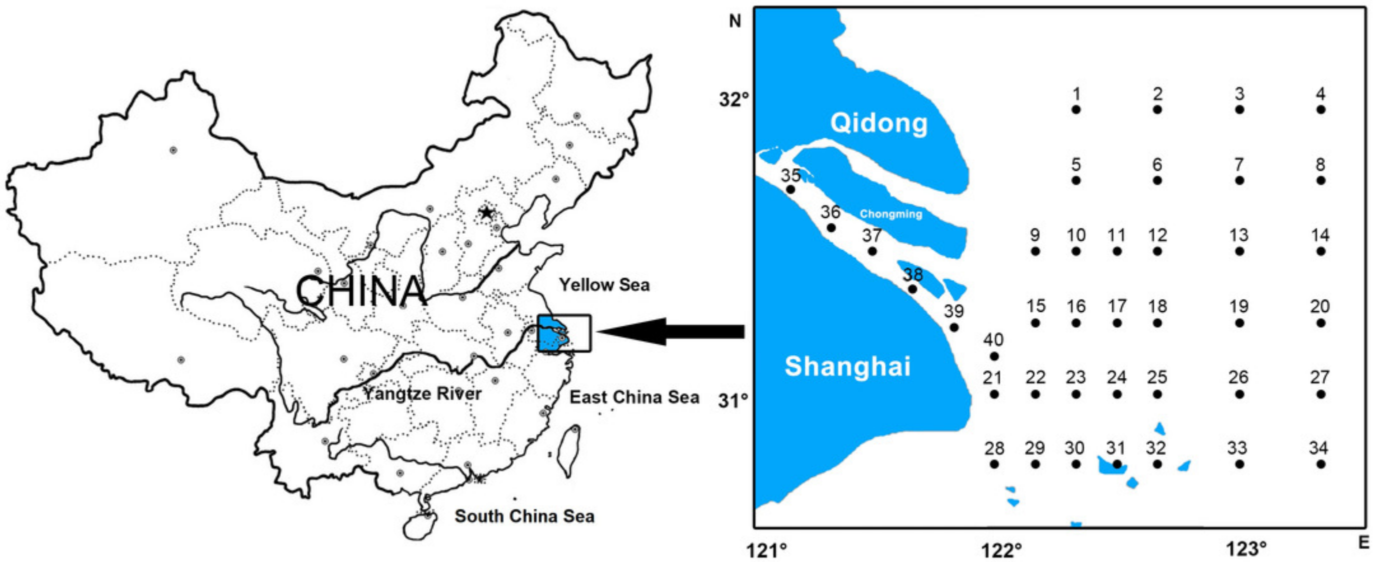

Figure 2

Distribution of ichthyoplankton abundance in the present study

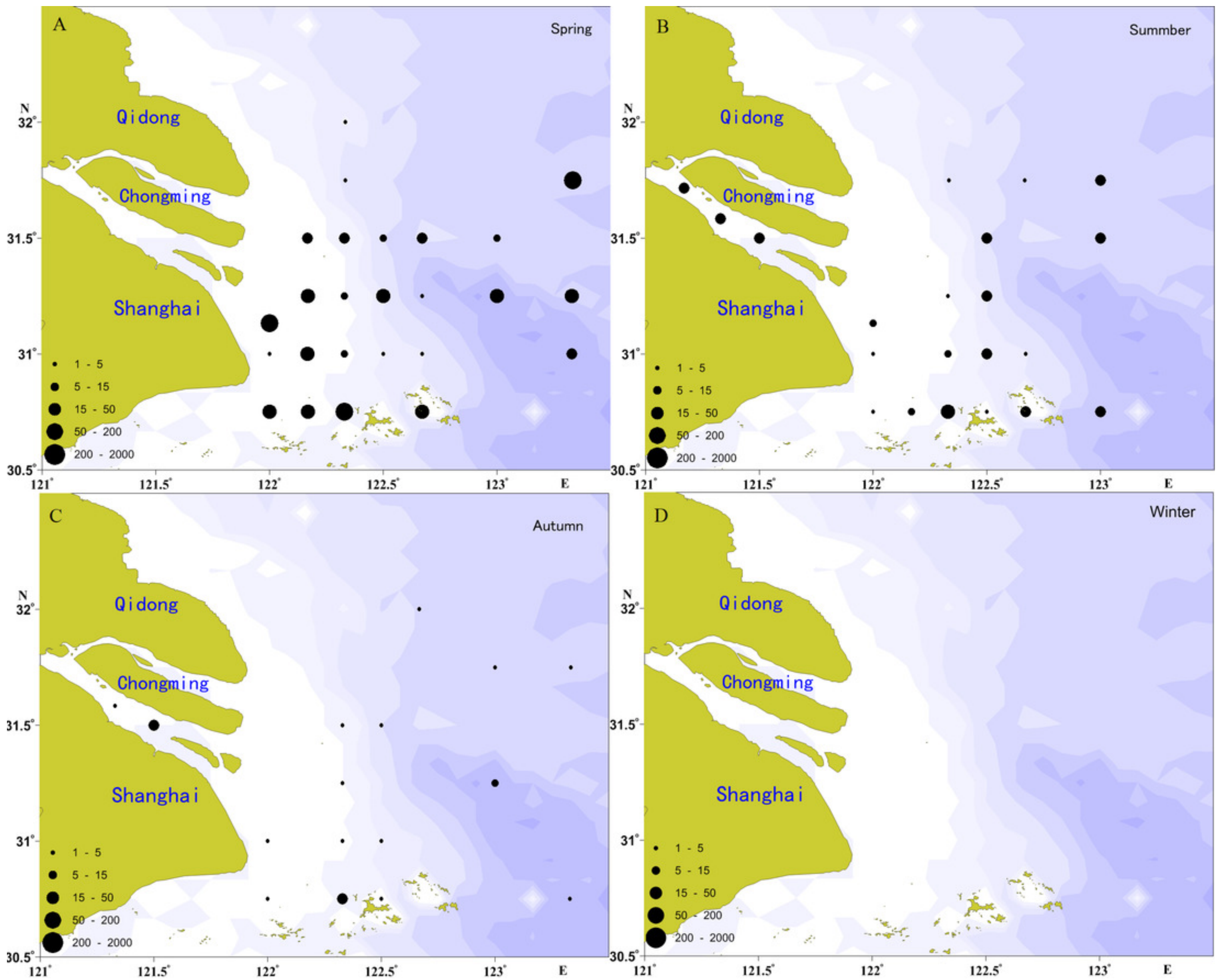

Figure 3

CCA biplot of sampling stations

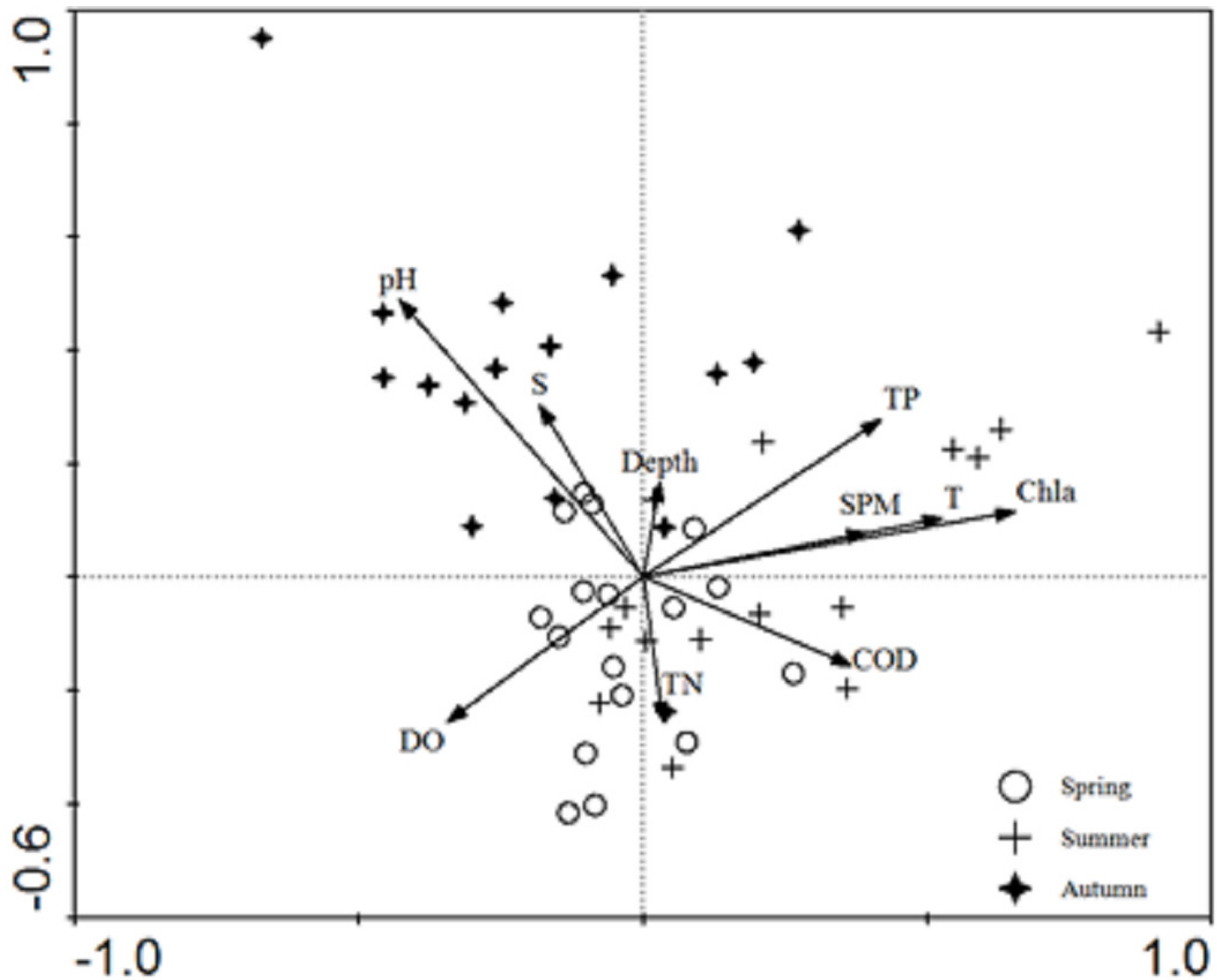

Figure 4

CCA biplot of ichthyoplankton species

The italic characters indicate the abbreviate name of the species as shown in Table 1

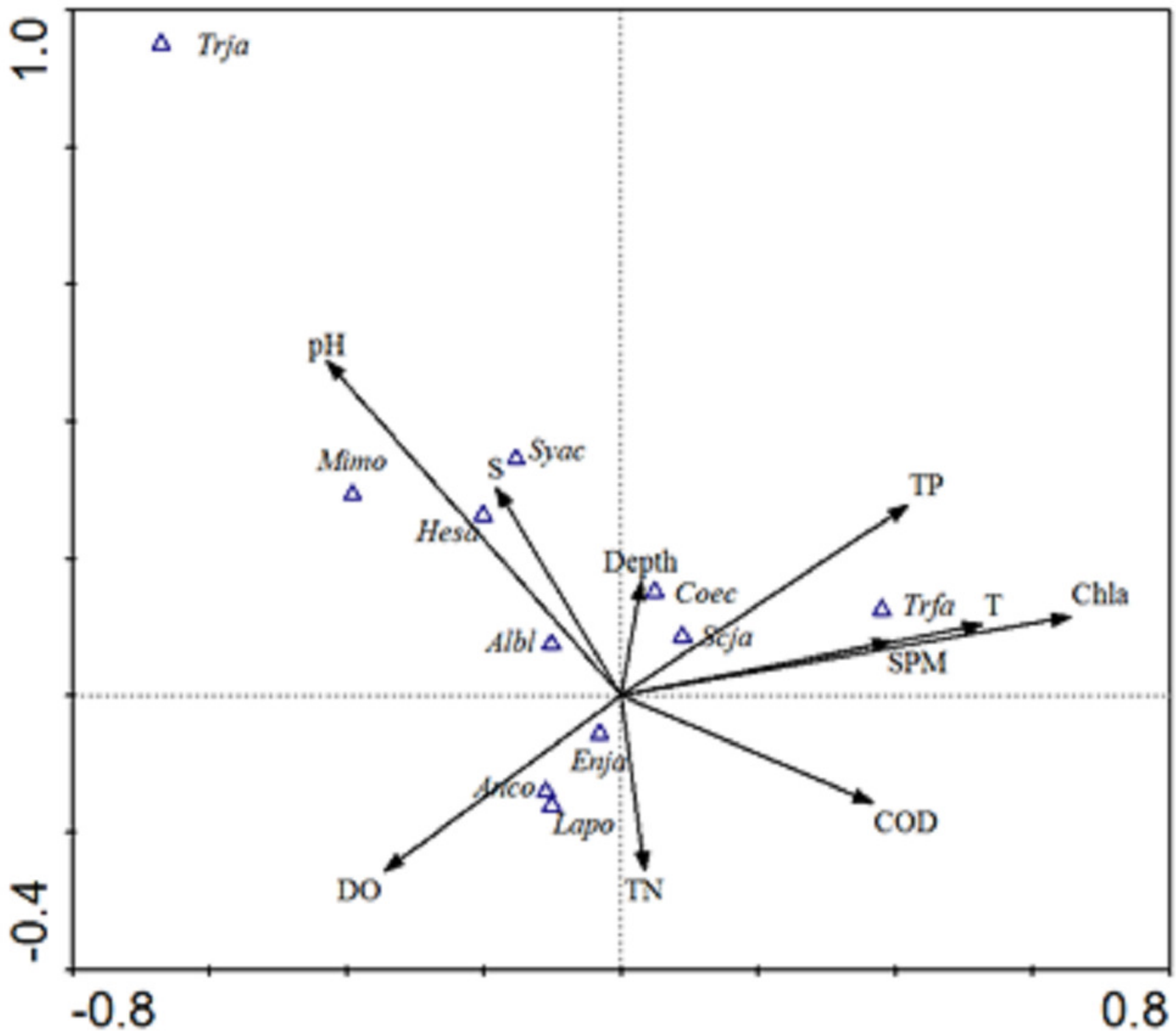

Figure 5

Spring long-term variation of the ichthyoplanton in Yangtze Estuary

The data for 1999-2011 was referenced from Zhang et al 2015 and 2016.