



Zhaomin Chen^{1,2,3}, Cui Liang^{1,2,4,*} and Weiwei Xian^{1,2,4,*}

- ¹ CAS Key Laboratory of Marine Ecology and Environmental Sciences, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China; chenzhaomin@qdio.ac.cn
- ² Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China
- ³ University of Chinese Academy of Sciences, Beijing 100043, China
- ⁴ Center for Ocean Mega-Science, Chinese Academy of Sciences, Qingdao 266071, China
- * Correspondence: liangc@qdio.ac.cn (C.L.); wwxian@qdio.ac.cn (W.X.); Tel.: +86-139-6422-6627 (C.L.); +86-138-6982-4990 (W.X.)

Abstract: The offshore waters of the Yangtze Estuary are an important fish habitat, and the large gradient of environmental conditions leads to different fish assemblages. We studied the spatial and temporal variations in fish assemblages and their relationships with environmental factors in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016. The fish assemblage consisted of 64 fish species from 39 families, of which 6 species were dominant. There were significant interannual differences in fish abundance, biomass, and species composition, with the highest abundance and biomass in 2013, the lowest abundance in 2016, and the lowest biomass in 2015. Redundancy analysis revealed that total suspended particles and dissolved oxygen drove interannual variation in fish abundance, biomass, and species composition, and depth drove spatial variation in the fish assemblage. According to the depth, the fish were classified as shallow assemblage and deep assemblage. Understanding the spatial and temporal patterns of fish assemblage in the offshore waters of the Yangtze Estuary is beneficial to the conservation of fish and the sustainable use of fishery resources in the offshore waters of the Yangtze Estuary.

Keywords: assemblage structure; environmental factors; annual variation; spatial characteristic; multivariate analysis

1. Introduction

The estuarine ecosystem is a transition zone between freshwater and the ocean, and a channel for land–sea interaction [1]. As a typical intersection of two ecosystems, estuaries have great ecological importance [2–4]. The Yangtze Estuary is the largest mixed zone of inlet rivers and seawater in China, with high run-off and sand transport, influenced by external currents, wind, waves, and other factors, with complex and variable hydro-dynamic effects, complex sediment transport, and strong land–sea interaction [5]. The environmental factors in the Yangtze Estuary are complex and variable, and the spatial distribution gradient is large. The Yangtze River runoff continuously delivers large amounts of nutrients to the estuary, and the high primary productivity facilitates the survival of marine organisms such as fish and invertebrates, making this area a very important fishery site in China [6]. However, excessive nutrients can also lead to the eutrophication of seawater, and the eutrophication, stratification, currents, and other parameters further causing bottom hypoxia [7–9].

The fish of the Yangtze Estuary can be classified ecologically into freshwater fish, brackish fish, reef-associated fish, and marine fish, among which there are more than 50 species of economic fish [10]. Although the study of fish resources in the Yangtze Estuary has a



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Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). history of more than 170 years, the research work has long lacked systematization and completeness; in particular, basic research is very weak, and long-term monitoring of fish and the environment in the Yangtze Estuary is critical [10]. The community dynamics [11,12], fish species models [13], and taxonomic diversity [14] of the Yangtze Estuary have been reported, but most studies using correlation analysis on interannual variation in fish assemblage structure and the relationship between the estuary and the environment were conducted before 2012 [1,15,16]. The data and research work from recent years need to be updated urgently. Fish in the Yangtze Estuary tend to reach their highest abundance and biomass in the autumn after breeding and fishing moratorium periods. Fish monitoring during the autumn is an important part of the assessment of local fishery resources and could also provide a basis for the assessment of the benefits of the fishing ban in the Yangtze Estuary [17]. The study of fish resources in the Yangtze Estuary is important, since the ecological environment is undergoing drastic changes, and the fish resources are in a highly volatile condition. The conservation of fish resources in the Yangtze Estuary must be supported by a large amount of basic research work [16,18].

To strengthen the basic research on fish resources in the Yangtze Estuary, we report the temporal and spatial variations in assemblage structure and the environmental variables affecting it in autumn (November) in the years from 2012 to 2016. This contribution can provide a scientific basis for improving the management of fishery resources in the offshore waters of the Yangtze Estuary, as well as be a reference for the sustainable use of fishery resources in the area.

2. Materials and Methods

2.1. Sample Collection

Biological and oceanographic data were collected yearly during the fall in fishery evaluation cruises (November 2012, November 2013, November 2014, November 2015 and November 2016). Due to the fishing prohibition policy, water depth requirements, and safety considerations for trawlers, our trawlers could not enter the shallow water (usually with low salinity) within the Yangtze Estuary for trawl sampling (Figure 1). Weather and equipment failure resulted in not all of the sites being sampled for each survey. Sampling was conducted at 14 stations in 2013 and 15 stations in the remaining years.

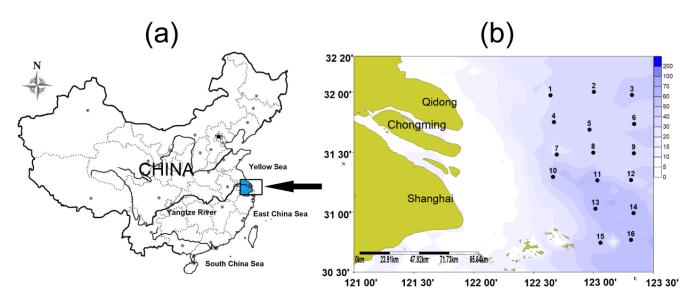


Figure 1. (**a**): the geographical location of the Yangtze Estuary; (**b**): sampling stations in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016, the numbers and dots in the figure represent the different stations (2012: missing station 10; 2013: missing stations 7 and 10; 2014: missing station 7; 2015: missing station 1; 2016: missing station 10). The background color indicates the depth variation.

The survey vessel was a 255 kW bottom double trawler, the survey gear was a 150.5 m \times 96.5 m trawl, the perimeter of the net mouth was 150.5 m, the upper line length was 66 m, the lower line length was 73 m, the maximum mesh of the net was 200 mm, the mesh size decreased from the net body to the cod-end, and the cod-end was 30 mm. When measured at sea, the net headline height during trawling was 9–11 m, the average distance between the net sleeves was 18 m, and the trawling speed of each station was 2–3 Kn and the trawling time was 0.5–1 h.

All catches were cryopreserved and sent back to the laboratory for identification and classification. Each fish was measured in length and weighed. Conductivity, temperature, and depth (CTD) equipment recorded salinity (S), temperature (T), and depth (D) at each station. Surface water was collected, cryopreserved, and brought back to the laboratory for analysis (pH, total suspended particles (TSP), dissolved oxygen (DO), chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), and chlorophyll a (Chl a)). Total nitrogen and total phosphorus were measured by a QuAAtro continuous-flow analyzer, total suspended matter was measured by filtration, chemical oxygen demand and dissolved oxygen were measured by iodine titration, and chlorophyll a was measured by fluorescence extraction. All of these were determined in the study in accordance with GB/T 12763–2007, Specifications for Oceanographic Surveys.

2.2. Data Analysis

Species abundance and biomass at each station were expressed as 10³ individuals per km² (10³ ind. km⁻²) and kilogram per km² (kg km⁻²), respectively. The dominant fish species were classified using the index of relative importance (IRI), calculated as:

$$IRI = (N\% + W\%) \times F\% \times 10,000, \tag{1}$$

where N% and W% are the relative abundance and biomass, respectively. F% is the percentage frequency of occurrence of the species in all stations.

Only species occurring in at least two stations were included in the follow-up data analysis [19].

When SPSS version 27.0 was used to calculate the significance of interannual differences in the 10 environmental factors (S, D, T, pH, COD, TSP, DO, TN, TP, and Chla), Levene's and Kolmogorov–Smirnov tests were applied to determine data homoscedasticity and normality, respectively. For environmental data that could not meet both requirements, square root ($x^{1/2}$) or log transformation ($\log_{10}(x + 1)$) were performed, and for those that could meet both conditions after transformation, one-way ANOVA was performed on the transformed data; for those that still could not meet the requirements, the non-parametric Kruskal–Wallis test was used. The significance of differences in environmental data among years was compared using the paired comparison method.

The spatial distribution of abundance and biomass was visualized by drawing distribution maps using SURFER version 12.0. Annual and spatial variation in fish assemblage structure was analyzed by PRIMER version 6.0 [20,21]. First, fish species with frequencies greater than 5% were used to analyze the assemblage structure and its relationship with environmental factors to reduce the influence of rare species. Based on $log_{10}(x + 1)$ abundance data (to reduce the effect of dominant fish species), the Bray–Curtis similarity matrix was constructed. Non-metric multidimensional scaling (NMDS) was then used to identify annual variation in fish assemblage structure. Non-parametric similarity analysis was performed using ANOSIM to determine the significance of inter-annual and spatial variation in fish assemblage structure. The similarity percentage analyses based on the abundance of fish species were performed with SIMPER to detect typical species contributing most to the similarity in each observed assemblage (cumulative percentage above 90%) and its contribution percentage.

Canoco5.0 was used to study the relationship between environmental variables and fish assemblages (canonical correspondence analysis (CCA) or redundancy analysis (RDA)). Detrended correspondence analysis (DCA) was first performed to determine a suitable

response according to the maximum gradient length of the first DCA axis (>4, CCA; >4 and <3, CCA or RDA; <3, RDA) [18]. The maximum gradient length is 2.3 SD, so RDA was chosen. Log (x + 1) conversion of abundance and environment data was carried out to minimize the effects of extreme values and zeros. The Monte Carlo permutation test was used to identify the key factors that significantly affect the fish assemblages (p < 0.05).

3. Results

3.1. Faunal Composition

In total, 64 fish species referable to 14 orders and 39 families were identified. Of these, 20 species (31.25%) occur in all years (Figure 2a) and 24 (37.5%) occur in singular years. Aulopiformes, Perciformes, and Clupeiformes account for the highest proportion of the overall fish abundance (Figure 2b). All Aulopiformes captured from the autumns of 2012–2016 were *Harpadon nehereus*, Perciformes contained 27 fish species, and Clupeiformes contained 10 fish species. The remaining orders account for a very low proportion of fish abundance. The depth range, climate zone/temperature range, and distribution of all fish caught in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016 are listed in Table S1.

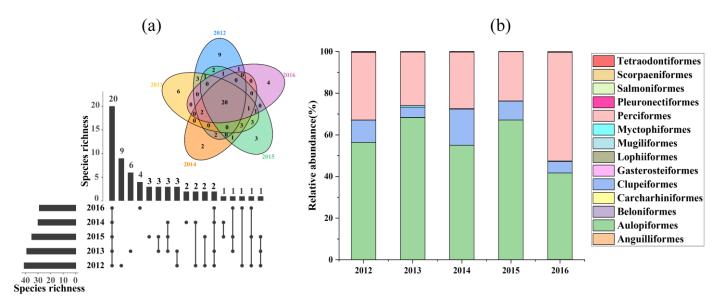


Figure 2. Species richness (**a**) and relative abundance of taxa (**b**) in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016. (**a**): in the Venn plot, different colors indicate fish assemblages in different years, and numbers indicate the number of species that are unique or common in a year; UpSet plot showing the fish composition from 2012 to 2016 (e.g., 9 species were found only in 2012 (left), whereas 20 species occurred in the 5 different years).

3.2. Environment Variables

Of the ten environmental factors examined, nine differ significantly among the years, while no significant difference is detected in depth (Table 1). Station depth varies from 23 m to 62 m. Salinity is significantly higher in 2013 than in 2014–2016, while the temperature is significantly lower in 2012 and 2014 than in the other years. Both dissolved oxygen and pH peak in 2012, with significant inter-annual variation, but with different trends over time. Chemical oxygen demand, on the contrary, has the lowest value in 2012 and significantly lower than in other years. Both total nitrogen and total phosphorus show their lowest values in 2016, with the difference being that total nitrogen peaks in 2014 and total phosphorus peaks in 2015. TSP is significantly lower in 2014 and 2015 than in other years, while chlorophyll a is significantly lower in 2012 and 2014 than in the other years.

Environmental Factors	2012	2013	2014	2015	2016
Depth (D)	42.6 ± 11.12	41.14 ± 11.93	38.47 ± 8.41	37.67 ± 8.05	40.4 ± 9.53
Salinity (S)	32.3 ± 0.57 $^{\mathrm{bc}}$	$33.51\pm0.48~^{\rm a}$	$30.36\pm2.32~^{\rm c}$	$31.01 \pm 3.12^{\text{ b}}$	$32.56 \pm 1.12^{\text{ b}}$
Temperature (T)	$19.58\pm0.56~^{\rm c}$	$21.36\pm0.34~^{a}$	$20.6\pm0.51~^{\rm b}$	$21.32\pm0.97~^{a}$	$21.56\pm0.86~^{\rm a}$
Dissolved oxygen (DO)	7.4 ± 0.41 $^{\rm a}$	7.26 ± 0.17 $^{ m ab}$	$7.37\pm1.14~^{\rm b}$	6.41 ± 0.74 ^b	7.13 ± 0.55 ^b
pH	8.46 ± 0.09 $^{\rm a}$	$8.23\pm0.05~^{\rm b}$	$7.99 \pm 0.29 \ ^{ m bc}$	$7.93\pm0.05~^{\rm c}$	8.36 ± 0.13 $^{\rm a}$
Chemical oxygen demand (COD)	0.63 ± 0.31 ^b	0.93 ± 0.26 $^{\rm a}$	1.22 ± 0.82 ^a	1.38 ± 0.83 ^a	1.12 ± 0.38 ^a
Total nitrogen (TN)	13.22 ± 4.5 ^b	16.52 ± 9.16 ^b	$28.45\pm15.05~^{\rm a}$	$19.17\pm11.48~^{\mathrm{ab}}$	$8.37\pm10.21~^{\rm c}$
Total phosphorus (TP)	0.69 ± 0.2 ^{cd}	$0.9\pm0.45~\mathrm{^{bc}}$	$0.95\pm0.21~^{ m ab}$	$1.13\pm0.31~^{\mathrm{a}}$	0.6 ± 0.28 ^d
Total suspended particles (TSP)	7.28 ± 5.18 $^{\rm a}$	13.53 ± 17 a	$2.66\pm0.85~^{\rm b}$	$2.05\pm1.53~^{\rm b}$	5.21 ± 2.15 a
Chlorophyll a (Chl a)	$0.2\pm0.04^{\text{ b}}$	0.52 ± 0.15 $^{\rm a}$	$0.32\pm0.5~^{\rm b}$	0.55 ± 0.41 a	0.37 ± 0.16 a

Table 1. Temporal variation in environmental factors in the offshore waters of the Yangtze Estuaryduring the autumns of 2012–2016 (Mean \pm SD).

Values with different letters $(^{a-d})$ indicate significant differences among the years; values with the same characters indicate insignificant differences.

3.3. Annual Variation in Fish Assemblages and Relationships with Environmental Factors

Annual variations in fish abundance and biomass are shown in Figure 3a. Abundance is highest in 2013 and gradually decreases, and biomass is highest in 2013 and lowest in 2015, with partial differences between the abundance and biomass trends. Annual variation in fish assemblage structure is portrayed in the NMDS ordination plot, one-way ANOSIM (R-value and significance level), and SIMPER analysis (Figure 3b; Table 2). The year 2015 is significantly different from all other years (p < 0.05). The years with no significant difference on the NMDS plot are close in position or even overlap, and the years with significant differences are clearly distinguished by fish assemblages.

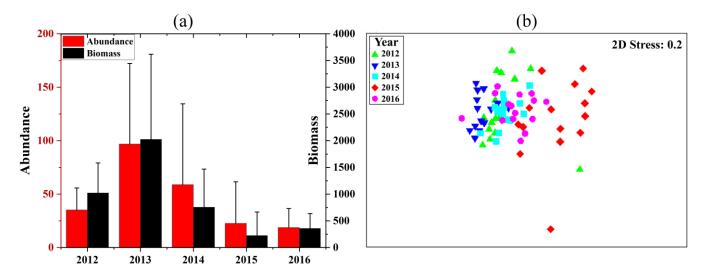


Figure 3. Annual variation in fish mean abundance and mean biomass (**a**), where error bars indicate standard deviations, and the NMDS plot of the fish assemblage structure (**b**) in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016.

Harpadon nehereus is the most dominant fish species, with the highest IRI values in the five years (Table 3). *Trichiurus japonicus* is the second dominant species after *Harpadon nehereus*. These two species are the dominant species in each year from 2012–2016, occupying an absolutely dominant position in the studied area. In 2016, the most dominant species consists of five species, with four dominant species in 2013 and 2014, and three dominant species in 2012 and 2015.

Groups	ANO	SIM	SIMPER	
	R	Р	Average Dissimilarity	
2012 vs. 2013	0.073	0.057	54.32	
2012 vs. 2014	-0.005	0.457	54.64	
2012 vs. 2015	0.188	0.006	76.42	
2012 vs. 2016	0.095	0.04	62.34	
2013 vs. 2014	0.149	0.01	51.64	
2013 vs. 2015	0.433	0.001	77.9	
2013 vs. 2016	0.374	0.001	62.67	
2014 vs. 2015	0.28	0.001	73.68	
2014 vs. 2016	0.055	0.097	56.15	
2015 vs. 2016	0.164	0.005	72.66	

Table 2. Temporal variation in environmental factors in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016 (mean \pm SD).

Table 3. The dominant fish species in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016. The dominant species in each year are marked in bold.

			IRI		
Dominant Species	2012	2013	2014	2015	2016
Collichthys lucidus	253.12	533.08	337.46	1.08	327.92
Larimichthys polyactis	391.90	88.95	289.55	2940.67	603.06
Trichiurus japonicus	4437.75	2871.92	3692.76	647.09	5590.32
Setipinna taty	1956.62	866.09	3269.56	207.99	672.35
Pampus argenteus	108.01	116.63	579.06	427.41	929.86
Harpadon nehereus	10,420.92	14,588.79	10,384.48	8580.33	9031.50

The relative abundance, biomass, and species evenness of fish assemblages from 2012–2016 are reflected by the rank–abundance curves (Figure 4). The longer span on the horizontal axis for the curves in 2012 and 2013 compared to the other years indicates higher species richness. Both the abundance and biomass curves have large gradients with steep descending slopes, indicating low evenness and a large proportion of dominant species in total abundance (96.72%) and total biomass (95.88%).

Based on the Monte Carlo tests of the F-ratios (p < 0.05), total suspended particles, depth, and dissolved oxygen are the most significant environmental variables driving the spatiotemporal variation in fish assemblages. The RDA results relate the annual variation in fish assemblages in the Yangtze Estuary to the environmental descriptors considered, such as total suspended particles, depth, and dissolved oxygen (Figure 5a). Total suspended particles and dissolved oxygen are the main environmental factors driving the interannual variation in fish assemblages. According to the results of the ANOSIM and SIMPER analyses, fish assemblages in 2015 are significantly different from other years, associated with low total suspended particles and low dissolved oxygen. Fish assemblages in 2012 are in high total suspended particles and high dissolved oxygen positions. Fish assemblages in 2013, 2014, and 2016 are associated with moderate total suspended particles and dissolved oxygen.

The location of species on the RDA diagram indicates their habitat preferences on an environmental gradient [22] (Figure 5b). Most fish are in the medium range of dissolved oxygen and total suspended particles, and a few are in the high or low range of dissolved oxygen and total suspended particles. *Uranoscopus japonicus, Takifugu obscurus,* and *Engraulis japonicus* are more abundant at sites with lower total suspended particles and dissolved oxygen, whereas *Collichthys lucidus, Harpadon nehereus, Coilia nasus,* and *Setipinna taty* are more abundant at sites with higher total suspended particles and dissolved oxygen. The degree of interpretation of the RDA results is shown in Table 4.

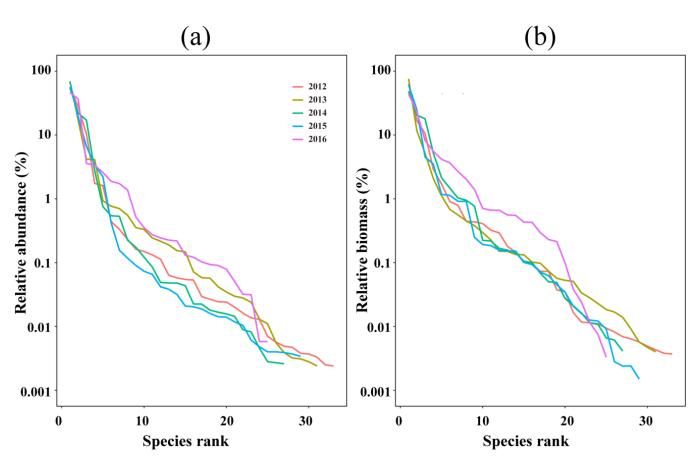


Figure 4. The relative abundance (**a**) and biomass (**b**) of the fish in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016 are reflected in the curve length on the horizontal axes and the evenness of the curve shape.

Table 4. Results of the redundancy analysis relating the fish abundance data to environmental factors
in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016.

Axis	1	2	3	Total Inertia
Eigenvalues	0.1538	0.0182	0.0092	
Species-environment correlations	0.6058	0.2833	0.3755	
Cumulative percentage variance				
of species data	15.38	17.2	18.12	
of species–environment relation	84.89	94.91	100	
Sum of all eigenvalues				1
Sum of all canonical eigenvalues				0.18

Correlations between environmental factors during the autumns of 2012–2016 are demonstrated in Figure 5c, with the strongest positive correlations between temperature and salinity. The correlations between total suspended particles and dissolved oxygen, the main environmental factors driving temporal variation in fish assemblages, are not strong. Unlike Figure 5a, which shows the driving environmental factors that cause interannual variation in fish assemblages, Figure 5c shows the relationship between a particular fish assemblage itself and environmental factors. Among them, depth, dissolved oxygen, and pH are significantly correlated with the annual fish assemblage.

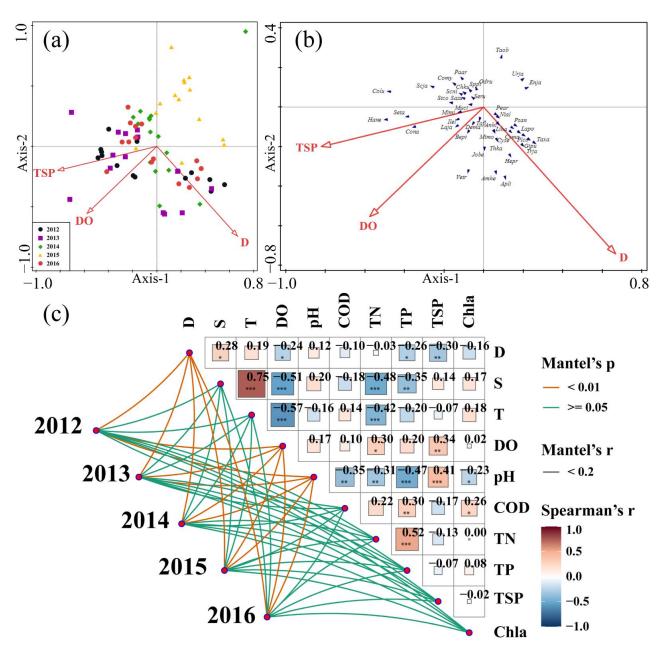


Figure 5. RDA plots of fish assemblages (**a**) and species (**b**) associated with environmental variables and correlations between fish assemblages and environmental factors (**c**) in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016, *, **, *** indicates different degrees of correlations. *Colu: Collichthys lucidus; Lapo: Larimichthys polyactis; Sppi: Sphyraena pinguis; Scni: Scomberomorus niphonius; Trja: Trichiurus japonicus; Seta: Setipinna taty; Chku: Chelidonichthys kumu; Paar: Pampus argenteus; Hane: Harpadon nehereus; Cona: Coilia nasus; Liha: Liza haematocheila; Laja: Lateolabrax japonicus; Mimo: Minous monodactylus; Sazu: Sardinella zunas; Jobe: Johnius belangerii; Muci: Muraenesox cinereus; Dema: Decapterus maruadsi; Tafl: Takifugu flavidus; Cyse: Cynoglossus semilaevis; <i>Psan: Psenopsis anomala; Comy: Coilia mystus; Thka: Thryssa kammalensis; Anhi: Antennarius hispidus; Taxa: Takifugu xanthopterus; Apli: Apogonichthys lineatus; Enja: Engraulis japonicus; Pear: Pennahia argentata; Hepr: Hemisalanx prognathus; Plco: Pleuronichthys cornutus; Mimi: Miichthys miiuy; Stco: Stolephorus commersonii; Bept: Benthosema pterotum; Scja: Scomber japonicus; Vetr: Vespicula trachinoides; Seru: Secutor ruconius; Cyma: Cynoglossus macrolepidotus; Taob: Takifugu obscurus; Odru: Odontamblyopus ubicundus; Nial: Nibea albiflora. The environment factor code is shown in Table 1.*

3.4. Spatial Characteristics of the Fish Assemblages

Due to the large environmental gradient in the offshore waters of the Yangtze Estuary, there are significant spatial differences in the abundance, biomass, and assemblage structure of fish assemblages in the autumns of 2012–2016 (Figure S1). Among the environmental factors affecting the structure of fish assemblages, total suspended particles and dissolved oxygen drive the interannual variation of fish assemblages, while depth drives their spatial structure variation. Based on the position of the fish assemblages on the RDA ordination plots (Figure 6a), the fish assemblages are separated into two groups, corresponding to shallow assemblage and deep assemblage, and the corresponding spatial distribution locations are shown in Figure 6c. The ANOSIM analysis reveals significant differences in the shallow and deep fish assemblages are 43.82% and 32.48%, respectively, while the average dissimilarity between them is 64.89% (indicating that the fish assemblages differ between the areas).

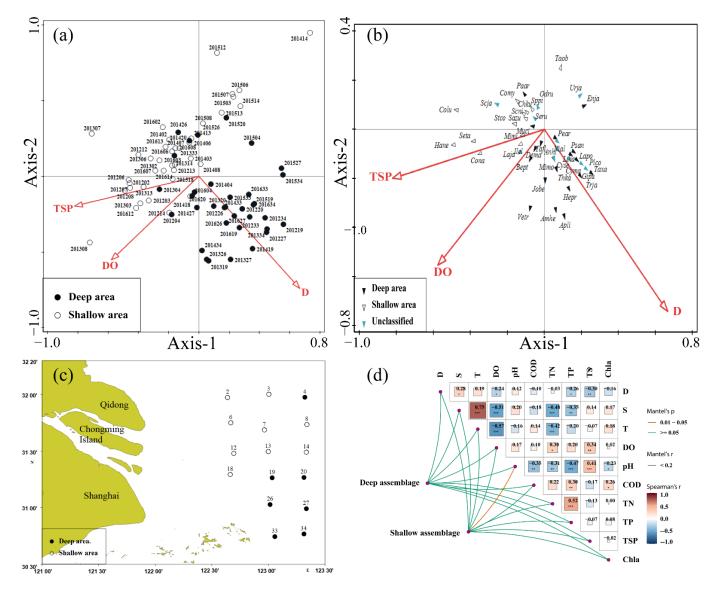


Figure 6. RDA ordination plots depicting correlations between fish assemblages (**a**), fish species (**b**), and key environmental factors, and the distribution of different assemblages (**c**) and the correlations between the fish assemblages and environmental factors (**d**) in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016, *, **, *** indicates different degrees of correlations.

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According to the contribution of the different fish species to the two assemblages, the fish species can be divided into deep and shallow typical species (Table 5). The RDA analysis of the two groups of fish and environmental factors shows that most of the typical deep assemblage fish species are mainly located in the bottom-right of the plot, which is positively correlated with depth, and the typical shallow assemblage fish species are mainly located in the top-left of the plot, which shows a negative correlation with depth (Figure 6c). Figure 6d shows the relationship between the deep and shallow fish assemblages themselves and environmental factors. Among them, the shallow fish assemblage is significantly correlated with pH.

Table 5. Typical species (contribution of more than 5%) as determined by SIMPER in the different

waters adjacent to the Yangtze Estuary during the autumns of 2012–2016.

Contribution Percentage for Average Similarity (%) **Typical Species** Deep Area Shallow Area Trichiurus japonicus 36.47 16.65 Harpadon nehereus 30.96 52.27 Setipinna taty 9.72 17.36 Larimichthys polyactis 8.63 2.91Apogonichthys lineatus 5.13 0.14

Among all environmental variables, the depth and total suspended particles are significantly different between shallow and deep areas, and the total suspended particles are significantly higher in the shallow waters (F = 6.137, p < 0.05) (Table 6). There are no significant differences in the remaining environmental factors between shallow and deep areas.

Table 6. Mean value and range of the environmental factors in the different offshore waters of the Yangtze Estuary during the autumns of 2012–2016.

Environmental Factors	Deep	Area	Shallow Area		
	Mean \pm SD	Range	Mean \pm SD	Range	
Depth (D)	$47.97\pm9.15~^{\rm a}$	30–62	32.95 ± 3.23 ^b	23–41	
Salinity (S)	32.04 ± 2.37	24.93-33.82	31.85 ± 1.92	23.72-33.93	
Temperature (T)	21.16 ± 1.05	18.92-22.94	20.62 ± 0.90	18.60-22.04	
Dissolved oxygen (DO)	7.13 ± 0.56	6.24-8.86	7.10 ± 0.94	3.43-8.57	
pH	8.18 ± 0.28	7.46-8.61	8.20 ± 0.23	7.56-8.58	
Chemical oxygen demand (COD)	1.12 ± 0.70	0.26-3.66	1.01 ± 0.57	0.34-3.40	
Total nitrogen (TN)	17.60 ± 14.02	2.16-66.43	16.75 ± 11.21	3.06-45.55	
Total phosphorus (TP)	0.77 ± 0.34	0.27-1.65	0.93 ± 0.35	0.46-2.04	
Total suspended particles (TSP)	3.22 ± 2.47 ^a	0.26-9.90	8.59 ± 11.37 ^b	0.51-71.56	
Chlorophyll a (Chl a)	0.38 ± 0.36	0.01-1.79	0.40 ± 0.31	0.02-1.59	

Values with different letters (a-b) indicate significant differences between deep area and shallow area.

4. Discussion

4.1. Composition of the Fish Assemblages

In the present study, a total of 64 fish species were caught in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016, which is clearly at a low level compared to the autumns of 2002–2005 [17]. The number of fish species caught in our study also decreases from 41 in 2012 to 29 in 2016. This is closely related to the effects of high-intensity fishing and changes in the offshore marine environment on fish spawning and bait grounds in the last decade [7,23]. The ichthyoplankton assemblage in the Yangtze Estuary has also declined from 94 species in the 1980s to 26 species in 2013–2020, and the replenishment capacity of the fishery resources has decreased substantially [24,25]. Tropical and subtropical estuaries have higher species richness than temperate systems [26].

number of fish species in the waters of the Pearl River Estuary is much greater than in the waters of the Yangtze River estuary [27]. In Zuari, a well-mixed tropical estuary located along the southwest coast of India, the number of fish species can reach 224 in the same period [28].

Combinations of a large number of rare species and a few highly abundant species are common features of temperate estuarine faunae [29,30]. Due to the mixed water masses in the Yangtze Estuary, not all of the species are adapted to the local conditions [31]. The sensu stricto freshwater and marine species do not occur in the offshore waters of the Yangtze Estuary or occur in small numbers at very few stations for only a short period of time. For example, the Yangtze River's abundant freshwater fish such as *Mylopharyngodon piceus* and *Ctenopharyngodon idella* do not appear in the offshore waters of the Yangtze Estuary, while Johnius distinctus, which is a marine fish, only occurs in two stations in 2015 [32]. In the present study, only six of the species caught in the Yangtze Estuary dominate with IRI values > 500. The dominant species account for a significant proportion of total abundance (96.72%) and total biomass (95.88%), and species homogeneity is low. In comparison with the autumn fish in the Yangtze River estuary from 2002 to 2005, the dominant species do not differ much, but the dominance of the traditionally dominant species decreases, and the species with absolute dominance changes from *Trichiurus japonicus* to *Harpadon nehereus* [17]. The structure of fishery resources in the Yangtze Estuary has changed significantly, with the decline in traditional fishery resources and the miniaturization of fish [33]. This phenomenon is manifested in the offshore waters of the Yangtze Estuary by the decline in traditional large economic species resources and the gradual dominance of small secondary economic species fish [34]. The protection and restoration of fishery resources urgently need to be strengthened.

4.2. Annual Variation in Fish Assemblages

Estuaries are highly dynamic ecosystems with complex physical, chemical, and hydrological conditions that change dramatically over time [26,35,36]. In this study, all environmental factors except depth exhibit significant interannual differences, which further influences the abundance, biomass, and composition structure of the fish communities. In the autumns of 2002–2005, water temperature and salinity were the main environmental factors causing the interannual variation of fish in the Yangtze Estuary [17]. In the spring of 2004 and 2007, the temperature was still the main environmental factor driving fish community variation in the Yangtze Estuary [1]. In addition to the Yangtze Estuary, in a small macrotidal estuary (the Canche, France), temperature, salinity, and Crangon crangon (a potential predator for young-of-the-year fish or prey for older ones) were the three most important factors influencing fish species richness and abundance [37]. Temperature and salinity have relatively strong effects on the temporal variation of most estuarine fish, but there are exceptions. In a sample survey of the subtidal fish assemblage of the Tagus estuary coastal area, depth and sediment type were the main structural factors in the fish assemblage. Temperature and salinity were less important to the overall assemblage structure, although this may be due to the particular climatic regime of the sampling year [38]. In this survey, due to the fixed sampling season, temperature and salinity show little difference in the autumn and do not become the main influencing factors. Total suspended particles and dissolved oxygen are the main environmental driving factors for the temporal variation of fish assemblage structure in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016, and the correlation between them is not strong.

During the spring and fall of 1998–2001 and the autumn of 2001, there was no significant intervention effect of total suspended particles on the variations in fish assemblages [15,39]. In this study, total suspended particles are the dominant environmental factor driving fish assemblages in the Yangtze Estuary in the autumn. Total suspended particle concentrations are mainly influenced by benthic sediment resuspension, riverine inputs, upwelling, and plankton [40,41]. Wind direction also plays a significant role in the dispersal of total suspended particle concentrations, with southerly winds favoring the dispersal of high total suspended particle concentrations offshore and easterly winds inhibiting expansion [42]. In 2012–2016, the cumulative effect of Yangtze River input and the diffusion effect of higher wind speeds and southerly winds resulted in the highest level of total suspended particles in 2013 [42]. There was a good log–linear relationship between the total suspended matter concentration and turbidity in the offshore waters of the Yangtze Estuary, and the influencing mechanism on fish distribution was consistent [43]. High turbidity increases the survival rate of estuarine fish by keeping juveniles and small adults away from visual predators and providing more available food [44]. There are many small fish in the turbidity waters, which are an important food for marine carnivorous fish. Carnivorous fish may choose their habitat based on a balance of factors such as the consumption and time of predation in turbidity waters [45]. The changing trend of fish biomass in the autumns of 2012–2016 is basically consistent with the change in total suspended particles, that is, the higher the concentration of total suspended matter, the higher the fish biomass, which is consistent with the above results. In terms of fish assemblage structure, RDA shows that fish such as Harpadon nehereus and Collichthys lucidus are associated with high total suspended matter concentrations. Harpadon nehereus show stronger feeding in the autumn, with the highest stomach fullness coefficient, and, therefore, prefer areas with higher bait organisms [10,31]. In the present study, high total suspended matter concentrations in the Yangtze Estuary in the autumn of 2013 provide more feeding opportunities for *Harpadon nehereus*, and, thus, their abundance and biomass reach their highest values in 2013.

Dissolved oxygen is another main factor driving the interannual variation of fish aggregation in the Yangtze Estuary from 2012 to 2016. Dissolved oxygen content in seawater is an important reference item to determine the biological growth level and seawater pollution level in the sea area [46]. Dissolved oxygen, as a basic condition for the survival of aquatic animals, is an important environmental factor affecting the growth, respiration, material, and energy metabolism of aquatic animals [47]. When the dissolved oxygen concentrations drop to 5.0 mg/L, some fish experience respiratory distress, and when dissolved oxygen levels fall below 2.0 mg/L or below 3.0 mg/L, low-dissolved-oxygen sea zones can form [48]. The hypoxic zone in the Yangtze Estuary is mostly formed in the summer, and the hypoxic phenomenon soon subsides after September, and dissolved oxygen content increases, so there is no hypoxic zone in the autumn [49]. Hajisamae et al. found a significant positive correlation between dissolved oxygen in the water and fish biomass [50]. Keller et al. reported a significant decrease in biomass and species diversity of benthic organisms along a low oxygen gradient [51]. Hypoxia may also reduce growth, reproductive success, and recruitment success [52]. In this study, dissolved oxygen reaches its highest value in 2012 and its lowest value in 2015, with significant differences. There is no positive correlation between dissolved oxygen and fish biomass, which may be influenced by the synergistic effect of other environmental factors and human activities. The different preferences and adaptability of different fish species to dissolved oxygen also affect the species composition of fish in the different years [53–55]. The RDA shows that fish such as Benthosema pterotum, Vespicula trachinoides, Decapterus maruadsi, and Lateolabrax japonicus are associated with high dissolved oxygen levels. Benthosema pterotum and Decapterus maruadsi, as pelagic fish, require higher dissolved oxygen content [10,56]. Lateolabrax japonicus is sensitive to dissolved oxygen, and low oxygen stress can cause significant oxidative damage to the juvenile *Lateolabrax japonicus* organism; therefore, *Lateolabrax japonicus* prefers areas of high dissolved oxygen, which in the Yangtze Estuary only occurred in 2012 when dissolved oxygen was high [57].

In addition to environmental changes, high-intensity fishing is also an important reason for the interannual variation of fish assemblages. With the increase in fishing efforts, fishing intensity far exceeds resource replenishment capacity, and major economic fish in the Yangtze River estuary are now overfished [10]. In this study, fish total abundance decreases year by year after reaching its peak in 2013, and it reaches its lowest value in 2016, which is only 19.25% of the abundance in 2013. Therefore, it is imperative to protect the

ecological environment of the Yangtze Estuary, limit the fishing intensity, and standardize the use of fishing gear.

4.3. Spatial Characteristics of the Fish Assemblages

Coastal areas are important habitats for many marine fish, and there are often large gradients in environmental conditions that result in different species assemblages [58]. Depth is an important variable in estuarine ecosystems and is closely related to the distribution of marine organisms, light intensity, food availability, and temperature [59]. In this study, the range of depths in the Yangtze Estuary is 23–62 m, with large spatial variation driving spatial variation in fish assemblages, but no significant interannual variation in time. This spatial variability is largely a product of the different spatial patterns exhibited by different species [60]. The fish are classified into shallow assemblage and deep assemblage according to depth. The species have their own preferences for the water layers, with 19 species preferring to live in the deeper areas, 13 species preferring shallow areas, and deep assemblage having a higher species diversity.

Depth has historically been an important factor, but it is not the only factor driving spatial variability in the Yangtze Estuary. In this study, total suspended matter also differs significantly in the deep areas and shallow areas, suggesting that total suspended matter drives interannual variation along with spatial variation in fish assemblages. Total suspended matter concentrations are lower in the deep area near the outer sea and higher in the shallow area near the estuary. In 2004 and 2007, depth, salinity, and total suspended matter together drove variation in the spatial structure of spring fish assemblages in the Yangtze Estuary, dividing fish into southern and northern assemblages [1]. The invertebrate assemblage in the Yangtze Estuary was also divided into shallow assemblage and deep assemblage according to depth [61]. In addition to depth, the spatial variation in fish assemblages in the Yangtze Estuary is also driven by other environmental factors at different times. In 2018, the spatial variation in fish in the Yangtze Estuary was driven by salinity, which was divided into high-salt assemblage and low-salt assemblage depending on salinity [18]. With the exception of the Yangtze Estuary, significant spatial variability is an essential feature of most estuaries (except those with high mixing uniformity) [28,62,63]. On the Alaska Beaufort Shelf, significant interactions between depth and along-shelf position helped define six geographic regions [64]. Continuously changing environmental gradients underlie the spatial variability in estuarine fish communities.

Biotic interactions and human activities may act in conjunction with environmental preferences in the spatial selection of fish. Fish select their preference for environmental conditions while also integrating the effects of solicitations, interspecific competition, predator avoidance, and human activities [58]. Flow alterations caused by dam construction, pollution, habitat destruction, and other human activities can all lead to variations in fish biomass and community structure. Therefore, further studies should quantify the importance of biological effects and human activities on the spatial and temporal distribution of fish to generate more reliable assessments of fish assemblage dynamics and facilitate our development of more rational and effective fisheries conservation strategies.

5. Conclusions

We reported on the spatial and temporal variability in fish assemblages in the Yangtze Estuary over five years in the autumn and their relationships with environmental factors. The fish assemblage in the offshore waters of the Yangtze Estuary is characterized by a few highly abundant species and many rare species. The spatial and temporal variability in fish assemblages is significant. Total suspended particles and dissolved oxygen drive interannual variation in fish abundance, biomass, and species composition, and depth drives spatial variation in the fish. Depending on the depth, the fish were classified and divided into shallow assemblage and deep assemblage. It is worth noting that interactions between fish organisms and human activities may also influence species distribution and could be considered further in future studies.

Supplementary Materials: The following supporting information can be downloaded at: https: //www.mdpi.com/article/10.3390/d15050669/s1, Figure S1: Temporal and spatial variation of fish abundance and biomass in the adjacent waters of the Yangtze Estuary during autumn of 2012–2016; Table S1: The depth range, climate zone/temperature range, and distribution of all fish caught in the offshore waters of the Yangtze Estuary during the autumns of 2012–2016.

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