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Assemblage Structure of Ichthyoplankton and Its Relationship with Environmental Factors in Late Summer-Autumn and Winter in the Beibu Gulf, China

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Abstract: Being a biologically diversed hotspot in the global marine ecosystem, the Beibu Gulf is inhabited by a high diversity of fish and serves as a vital fishing ground in China. Due to continuous overfishing, the fishery resource has drastically declined in the Beibu Gulf. However, information about the ichthyoplankton assemblages in this area is still lacking. In this present study, ichthyoplankton diversity, spatial and temporal distribution patterns, and assemblage structures were examined using the specimens collected in the late summer–autumn and winter of 2022 in the Beibu Gulf, and the relationship between ichthyoplankton assemblage and environmental variables was studied. A total of 117 ichthyoplankton taxa, belonging to 13 orders and 42 families, were recorded. The most abundant families were Gerreidae, Leiognathidae, and Sillaginidae in late summer–autumn, accounting for 38.74%, 27.95%, and 9.94%, respectively. Sparidae, Platycephalidae, and Sillaginidae were the most abundant families in winter, accounting for 34.03%, 17.15%, and 8.20%, respectively. Cluster analysis identified five assemblages in late summer-autumn and four assemblages in winter. The most characteristic species in each cluster were *Terapon jarbua*, *Sillago sihama*, *Leiognathus brevirostris*, *Mene maculate*, and *Scomberoides tol* in late summer-autumn and *Scomberomorus commerson*, *Acanthopagrus latus*, *Sillago* sp., and *Evynnis cardinalis* in winter. The results of the canonical correspondence analysis indicated that pH, chlorophyll-a (Chl-a), depth, dissolved oxygen (DO), sea surface salinity (SSS), total nitrogen (TN), and total phosphorus (TP) were the major environmental variables affecting the ichthyoplankton assemblage structure in the Beibu Gulf. The finding of this study will provide valuable information in conserving fish spawning grounds and developing fishery management practices to protect fishery resources in the Beibu Gulf.

Keywords: ichthyoplankton; assemblage structure; spatial and temporal distribution; environmental variables; Beibu Gulf

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1. Introduction

Knowledge regarding the spatial and temporal distribution patterns of ichthyoplankton assemblage structures is vital to infer the location of spawning sites and develop fishery management strategies to protect them [1–3]. Ichthyoplankton commonly refers to fish eggs and larvae stages. The assemblage structures of these stages are affected and regulated by the biological and physical processes, and the spatial and temporal distribution patterns are vital in detecting spawning sites [4,5]. Ichthyoplankton assemblage distribution can also be correlated with the spawning phases of adult fishes and biotic/abiotic factors, the conditions suitable for adult reproductive activities, and the mechanisms affecting early planktonic stages can be identified [6,7].

The Beibu Gulf can be described as a semi-enclosed bay situated in the northwestern South China Sea (17°00′–21°75′ N, 105°67′–110°17′ E), covering an area of 1.28×10^5 km^2 with an average depth of 38 m. It is surrounded by Vietnam in the west, China's Guangxi Zhuang Autonomous Region in the north, and Leizhou Peninsular and Hainan Island in the east. With unique climate and geographical conditions, the Beibu Gulf waters are inhabited by 1519 fish species, with Perciformes being the order comprising the highest number (907) species [8]. This region is one of the four principal Chinese ocean fishing grounds due to the abundant fishery resources. However, due to long-term high-intensity trawling operations, the fishery resources have seriously declined, and the fishery catch structure was characterized by a reduction in the fish species diversity, following the abundance of high-value and large-size economic fish species, and low economic value, low trophic level, small size, and low-graded bait fishes [9–13]. Therefore, it is necessary to investigate the spatial–temporal patterns of the ichthyoplankton assemblage and detect the relationship between environmental variables and the ichthyoplankton.

Numerous studies concerning ichthyoplankton assemblage structures have been conducted in the Beibu Gulf [14–16]. The earliest studies took place in 1959–1962 and 1997–1999 [14]. Lin et al. (2004) [14] also described the diversity of fish eggs and larvae in autumn and winter surveys using the samples from 2001 to 2002. Zhou et al. (2008) [15] reported the abundance of fish eggs and larvae in summer, and Zhou et al. (2011) [16] studied the taxonomic composition, abundance, and distribution of the ichthyoplankton and their relationships with environmental factors such as surface temperature, salinity, and chlorophyll a (Chl-a), during four cruises from 2006 to 2007. Hou et al. (2021) [17] and Hou et al. (2022) [18] molecularly identified 53 species of formalin-fixed fish larvae from 2013 to 2017 and 32 eggs of fish species, respectively. However, due to the difficulties in identifying ichthyoplankton, especially fish eggs, information on the location of spawning grounds of fishes is still limited.

In this study, we set for two ichthyoplankton cruises in late summer-autumn and winter 2022. Our objectives were to (i) describe the characteristics of ichthyoplankton assemblage and (ii) reveal relationships between the spatial and temporal distribution patterns of ichthyoplankton assemblage structure and environmental variables. The results can provide a view on ichthyoplankton from a scientific basis for the better protection and management of the fishery resources in the Beibu Gulf.

2. Materials and Methods

2.1. Study Area and Sample Collection

Two surveys were conducted at 55 stations in late summer-autumn (August-September) and 60 stations in winter (December) of 2022 in the waters of the Beibu Gulf (Figure 1). The water depth of sampling stations was from 10 to 136 m, with the minimum depth occurring at station 5 and the maximum depth at station 55. Ichthyoplankton samples were collected using zooplankton nets, with a length of 80 cm in diameter for the net mouth, 2.7 m long and 505 µm for the mesh, and 400 µm for the cod-end container mesh size, and horizontally hauled for 10 min at nearly 2.0 knots. Specimens were then immediately preserved in a 5% formalin solution. The investigation was under the guidance of 'Specification of Oceanographic Investigation' [19].

A plexiglass water sampler was used to the collect surface seawater samples during plankton sampling at each station. Sea surface temperature (SST), sea surface salinity (SSS), pH, and dissolved oxygen (DO) were measured using a YSI professional plus handheld multi-parameter water quality analyzer (YSIINC, Ohio USA) [20]. The water depth of each station was measured via a Conductivity, Temperature and Depth instrument (CTD). A 50 mL seawater sample was taken from the surface of each station, passed through a 0.45 µm mixed fiber filter membrane that was treated with 1% hydrochloric acid, and transferred to a polyethylene bottle treated with 1% hydrochloric acid prior to sampling. All samples were taken in triplicate and kept frozen at −20 °C before analysis. The five environmental variables, i.e., total nitrogen (TN), total phosphorus (TP), silicate (SiO_3^{2-}), nitrate (NO_3^-), and nitrite (NO_2^-), were measured using a flow injection auto-analyzer (AA3, BRAN + LUEBBE, Hamburg, Germany) [21]. Chl-a in each sample was analyzed in the ship laboratory via fluorescence spectrophotometry within 24 h. Zooplankton samples were vertically collected by the standard plankton net and were fixed in a formaldehyde solution with a final concentration of 5% on board.

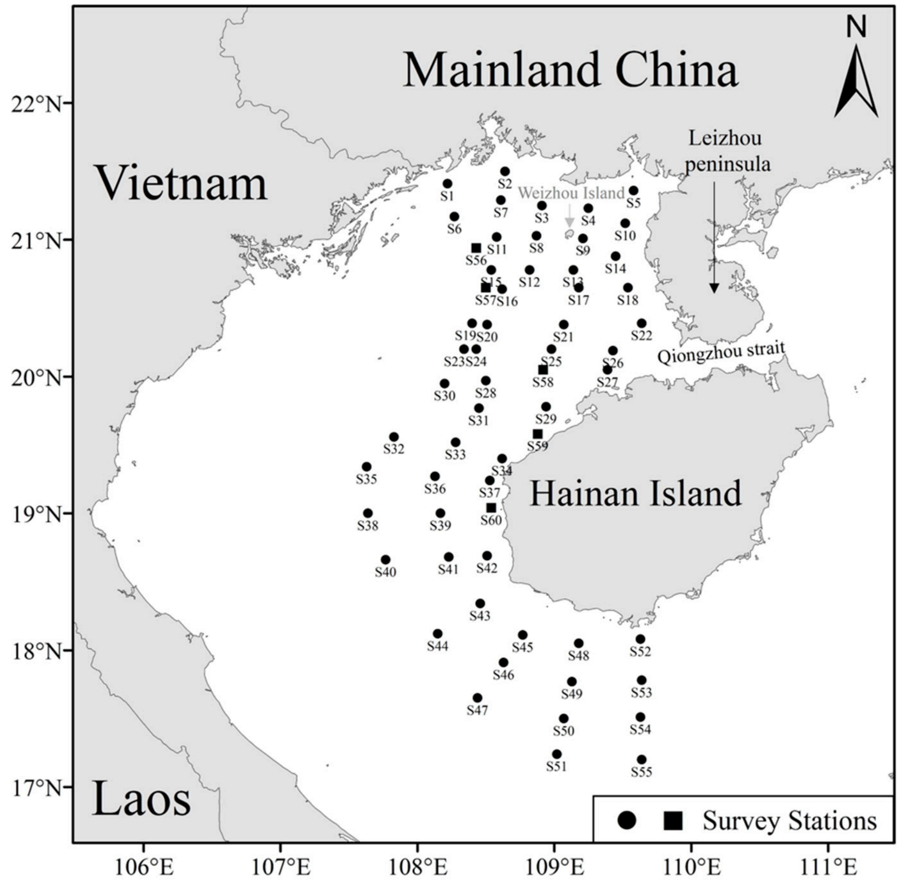

Figure 1. Sampled stations of ichthyoplankton in the Beibu Gulf.

2.2. Data Analysis

In the laboratory, ichthyoplankton specimens were identified to the lowest taxonomic level based on their morphological characteristics [11,18,22,23]. Each ichthyoplankton taxon was assigned to an ecotype according to its habitat type based on the fishbase platform (https://fishbase.mnhn.fr/search.php; accessed on 13 March 2023). Species density was calculated as the abundance per 100 m^3 for each station. The index of relative importance (*IRI*) was used to determine the dominant taxa of ichthyoplankton [24], estimated as follows:

$$IRI = N\% \times F\% \times 10{,}000$$

where $N\%$ is the relative abundance of the total catch, and $F\%$ is the occurrence frequency. Each taxon was defined based on IRI as a dominant species ($IRI \geq 100$), common species ($10 \leq IRI < 100$), or rare species ($IRI < 10$).

A one-way ANOVA test was conducted to analyze the significance between seasonal changes and the environmental variables. The species/taxa accounting for IRI index of less than 10 were then used in each seasonal assemblage structure analysis by standardizing the data with a log(x + 1) transformation to enhance the weighting of rare species. [25], then clustered using the Bray–Curtis similarity and Non-metric Multi-Dimensional Scaling (NMDS; Primer Software, Version 7.0). The differences within the clusters of each season were examined via nonparametric multivariate analysis (ANOSIM) [26]. Similarity percentage

(SIMPER) analysis was used to calculate the contribution of each ichthyoplankton taxon to the Bray–Curtis similarity within clusters, and dissimilarity among clusters [26]. Canonical correspondence analysis (CCA) was applied to explain relationships between environmental variables and ichthyoplankton assemblages (CCA, CANOCO Software, Version 5.0). The rare species were down-weighted. The statistical significance of environmental variables was tested, by permutation test using a Monte Carlo approach (999 permutations) [27] after a forward selection. The variation inflation factor (VIF) was calculated to detect if collinearity existed between the independent environmental variables, with an upper cutoff value of 4 [28]. The CCA ordination diagrams were plotted to analyze the associations between the environmental variables and sampling stations/ichthyoplankton taxa.

3. Results

3.1. Environmental Factors

In this study, SST showed a significant decrease (F = 577.65, $p < 0.05$) from late summer-autumn to winter in 2022. The pH values showed a significant downward trend (F = 146.30, $p < 0.05$), while Chl-a (F = 41.69, $p < 0.05$), DO (F = 102.21, $p < 0.05$), SST (F = 101.12, $p < 0.05$), NO_2^- (F = 24.91, $p < 0.05$), and NO_3^- (F = 19.96, $p < 0.05$) showed significant upward trends (Table 1).

Table 1. Temporal variation of environmental variables in late summer-autumn and winter in the Beibu Gulf.

Factors	Unit	Late Summer-Autumn	Winner
pH		8.30 ± 0.07^{a}	8.13 ± 0.08^{b}
Chlorophyll a (Chl-a)	μg/L	0.67 ± 0.67^{a}	1.79 ± 1.06^{b}
Dissolved oxygen (DO)	mg/L	6.52 ± 0.48^{a}	7.27 ± 0.28^{b}
Sea Surface Salinity (SSS)		30.84 ± 2.66^{a}	32.80 ± 0.93^{b}
Sea Surface Temperature (SST)	°C	30.69 ± 0.71^{a}	22.16 ± 2.29^{b}
Total Nitrogen (TN)	μmol/L	5.74 ± 2.41^{a}	7.70 ± 4.52^{a}
Total Phosphorus (TP)	μmol/L	0.30 ± 0.15^{a}	0.22 ± 0.22^{a}
NO_2^-	μmol/L	0.10 ± 0.16^{a}	0.44 ± 0.47^{b}
NO_3^-	μmol/L	0.52 ± 0.97^{a}	1.85 ± 1.96^{b}
SiO_3^{2-}	μmol/L	36.22 ± 25.33^{a}	27.50 ± 14.57^{a}
Zooplankton	ind/m^3	63.44 ± 135.63^{a}	31.31 ± 33.74^{a}

Note: Environment factors: mean ± SD, n = 55 stations (late summer-autumn), n = 60 stations (winter); values with different letters (a, b) indicate a significant difference at $p < 0.05$ among cruises.

3.2. Species Composition

A total of 20,907 individuals were collected, including 17,345 individuals from the late summer-autumn cruise and 3562 from the winter cruise. The samples were classified into 117 taxa, comprising 13 orders and 40 families, with 75 taxa identified to species level. In addition, the damaged specimens that could not be identified were categorized as unidentified taxa. The family Carangidae represented the greatest number of taxa (8), followed by Leiognathidae (7), Gobiidae (7), Engraulidae (6), Sciaenidae (6), Muraenidae (5), Trichiuridae (5), and Cynoglossidae (5) (Table S1) in the two surveys. Particularly noteworthy are the families Carangidae, Trichiuridae, and Sciaenidae, in which genera *Decapterus*, *Trichiurus*, and *Pennahia* contain the economically important fishes of the marine fishery catch in the Beibu Gulf. The most abundant families were Gerreidae, Leiognathidae, and Sillaginidae in late summer-autumn, accounting for 38.76%, 27.95%, and 9.94%, respectively (Table 2).

Table 2. The dominant and common ichthyoplankton taxa in IRI.

Species	Late Summer-Autumn		Winter	
	IRI	Percentage of Total (%)	IRI	Percentage of Total (%)
Gerres limbatus	634.33	38.76		
Leiognathus ruconius	454.68	13.16		
Sillago sihama	302.81	9.80	30.42	1.30
Leiognathus equulus	245.38	12.27		
Mene maculata	72.96	2.51	21.82	2.18
Sardinella gibbosa	71.34	3.92		
Encrasicholina heteroloba	22.77	4.18		
Leiognathus brevirostris	20.87	1.91		
Stolephorus commersonnii	20.45	2.25		
Pristigenys niphonia	14.60	0.80		
Scomberoides tol	14.16	0.87		
Acentrogobius sp.	10.28	0.81		
Terapon jarbua	10.05	0.46	14.21	0.95
Acanthopagrus latus			1255.97	19.83
Nematalosa japonica			649.12	16.23
Evynnis cardinalis			445.76	12.74
Sillago sp.			187.44	6.25
Trichiurus sp.1			64.39	2.58
Acropoma japonicum			63.19	2.53
Platycephalus cultellatus			59.63	2.56
Callionymus curvicornis			28.10	2.11
Alepes kleinii			26.29	2.63
Inegocia japonica			15.61	0.85
Psenopsis anomala			15.29	1.53
Photopectoralis bindus			14.11	1.41
Bregmaceros sp.			12.96	1.11
Scomberomorus commerson			12.82	0.96
Champsodon atridorsalis			12.71	0.69
Encrasicholina punctifer			12.32	1.23
Johnius macrorhynus			12.18	0.91
Acanthopagrus schlegelii			11.28	0.97
Branchiostegus albus			10.74	1.29
Parapercis lutevittata			10.50	1.26

Note: Trichiurus sp.1 indicated this is one of the two taxa in Genus Trichiurus.

Sparidae, Platycephalidae, and Sillaginidae were the most abundant families in winter, accounting for 34.03%, 17.15%, and 8.20%, respectively (Table S1). Thirty-seven taxa occurred only in late summer-autumn, fifty-one taxa occurred only occurred in winter, and twenty-nine taxa occurred in both surveys. Among these taxa, *Gerres limbatus* Cuvier, 1830, *Leiognathus ruconius* (Hamilton, 1822), *Sillago sihama* (Forsskål, 1775), and *Leiognathus equula* (Forsskål, 1775) were the dominant species in late summer-autumn, while *Acanthopagrus latus* (Houttuyn, 1782), *Escualosa thoracata* (Valenciennes, 1847), *Evynnis cardinalis* (Lacepède, 1802), and *Sillago* sp. were dominant in winter. According to the habitats for adult fish species, the ichthyoplankton samples were categorized into six ecotypes at the species level: 34, 10, 18, 10, 2, and 1. Moreover, the species were categorized as demersal, pelagic-neritic, reef associated, benthopelagic, bathypelagic, and pelagic-oceanic, respectively (Table S1).

3.3. Spatial-Temporal Variation in Late Summer-Autumn and Winter

The mean total abundance of ichthyoplankton was 253.07 ind./100 m^3 in late summer-autumn cruise, with a range of 0–5, 257.08 ind./100 m^3, and the mean abundance was 25.11 ind./100 m^3 in the winter cruise, with a range of 0–285.42 ind./100 m^3.

In late summer-autumn, the spatial distribution pattern of ichthyoplankton showed a general trend of higher abundances in the north and lower abundances in the south, and the abundance decreased significantly with increasing water depths and decreasing latitudes ($p < 0.05$). The abundance of ichthyoplankton was concentrated in three sea areas of the Beibu Gulf and scattered in the other sea areas (Figure 2). The first concentrated area was mainly in the northern waters of the Beibu Gulf around Weizhou islands, with the three most abundant stations being S4 (5257.08 ind./100 m^3), S3 (1415.58 ind./100 m^3), and S9 (1101.47 ind./100 m^3), respectively. The second concentrated area was mainly in the northwest corner of Hainan Island near the Qiongzhou Strait and the LinGao coral reef, with station S27 (1745.60 ind./100 m^3) having the highest abundance in the Beibu Gulf. The third area was mainly concentrated in the southwest of Hainan Island from Dongfang to Yinggehai sea areas, near the mouth of the Beibu Gulf, with the two most abundant stations being S41 (500.87 ind./100 m^3) and S45 (185.80 ind./100 m^3), respectively (Figure 2). For stations in other areas, the abundance values were below 100 ind./100 m^3 except for two stations which had zero abundance.

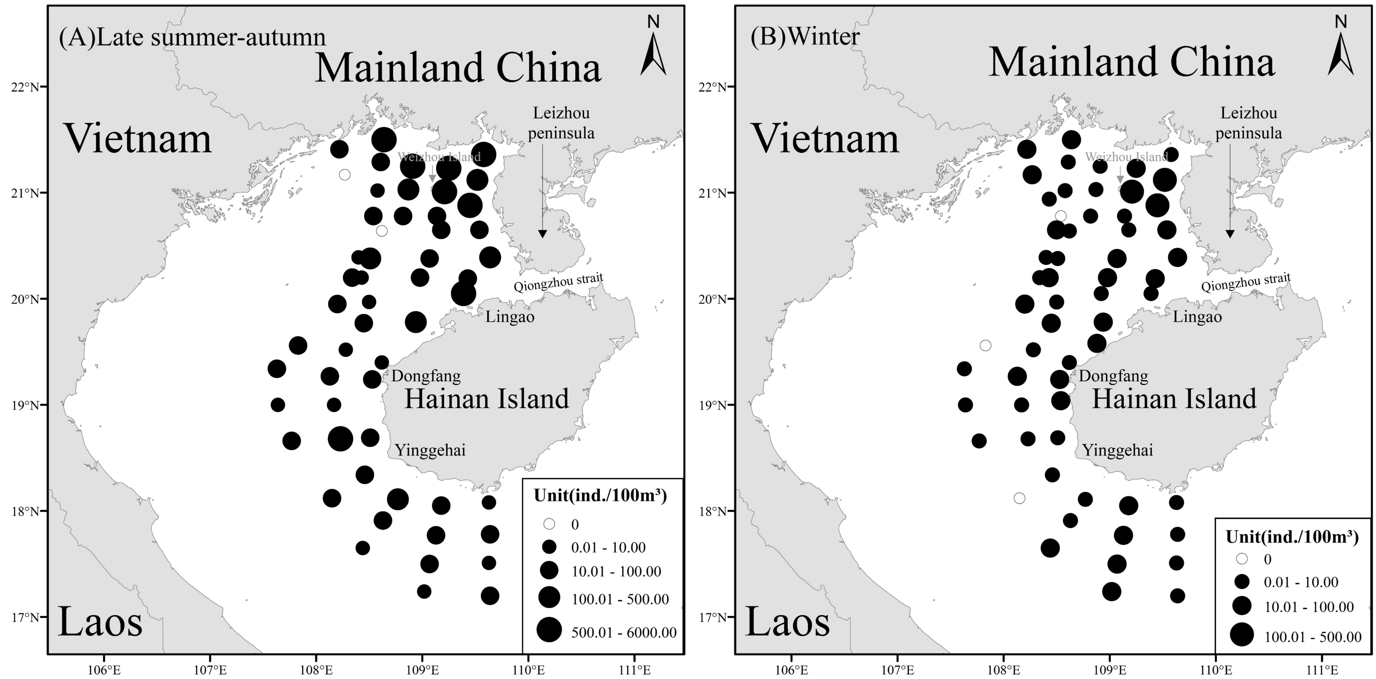

Figure 2. Spatial and temporal distribution of ichthyoplankton abundance in late summer-autumn (**A**) and winter (**B**).

In winter, the spatial distributional pattern of ichthyoplankton showed a lower density without an obvious aggregation area (Figure 2), which indicated that the survey season was not the main spawning period. The abundances of the following three stations were more than 100 ind./100 m^3: S14 (285.42 ind./100 m^3), S10 (243.51 ind./100 m^3), and S9 (171.92 ind./100 m^3). The three stations were all located at the east of the Weizhou Island and west of Leizhou Peninsula. For the other stations, the abundance values were below 100 ind./100 m^3 except for two stations which had zero abundance.

In this study, the stress coefficients of the NMDS analysis were 0.17 and 0.17 in the late summer-autumn and winter surveys, which indicated that the discrimination between groups was not significant. The ANOSIM test indicated that there was significant difference among ichthyoplankton clusters in late summer-autumn, except for groups a and c ($R > 0.49$, $p > 0.05$), and winter (ANOSIM, $R > 0.58$, $p < 0.05$). SIMPER analysis also indicated that the average dissimilarities between clusters in the two cruises were high (average dissimilarity $\geq$ 81.09%, Table 3). Moreover, the SIMPER analysis indicated that the most dominant characteristic taxa in each cluster were *Terapon jarbua* (Forsskål, 1775), *S. sihama*, *Leiognathus brevirostris* (Valenciennes, 1835), *Mene maculate* (Bloch & Schneider, 1801), and *Scomberoides tol* (Cuvier, 1832) in late summer-autumn and *Scomberomorus commerson* (Lacepède, 1800), *A. latus*, *Sillago* sp., and *E. cardinalis* in winter (Table S2). In late summer-autumn, five clusters were formed comprising 13 categories of ichthyoplankton

(Figure 3A,C). In winter, four clusters accounted for 23 categories of ichthyoplankton (Figure 3B,D).

Table 3. The comparison of the assemblage structure according to one-way ANOSIM and SIMPER analysis.

		ANOSIM		SIMPER
Seasons	**Groups**	**R**	***p***	**Average Dissimilarity %**
Late summer-autumn	Global	0.681	0.001	
	a & b	0.729	0.003	94.01
	a & c	1.000	0.100	100.00
	a & d	0.757	0.001	92.75
	a & e	0.778	0.012	94.30
	b & c	0.731	0.006	94.08
	b & d	0.731	0.001	93.37
	b & e	0.495	0.001	84.59
	c & d	0.659	0.005	88.75
	c & e	0.656	0.036	92.13
	d & e	0.610	0.001	83.79
Winter	Global	0.717	0.001	
	a & b	0.884	0.003	97.62
	a & c	1.000	0.036	94.94
	a & d	0.920	0.003	95.75
	b & c	0.586	0.001	83.9
	b & d	0.724	0.001	85.09
	c & d	0.708	0.001	81.09

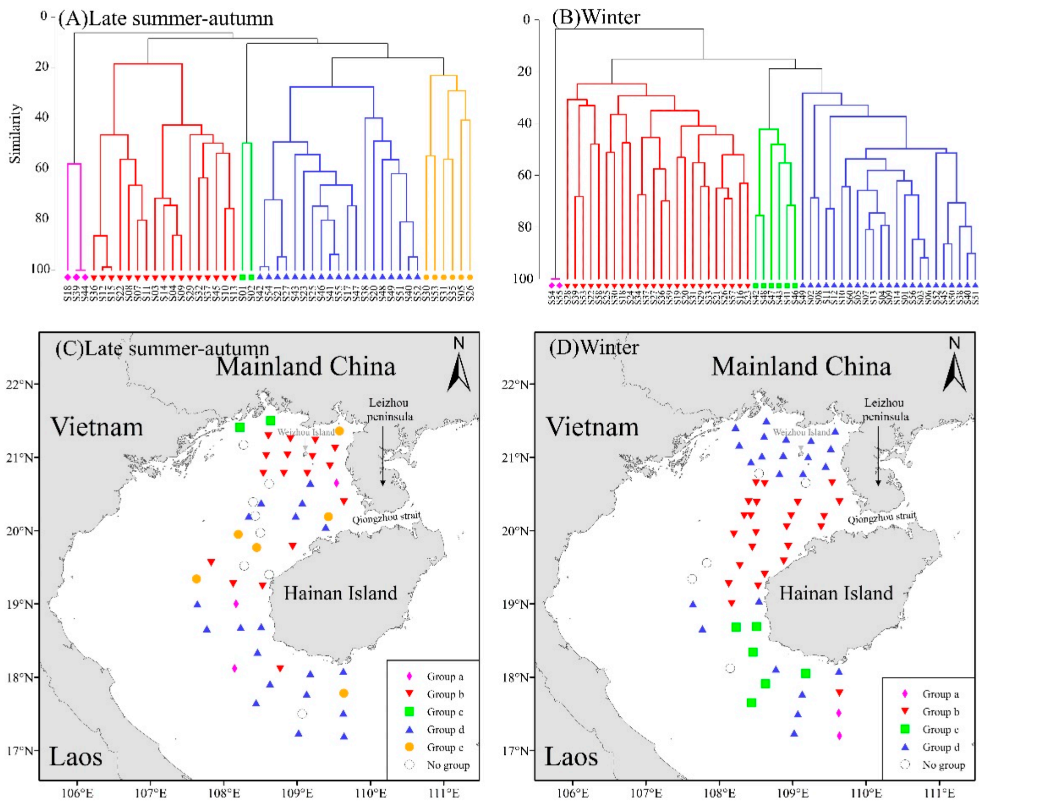

Figure 3. Group average clustering and spatial pattern in late summer-autumn (**A**,**C**) and winter (**B**,**D**).

3.4. Relationships of Assemblage Structure to Environmental Factors

In this study, VIF tests indicated that sea surface temperature (SST) showed multicollinearity with the other environmental variables (VIF = 5.39). Hence, we excluded it from the subsequent CCA analysis. The results of Monte Carlo permutation tests showed that four environmental variables (SiO_3^{2-}, NO_3^-, NO_2^-, and zooplankton) were excluded ($p > 0.05$), and seven environmental variables significantly contributed to explain the assemblage structures of ichthyoplankton, whereby pH, Chl-a, and depth were the top three key environmental factors that affected the ichthyoplankton assemblage structures ($p < 0.05$, Table 4).

Table 4. Explanation and contribution of environmental variables in the canonical correspondence analysis.

Variables	Explanation %	Contribution %	Pseudo-*F*	*p*
pH	7.5	35.3	8.1	0.001
Chl-a	3.6	16.9	4.0	0.001
Dep	2.5	11.5	2.8	0.001
DO	2.3	10.9	2.7	0.001
SSS	2.0	9.4	2.3	0.001
TN	1.8	8.4	2.1	0.002
TP	1.6	7.5	1.9	0.005

In the CCA analysis, the eigenvalues of CCA1, CCA2, CCA3, and CCA4 were 0.737, 0.312, 0.229, and 0.164, respectively. In short, CCA1 axis accounted for 44.24% of the variance in the relationship between environment variables and species with a correlation efficiency of 0.907. CCA2 axis accounted for 18.74% of the variance in the relationship between environment variables and species, and with a correlation efficiency of 0.801 (Table 5).

Table 5. Results of CCA based on ichthyoplankton abundance.

Axes	CCA Axes				Total Variance
	1	2	3	4	
Eigenvalues	0.737	0.312	0.229	0.164	7.815
Explained variation (cumulative)	9.42	13.42	16.35	18.46	
Pseudo-canonical correlation	0.907	0.801	0.633	0.595	
Explained fitted variation (cumulative)	44.24	62.98	76.76	86.63	
Sum of all eigenvalues					1.665

The sum of all eigenvalues (1.665) accounted for 21.31% of the eigenvalues (7.815), which indicated that the restrictive effect existed in establishing the environmental relationships in CCA. In this study, the results of the first two axes of the plot were used to explain the relationships between species and environmental variables. The first axis was positively correlated with DO, negatively correlated with pH, and distinguished most of the sampling stations in late summer-autumn from the stations in winter. The second axis was negatively correlated with depth, distinguishing the sampling stations of the northern waters from the southern waters of the Beibu Gulf (Figure 4A). Correlations between ichthyoplankton taxa and environmental variables were plotted (Figure 4B). In this study, the ichthyoplankton taxa did not distribute near the place of origin in the CCA analysis (Figure 4B). Most ichthyoplankton taxa in late summer-autumn were negatively correlated with pH and TP. *Acanthopagrus schlegelii* (Acsc), *A. latus* (Acsc), *Alepes kleinii* (Alkl), and *Evynnis cardinalis* (Evca) were positively associated with DO, and *Photopectoralis bindus* (Phbi), *Callionymus curvicornis* (Cacu), and *Nematalosa japonica* (Neja) were positively correlated with Chl-a in winter. Other ichthyoplankton taxa, i.e., *Champsodon atridorsalis* (Chat), *Parapercis lutevittata*

(Palu), etc., were positively associated with TN and SSS in winter (Figure 4B). In addition, some outlier species, e.g., *L. brevirostris* and *S. commersonnii*, were separated from other species along the second axis, showing a negative correlation with depth (Figure 4B).

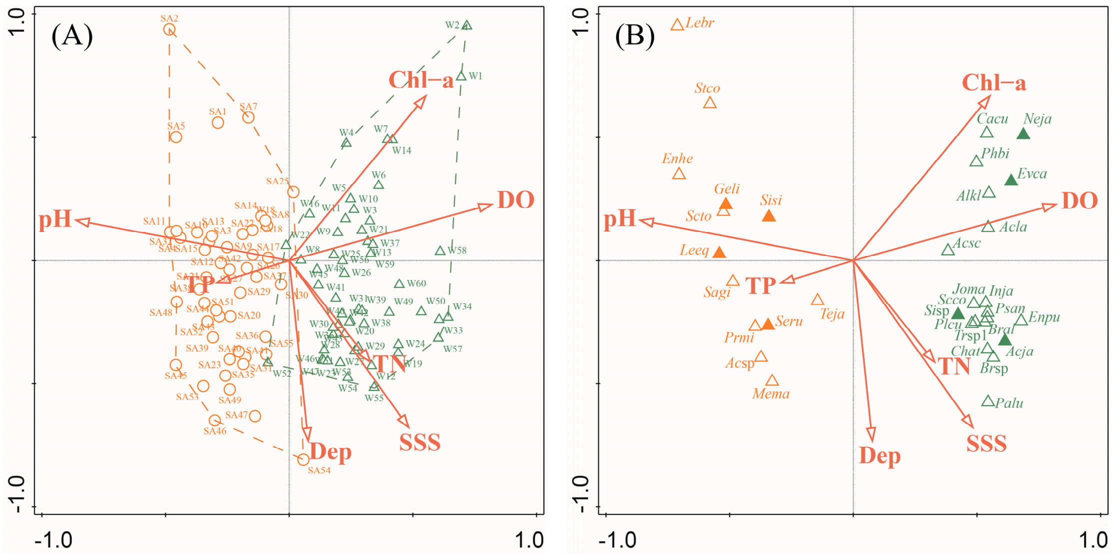

Figure 4. Canonical correspondence analysis with biplot of sampled stations (**A**) and species (**B**).

4. Discussion

4.1. Variations of Ichthyoplankton Composition

In the last two decades, some papers have reported on the diversity of ichthyoplankton in the Beibu Gulf. For instance, Lin et al. (2004) [14] reported 22 taxa in autumn and 16 taxa in winter (24 taxa overall) based on two cruises from 2001 to 2002. Among them, Sparidae and Carangidae were the most abundant for fish eggs, with an abundance percentage of 41.0% and 29.3%, respectively. *Stolephorus* sp. was the most abundant in fish larvae, at 56.1%. The study of Zhou et al. (2011) [16] reported 153 taxa based on four seasonal cruises from 2006 to 2007. Carangidae, Clupeidae, Sciaenidae, Engraulidae, Leiognathidae, and Nemipteridae were the most abundant in fish eggs, comprising 19.3%, 7.59%, 7.00%, 6.85%, 6.52%, and 5.15%, respectively. Engraulidae, Gobiidae, Bregmacerotidae, and Leiognathidae were the most abundant in fish larvae, at 33.78%, 8.49%, 7.59%, and 6.21%, respectively [16]. Hou et al. (2022) [29] reported 32 species of fish eggs in two surveys, where Carangidae, Sciaenidae, and Engraulidae accounted for most part of the taxonomic composition and abundance in families. In this study, 117 ichthyoplankton taxa were identified in late summer-autumn and winter. Gerreidae, Leiognathidae, and Sillaginidae were the most abundant families in late summer-autumn, and Sparidae, Platycephalidae, and Sillaginidae were the most abundant families in winter. The above studies revealed that the composition of ichthyoplankton changed significantly, i.e., the economic taxa suffered a decline, and the proportion of small fish increased. This was also reported to have changed the demersal fishery catch composition, with smaller-size and lower-value species being more abundant than before [9,12,30] In this study, 29 ichthyoplankton taxa that occurred in the samples from both surveys accounted for 24.78% of all taxa. This suggests that the majority of fish species spawned in the late summer-autumn or winter. This phenomenon corresponds to the southwest monsoon characteristics in the Beibu Gulf, with high water temperature and low Chl-a concentration in the late summer-autumn and northeast monsoon characteristics in winter, with low water temperature and high Chl-a concentration [31]. Preliminary studies of fish spawning period were mainly based on monitoring fish gonadal development in the Beibu Gulf [32,33]. Such an approach could infer spawning period but cannot accurately detect the location of spawning areas. Thus, the information we present on the Beibu Gulf provides clear evidence of the composition and distribution of ichthyoplankton in late summer-autumn and winter, and the presence of the fish spawning grounds in the study area.

4.2. The Spawning Season and Location

The abundance of fish larvae in the Beibu Gulf in late summer-autumn was significantly higher than that in winter ($p < 0.05$) due to the higher water temperature, short hatching period, and abundant food for larval growth in spring and summer [34]. The distribution of fish abundances in the Beibu Gulf was sporadic in winter, indicating that winter was not the peak spawning season. Zhou et al. (2011) [16] also reported that spring and summer are the main spawning seasons of fish plankton in the Beibu Gulf. In early autumn, fish larvae were mainly distributed in the coastal waters of the northern Beibu Gulf, such as the waters of Qinzhou Bay and from the north of Qiongzhou Strait to Tieshan Port. On the one hand, coastal dilutive water, mainland runoff, and the mixed water from the Qiongzhou Strait carry rich nutrients, which provide a suitable living environment for fish larvae and juveniles [35,36]. On the other hand, fish larval abundances were significantly positively correlated with chlorophyll a content ($p < 0.05$), and a high Chl-a content represents phytoplankton abundance and high secondary productivity [16], which provides abundant prey organisms conducive for fish reproduction and growth [37].

The Beibu Gulf is a semi enclosed bay. The hydrological condition in this area is relatively stable, and the ecology is relatively independent. Thus, the seasonal spatial distribution patterns of fish community are relatively stable, and the seasonal changes are not obvious in the Beibu Gulf. Wang et al. (2010) [10] divided the fish communities into five groups, and among them, four groups were considered to be relatively stable, and one group was unstable, which only appeared in spring. In this present study, the ichthyoplankton assemblage in the Beibu Gulf was divided into five groups in late summer-autumn and four groups in winter (Figure 3). The five groups in later summer–autumn were not regular, which could be due to the stronger effects of coastal water, open sea water, and mixed water in the Qiongzhou strait in late summer-autumn than in winter, thus leading to this irregular distribution phenomenon [38]. The four groups in winter were relatively regular, which may partially match the distributional patterns of adult fishes [10]. Particularly noteworthy were the group b in late summer-autumn and groups a and b in winter. These groups were mainly distributed in the northern waters of the Beibu Gulf (Figure 3), as the northern waters in the Beibu Gulf are mainly affected by coastal water, mixed water, and open sea water, especially along the coastal water and mixed water. The stable hydrological condition renders this area as the spawning ground of Clupeidae and Engraulidae.

4.3. Ichthyoplankton Assemblage Structure and the Relationship with Environmental Variables

Environmental factors have been shown to have a significant impact on the ichthyoplankton assemblage structure [39,40]. In this study, CCA indicated that pH, Chl-a, Dep, DO, SSS, TN, and TP significantly affected the distribution of ichthyoplankton in the Beibu Gulf. The pH level is a significant environmental factor that affects the distribution of an ichthyoplankton assemblage. On the one hand, due to the intake of freshwater, the pH level of the coastal waters decreases, and macronutrients are supplied, increasing the primary productivity and ichthyoplankton abundance. Chermahini et al. (2021) found that the entrance of freshwater input to the northern Persian Gulf supported a higher abundance of ichthyoplankton [41]. On the other hand, Vazzoler (1996) observed a positive correlation between pH and the density of some ichthyoplankton [42]. Some researchers found that the preference of certain ichthyoplankton species for waters which are slightly acidic pH may be an acquired behavior [43]. In short, the impact of pH on reproductive processes is not well understood now, but it has been observed to induce spawning in certain ichthyoplankton. In addition, salinity, temperature, and Chl-a are also considered as the major factors that affect the ichthyoplankton communities [5,16,44,45]. Salinity and temperature mainly affect fish reproductive activity, the spatial and temporal distribution of ichthyoplankton, and the assemblage structures [46,47]. Salinity can affect the hatching process and development rate [47], while temperature can affect the spawning fish's distribution, brood amount, and activity of hatching enzymes, which is strongly correlated with the duration of incubation [47]. Chl-a, associated with phytoplankton and zooplankton abundance, has an obvious influence on ichthyoplankton structure. Chl-a is associated with nutrient levels in the Beibu Gulf [31]. The high Chl-a level indicates high nutrient levels and rich food resources that are vital for the growth of fish larvae and juveniles. Water depth is mainly associated with the distribution of fish, which can affect the fish spawning activities and indirectly affect the distribution patterns of ichthyoplankton. However, sea surface water temperature (SST) was excluded from the CCA via the VIF test (VIF > 4). This also occurred in some studies, i.e., the ichthyoplankton assemblages in the estuary of Yangtze River [48], and the distribution of pelagic fish, i.e., jack mackerel *Trachurus japonicus* in the Beibu Gulf [49]. In this study, the SST ranged from 29.5 to 33.3 °C in the late summer-autumn and 13.8 to 25.9 °C in winter, and the low temperature may be a limiting factor that affects the fish spawning activities in subtropical tropical waters during winter. Lin (2004) [14] also indicated that autumn and winter are the inactive seasons for most fish spawning activities in the Beibu Gulf. However, SST was excluded in this study, which could be due to the spatial–temporal distribution of ichthyoplankton being more sensitive to other environmental factors or the selective threshold value of VIF < 4. In addition, other environmental factors also affected the ichthyoplankton assemblage, i.e., pH and DO represent water quality and TN and TP represent nutritional status.

In addition, the variation explained by the CCA's first four axes are 18.46%, which indicates that many other environmental factors can affect the ichthyoplankton assemblage structure in the Beibu Gulf, e.g., ocean currents, monsoons, and human activities [12,30,49].

5. Conclusions

In this study, 20,907 ichthyoplankton individuals were sampled in the Beibu Gulf, and a total of 117 taxa were identified, belonging to 13 orders and 40 families. Among them, 75 taxa were identified at the species level. The dominant families were Gerreidae, Leiognathidae, and Sillaginidae in late summer-autumn, and Sparidae, Platycephalidae, and Sillaginidae in winter. The spatial and temporal distribution of ichthyoplankton were divided into five clusters in late summer-autumn, and four assemblages in winter. pH, Chlorophyll-a (Chl-a), depth, dissolved oxygen (DO), sea surface salinity (SSS), total nitrogen (TN), and total phosphorus (TP) were the major environmental variables affecting the ichthyoplankton assemblage structure in the Beibu Gulf.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/jmse11091810/s1, Table S1: IRI index of ichthyoplankton taxa in late summer-autumn and winter of the Beibu Gulf; Table S2: The representative species and their contributions to the average similarity.

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References

1. Baumgartner, G.; Nakatani, K.; Gomes, L.C.; Bialetzki, A.; Sanches, P.V.; Makrakis, M.C. Identification of spawning sites and natural nurseries of fishes in the upper Paraná River, Brazil. *Environ. Biol. Fishes* **2004**, *71*, 115–125. [CrossRef]
2. Oliveira, E.; Ferreira, E. Spawning areas, dispersion and microhabitats of fish larvae in the Anavilhanas Ecological Station, Rio Negro, Amazonas State, Brazil. *Neotrop. Ichthyol.* **2008**, *6*, 559–566. [CrossRef]
3. Takeuchi, A.; Higuchi, T.; Watanabe, S.; Miller, M.J.; Yama, R.; Fukuba, T.; Okamura, A.; Okino, T.; Miwa, T.; Tsukamoto, K. Several possible spawning sites of the Japanese eel determined from collections of their eggs and preleptocephali. *Fish. Sci.* **2021**, *87*, 339–352. [CrossRef]
4. Rakocinski, C.F.; Lyczkowski-Shultz, J.; Richardson, S.L. Ichthyoplankton assemblage structure in Mississippi Sound as revealed by canonical correspondence analysis. *Estuar. Coast. Shelf Sci.* **1996**, *43*, 237–257. [CrossRef]
5. Hou, G.; Wang, J.; Liu, L.; Chen, Y.; Pan, C.; Lin, J.; Zhang, H. Assemblage structure of the ichthyoplankton and its relationship with environmental factors in spring and autumn off the pearl river estuary. *Front. Mar. Sci.* **2021**, *8*, 732970. [CrossRef]
6. Hare, J.A.; Fahay, M.P.; Cowen, R.K. Springtime ichthyoplankton of the slope region off the north-eastern United States of America: Larval assemblages, relation to hydrography and implications for larval transport. *Fish. Oceanogr.* **2001**, *10*, 164–192. [CrossRef]
7. Mesa, M.L.; Mesa, G.L.; Catalano, B.; Jones, C.D. Spatial distribution pattern and physical-biological interactions in the larval notothenioid fish assemblages from the Bransfield Strait and adjacent waters. *Fish. Oceanogr.* **2016**, *25*, 624–636. [CrossRef]
8. Wang, T.; Li, C.H.; Liu, Y.; Zhu, R. Biodiversity and conservation of fish in the Beibu Gulf. *Pak. J. Zool.* **2020**, 1–62.
9. Qiu, Y.; Zeng, X.; Chen, T.; Yuan, W.; Wang, Y. *Fishery Resources and Management in South China Sea*; The Ocean Press: Beijing, China, 2008.
10. Wang, X.; Qiu, Y.; Du, F.; Lin, Z.; Sun, D.; Huang, S. Fish community pattern and its relation to environmental factors in the Beibu Gulf. *J. Fish. China* **2010**, *34*, 1579–1586. [CrossRef]
11. Zhang, K.; Cai, Y.; Liao, B.; Jiang, Y.E.; Sun, M.; Su, L.; Chen, Z. Population dynamics of threadfin porgy *Evynnis cardinalis*, an endangered species on the IUCN red list in the Beibu Gulf, South China Sea. *J. Fish Biol.* **2020**, *97*, 1–11. [CrossRef]
12. Wang, X.; Qiu, Y.; Du, F.; Lin, Z.; Sun, D.; Huang, S. Spatio-temporal variability of fish diversity and dominant species in the Beibu Gulf. *J. Fish. Sci. China* **2011**, *18*, 427–436. [CrossRef]
13. Wang, X.; Qiu, Y.; Du, F.; Lin, Z.; Sun, D.; Huang, S. Dynamics of demersal fish species diversity and biomass of dominant species in autumn in the Beibu Gulf, northwestern South China Sea. *Acta Ecol. Sin.* **2012**, *32*, 333–342. [CrossRef]
14. Lin, Z.J. Fish eggs and larvae. In *Fishery Ecology Environment and Resource in the Exclusive Economic Zone and Continental Shelf of South China Sea*; Jia, X.P., Ed.; Science Press: Beijing, China, 2004; pp. 237–255.
15. Zhou, M.Y.; Lin, Y.S.; Hou, T.T. The abundance and distribution of ichthyoplankton in the Gulf of Tonkin in summer, 2006. In *Symposium of Marine Science Research in Beibu Gulf*; China Ocean Press: Beijing, China, 2008; pp. 264–271.
16. Zhou, M.Y.; Lin, Y.S.; Yang, S.; Cao, W.; Zheng, L. Composition and ecological distribution of ichthyoplankton in eastern Beibu Gulf. *Acta Oceanol. Sin.* **2011**, *30*, 94–105. [CrossRef]
17. Hou, G.; Chen, Y.; Wang, S.; Wang, J.; Zhang, H. Formalin-fixed fish larvae could be effectively identified by DNA barcodes: A case study on thousands of specimens in South China Sea. *Front. Mar. Sci.* **2021**, *8*, 634575. [CrossRef]
18. Hou, G.; Zhang, H. *Illustrations of Fish Eggs in the South China Sea (I)*; China Ocean University Press: Qingdao, China, 2022.
19. Ma, Y.; Xu, H.; Yu, T.; He, G.; Zhao, Y.; Zhang, G.; Shang, L.; Sun, Q.; Wu, Z.; Chen, S. *GB/T 12763.4-2007. Specifications for Oceanographia Survey—Part 4: Survey of Chemical Parameters in Sea Water*; Standards Press of China: Beijing, China, 2007.
20. Chen, X.; Zhao, H.; Jiang, G.; Tang, J.; Xu, Q.; Li, X.; Huang, Y.; Zou, S.; Dong, K.; Hou, W.; et al. Trophic states regulate assembly processes and network structures of small chromophytic phytoplankton communities in estuarine and coastal ecosystem. *Mar. Pollut. Bull.* **2022**, *175*, 113327. [CrossRef]
21. Han, A.; Dai, M.; Kao, S.; Gan, J.; Li, Q.; Wang, L.; Zhai, W.; Wang, L. Nutrient dynamics and biological consumption in a large continental shelf system under the influence of both a river plume and coastal upwelling. *Limnol. Oceanog.* **2012**, *57*, 486–502. [CrossRef]
22. Hou, G.; Zhang, H. *Illustrations of Fish Larvae and Juvenile in the South China Sea (I)*; China Ocean University Press: Qingdao, China, 2021.
23. Wan, R.J.; Zhang, R.J. *Fish Eggs, Larvae and Juveniles in the Offshore Waters of China and Their Adjacent Waters*; Shanghai Scientific and Technological Press: Shanghai, China, 2016.
24. Zhu, X.H.; Liu, D.; Sha, X.K. Relationship between community structure of ichthyoplankton and environmental factors in the Estuary of Changjiang River in spring. *Stud. Mar. Sin.* **2002**, *5*, 169–179.

25. Clarke, K.R. Non-parametric multivariate analyses of changes in community structure. *Austral. J. Ecol.* **1993**, *18*, 117–143. [CrossRef]
26. Clarke, K.R.; Richard, M.W. *Change in Marine Communities: An Approach to Statistical Analysis and Interpretation*, 2nd ed.; Plymouth Marine Laboratory: Plymouth, MA, USA, 2001.
27. Ye, S.; Lin, M.; Li, L.; Liu, J.; Song, L.; Li, Z. Abundance and spatial variability of invasive fishes related to environmental factors in a eutrophic Yunnan Plateau Lake, Lake Dianchi, southwestern China. *Environ. Biol. Fish.* **2015**, *98*, 209–224. [CrossRef]
28. Kabacoff, R. *R in Action: Data Analysis and Graphics with R*, 2nd ed.; Manning Publications: Shelter Island, NY, USA, 2015.
29. Hou, G.; Chen, Y.; Wang, J.; Pan, C.; Lin, J.; Feng, B.; Zhang, H. Molecular identification of species diversity using pelagic fish eggs in spring and late autumn-winter in the eastern Beibu Gulf, China. *Front. Mar. Sci.* **2022**, *8*, 806208. [CrossRef]
30. Yuan, W.W. Dynamics and succession of demersal resources in Beibu Gulf. *J. Fish. Sci. China* **1995**, *2*, 57–65.
31. Shen, C.; Yan, Y.; Zhao, H.; Pan, J.; Devlin, A.T. Influence of monsoonal winds on chlorophyll-α distribution in the Beibu Gulf. *PLoS ONE* **2018**, *13*, e0191051. [CrossRef] [PubMed]
32. Hou, G.; Zhu, L.X.; Lu, H.S. Growth, mortality and population composition of crimson sea bream, *Paragyrops edita* Tanaka in Beibu Gulf. *J. Guangdong Ocean Univ.* **2008**, *3*, 50–55.
33. Du, S.Q.; Feng, B.; Hou, G.; Lu, H.S.; Yan, Y.R. Age and growth of moonfish, *Mene maculata* from mouth of the Beibu Gulf, South China Sea. *J. Fish. China* **2012**, *36*, 576. [CrossRef]
34. Su, J.X. *Ichthology and Aquaculture of Marine Fishes*; China Agriculture Press: Beijing, China, 1980.
35. Liang, W.Z.; Tang, D.L. Distribution characteristics of phytoplankton size structure in the western South China Sea in summer. *J. Trop. Oceanogr.* **2017**, *36*, 93–101.
36. Cao, Z.Y.; Bao, M.; Guan, W.B.; Chen, Q. Water-mass evolution and the seasonal change in northeast of the Beibu Gulf, China. *Oceanol. Et Limnol. Sin.* **2019**, *50*, 532–542.
37. Xiao, Y.Z.; Wang, R.; Ou, Q. Relationship between abundance distribution of fish eggs, larvae and juveniles and environmental factors in the Pearl River Estuary waters in spring. *J. Oceanogr. Taiwan Strait* **2010**, *4*, 8.
38. Yuan, Y.Q.; Lv, X.N.; Wu, Z.X.; He, C.; Song, X.X.; Cao, X.H.; Yu, Z.M. Temporal and spatial distribution of main environmental factors in typical sea area of the beibu gulf and its influencing factors. *Oceanol. Limnol. Sin.* **2019**, *50*, 579–589.
39. Rodriguez, J.M. Assemblage structure of ichthyoplankton in the NE Atlantic in spring under contrasting hydrographic conditions. *Sci. Rep.* **2019**, *9*, 8363. [CrossRef]
40. Keskin, E.; Pauly, D. Species composition of ichthyoplankton assemblages: A response to seasonal temperature changes. *Mediterr. Mar. Sci.* **2019**, *20*, 222–226. [CrossRef]
41. Chermahini, M.A.; Shabani, A.; Naddafi, R.; Ghorbani, R.; Rabbaniha, M.; Noorinejad, M. Diversity, distribution, and abundance patterns of ichthyoplankton assemblages in some inlets of the northern Persian Gulf. *J. Sea Res.* **2021**, *167*, 101981. [CrossRef]
42. Vazzoler, A.E.A.M. *Biologia da Reprodução de Peixes Teleósteos: Teoria e Prática*; Eduem: Maringá, Brazil, 1996; p. 169.
43. Baumgartner, G.K.; Nakatani, M.C.; Baumgartner, M.S.T. Some aspects of the ecology of fish larva in the food plain of high Paraná River, Brazil. *Rev. Bras. Zool.* **1997**, *14*, 551–563. [CrossRef]
44. Jiang, Y.; Lin, B.A.; He, H.Y.; Ding, G.M.; Yan, L.T.; Zhang, G.; Liu, M.; Zheng, L.M. Species composition and assemblages of ichthyoplankton in Sansha Bay, Fujian Province, China. *Front. Mar. Sci.* **2021**, *8*, 758089. [CrossRef]
45. Wang, Y.; Liang, C.; Chen, Z.; Liu, S.; Zhang, H.; Xian, W. Spring ichthyoplankton assemblage structure in the Yangtze Estuary under environmental factors. *Front. Mar. Sci.* **2021**, *8*, 806096. [CrossRef]
46. Whitfield, A.K. Ichthyofaunal assemblages in estuaries: A South African case study. *Rev. Fish. Biol. Fish.* **1999**, *9*, 151–186. [CrossRef]
47. Fuiman, L.A. Special considerrations of fish eggs and larvae. In *Fishery Science: The Unique Contributions of Early Life Stages*; Fuiman, L.A., Werner, R.G., Eds.; Blackwell Science: Hoboken, NJ, USA, 2003; pp. 1–32.
48. Zhang, H.; Xian, W.; Liu, S. Seasonal variations of the ichthyoplankton assemblage in the Yangtze Estuary and its relationship with environmental factors. *PeerJ* **2019**, *7*, e6482. [CrossRef] [PubMed]
49. Feng, Y.T.; Shi, H.Y.; Hou, G.; Zhao, H.; Dong, C.M. Relationships between environmental variables and spatial and temporal distribution of Jack mackerel (*Trachurus japonicus*) in the Beibu Gulf, South China Sea. *PeerJ* **2021**, *9*, e12337. [CrossRef]