

Connectivity of nekton assemblages along artificial reefs and adjacent waters in Haizhou Bay

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Abstract:

The successful construction of marine protected areas (MPAs) in temperate waters largely depends on our understanding of the distribution and coexistence of organisms with varying habitat preferences, which helps us to better understand the community patterns mediated by connectivity in coastal areas. This study was conducted to examine the connectivity of nekton assemblages in artificial reefs and adjacent waters, which included five habitats: the artificial reef area (AR), aquaculture area (AA), natural area (NA), estuary area (EA) and comprehensive effect area (CEA), in Haizhou Bay in October 2020. Analysis of variance (ANOVA) showed that there were significant differences in the characteristics and abundances of nekton in each habitat ($P<0.05$). Approximately 38.2% of the individuals were found in at least three habitats, and very few species were present in only a single habitat. Several highly abundant nekton species were selected according to the kernel density estimates (KDEs), and their body lengths varied gradually among habitats, potentially indicating migration and diffusion during their life history. The results showed that artificial reefs and adjacent waters in Haizhou Bay are related by similar nekton assemblages and ontogenetic variation. Finally, this study has implications for the conservation and monitoring of nekton assemblages in artificial reefs and adjacent waters, highlighting that the principle of connectivity should be taken into consideration in the design of MPAs and MPA networks that can be applied in different stages of implementation and in different combinations of scenarios.

Key words: connectivity, nekton, artificial reef, adjacent sea area, Haizhou Bay

1 Introduction

The global trends of continuous processes associated with human activities and coastal development, such as overfishing, habitat destruction and marine environmental pollution, have led to the general degradation of the entire coastal ecosystem, which has imposed tremendous pressure on estuaries, harbors, gulfs and nearshore regions (Walker et al., 2014; Dance et al., 2015; Cordova et al., 2018; Reis et al., 2019). Over the years, artificial reefs have been constructed as human-made structures to increase environmental quality and species abundance in marine ecosystems for coastal ecological restoration (Seaman and Sprague 1991; Anne et al. 2015; Folpp et al. 2020) by creating suitable habitats and places for many marine organisms to grow, reproduce, forage and hide (Sherman et al., 2002). At present, ecological principles combining the planning, design and operation of artificial reefs have been extensively investigated in many coastal areas (Whitmarsh et al. 2008; Walker et al. 2014; Anne et al. 2015; Folpp

36 et al. 2020). Given that artificial reefs aim to retard marine habitat degradation, protect
37 endangered species and restore biodiversity, artificial reefs are regarded as conservation
38 and enhancement tools for marine environments and habitat recovery (Dafforn et al.,
39 2015; Becker et al., 2017).

40 Habitat connectivity provides an important perspective for further study of coastal
41 ecosystems (Dance et al., 2015; Diana et al., 2018). It includes two mechanisms:
42 movements of nekton in different stages of life history (Flitcroft et al., 2018) and
43 transport and exchange of nutrients (fundamental elements such as C, N, P, and S)
44 (Garcia et al., 2017; Laske et al., 2019), which play an important role in maintaining
45 population structures and regulating ecological processes. Most ecological studies on
46 habitat connections and distribution patterns of marine nekton focus on a limited
47 number of species or habitat types (Reis et al., 2019); thus, obtaining accurate and
48 different information on related species and habitats in various types of ecosystems
49 seems to be a major challenge.

50 Haizhou Bay, located in Lianyungang City, Jiangsu Province, is one of the
51 important fishing grounds in the Yellow Sea of China (Wang, 1993). Because of
52 numerous human activities, such as overfishing, port construction and waterway
53 transportation, since the 1980s, the habitat environment and fishery resources in
54 Haizhou Bay have been vastly and adversely affected, resulting in the fragmentation of
55 habitats and the destruction of ecosystem structure (Zhang et al., 2006; Zhang et al.,
56 2013). Since 2002, the local government has begun to build marine protected areas
57 (MPAs) dominated by artificial reefs for ecological restoration and resource
58 conservation in Haizhou Bay (Zhang et al., 2006; Wu et al., 2012). The construction of
59 artificial reefs affects aquatic biodiversity and food web ecology by affecting the flow
60 of water, sediments and organisms (Clark et al. 1999; Sherman et al. 2002). These
61 processes in turn affect the community structure and ecological pattern in adjacent
62 waters and finally alter the connectivity between habitats (Keller et al. 2017; Reeds et
63 al. 2018). However, the impacts of artificial reefs on marine ecosystems and
64 communities in adjacent waters, as well as ontogenetic changes and utilization patterns
65 of reef nekton, are largely unknown, especially in temperate seas (Diana et al. 2018).

66 To support the sustainable socioeconomic development of MPAs, therefore, it is
67 necessary to thoroughly explore the relationship between biodiversity and connectivity
68 in artificial reefs and adjacent waters.

69 In this study, we aim to (1) analyze the nekton assemblages and connectivity in
70 artificial reefs and adjacent waters in Haizhou Bay, (2) preliminarily explore the
71 influence of artificial reefs on the distribution pattern of nekton resources in adjacent
72 waters, and (3) identify ontogenetic shifts in nekton-habitat association patterns. Our
73 research will help better understand the connectivity of communities between different
74 habitats in temperate waters and provide a more scientific basis and improve specific
75 planning for improving the strategy, fishery management and construction of MPAs and
76 MPA networks in temperate coastal habitats in China.

77 2 Material and methods

78 2.1 Study area

79 Haizhou Bay, located west of the coast of Lianyungang city, Jiangsu Province,
80 north of the Qingdao Fishing Ground, and south of the Lvsi Fishing Ground, is mainly
81 composed of sandy and muddy habitats and represents an open bay with an area of
82 approximately 877 km²(Wang 1993). The climate and hydrology of Haizhou Bay are
83 greatly influenced by the mainland, and most fishing areas are controlled by coastal
84 currents (Luo et al. 2009). The tidal current in Haizhou Bay is mainly rotating flow,
85 with a velocity of 0.4-0.65 m/s (Xie et al. 2007). The environmental and fishery
86 resource surveys presented in this study were conducted in the coastal waters of
87 Haizhou Bay in autumn 2020.

88 2.2 Defining habitat types

89 According to the available geographical coordinate data sets in the study area
90 (34°49.20-34°55.00N, 119°16.167-119°59.50E), five major investigation areas ranging
91 from the Linhong Estuary to the artificial reef area were set, including the estuary area
92 (EA), the most polluted area and featuring a coarse sand substrate; the aquaculture area
93 (AA), an area mainly used for culturing shellfish and algae; the artificial reef area (AR),
94 a protected area consisting of a series of reefs on the sea bottom; the natural area (NA),

95 an area that has not been overly impacted by humans; and the comprehensive effect
96 area (CEA), an area in which several habitats co-occur. The distribution of the sampling
97 sites is shown in Figure 1.

98 To determine how the nekton utilized the habitat, the species were divided into the
99 following nine habitat groups: (1) AR species, which were present only in the artificial
100 reef area; (2) AA species, which were present in only the aquaculture area; (3) EA
101 species, which were present only in the estuary area; (4) NA species, which were
102 present only in the natural area; (5) AR-AA-EA species, which were present in the
103 artificial reef area, aquaculture area and estuary area; (6) AR-EA-NA species, which
104 were present in the artificial reef area, estuary area and natural area; (7) AR-AA-NA
105 species, which were present in the artificial reef area, aquaculture area and natural area;
106 (8) AA-NA-EA species, which were present in the artificial reef area, natural area and
107 estuary area; (9) AR-AA-EA-NA species, which were present in all four habitats (as a
108 comparison area, CEA was not included in the statistics) (Nakamura and Sano 2004).

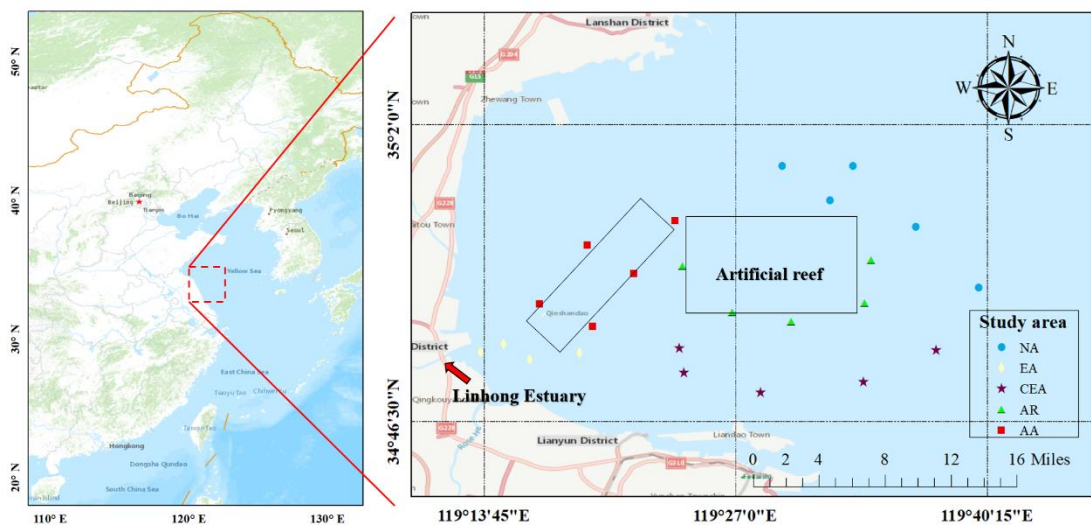


Figure 1. Study area and sampling site

AR, CEA, AA, EA and NA represent the artificial reef area, comprehensive effect area, aquaculture area, estuary area and natural area, respectively.

2.3 Sample collection

Environmental data were recorded by a CTD (conductivity, temperature, and depth) measuring system, sediment was collected using a sediment sampler, and nekton were sampled by a single ship with a wing single capsule bottom trawl (15/4×8 m, mesh size: 1×1 cm) at each site. The fishery investigation was conducted for approximately 30

118 minutes. Because a large number of reefs or culture nets and cages are commonly
119 present at the bottom of AR and AA, we could only sample around these habitats (see
120 the solid box of the study area in Figure 1). After identification, the samples were
121 packed into 100×150 cm PVC bags with fresh ice for preservation. The collection,
122 treatment and analysis of samples were in accordance with the relevant provisions of
123 the Marine Survey Code (General Administration of Quality Supervision, 2007). The
124 basic biological indicators (weight and length) and numbers of all samples were
125 recorded in the onshore laboratory (the total length of each sample was accurate to 0.1
126 cm). All nekton were identified to the lowest possible classification level
127 (<http://www.fishbase.org>).

128 2.4 Statical analysis

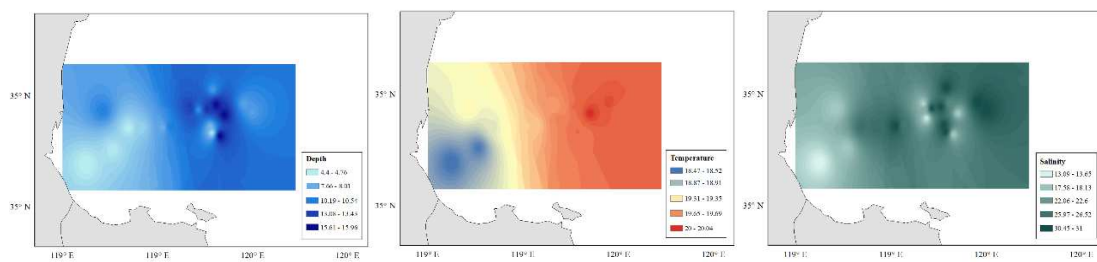
129 The larval to adult ratio and species abundance in each habitat were calculated to
130 evaluate the relationship between the use of different habitat types and nekton size. All
131 nekton were identified at the family level, and the numbers were counted. One-way
132 analysis of variance (ANOVA) was used to determine the differences in the number of
133 individuals and species and to analyze the differences in different body lengths of
134 nekton in each habitat. The similarity of nekton assemblages among different habitats
135 was calculated using continuous trawl data. To evaluate the distribution of species in
136 different habitats, samples were clustered based on Bray-Curtis similarity, and the
137 results were visualized by nonmetric multidimensional scale analysis (NMDS).
138 Nonparametric multivariate analysis of variance (NPMANOVA; $\alpha=0.05$) was used to
139 analyze the differences in nekton community composition in different habitats. To
140 verify the ontogenetic variation in the fish-habitat associations of a selection of species,
141 kernel density estimates (KDEs) were run by body length frequency data from each
142 habitat type. All statistical analyses were run in R software (Ver. 4.0.3).

143 3 Results

144 3.1 Characteristics of environment

145 The characteristics of the environment in the study area of Haizhou Bay are
146 illustrated in Figure 2. The depth ranged from EA (4.40 ± 2.91 m) to RA (12.56 ± 2.63 m),

147 and the mean salinity (23.76 ± 6.63) reflected the gradient from near the shore (EA:
 148 13.59 ± 1.56) to far from shore (NA: 30.87 ± 0.09). The particle size of sediments changed
 149 from EA ($16.86\ \mu\text{m}$) to NA ($62.52\pm 6.70\ \mu\text{m}$), which reflected the distribution of waters
 150 and sediments in Haizhou Bay. The mean temperature was $19.54\pm 0.39^\circ\text{C}$ and gradually
 151 increased from EA ($18.47\pm 0.04^\circ\text{C}$) to AR ($19.75\pm 0.13^\circ\text{C}$). The mean dissolved oxygen
 152 levels were stable along this horizontal gradient ($8.10\pm 0.35\ \text{mg/l}$). At the habitat level,
 153 the mean salinity, temperature and dissolved oxygen levels could represent the coastal
 154 environment in Haizhou Bay.



155
 156 Figure 2. The depth, temperature and salinity distribution of the study area in Haizhou Bay

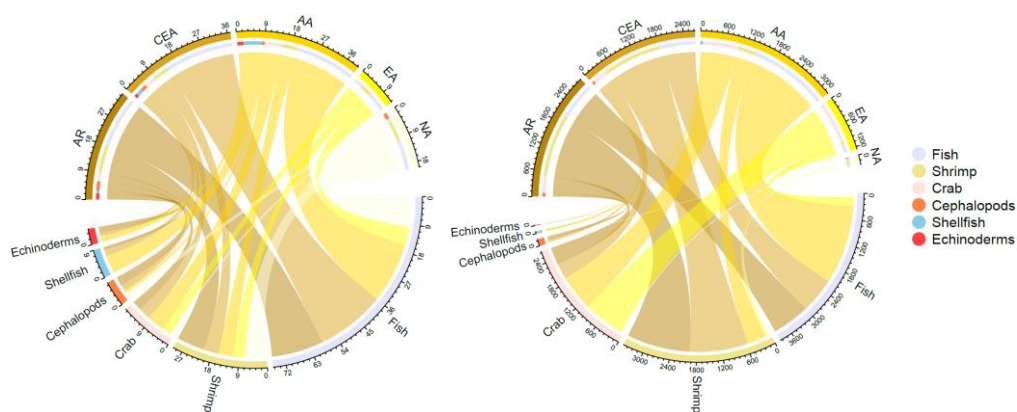
157 3.2 Nekton community characteristics

158 A total of 10,234 individuals were collected from five habitats, including 68
 159 species belonging to 42 families (Appendix 1). A total of 2867 individuals belonging to
 160 21 families and 35 species were collected in AR, 3213 individuals from 29 families and
 161 39 species were collected in AA, and 2664 animals belonging to 22 families and 39
 162 species were collected in CEA. In contrast, there were fewer species found in EA (1263
 163 animals belonging to 13 species in 8 families) and NA (227 animals belonging to 20
 164 species in 15 families) (Figure 3, Appendix 1).

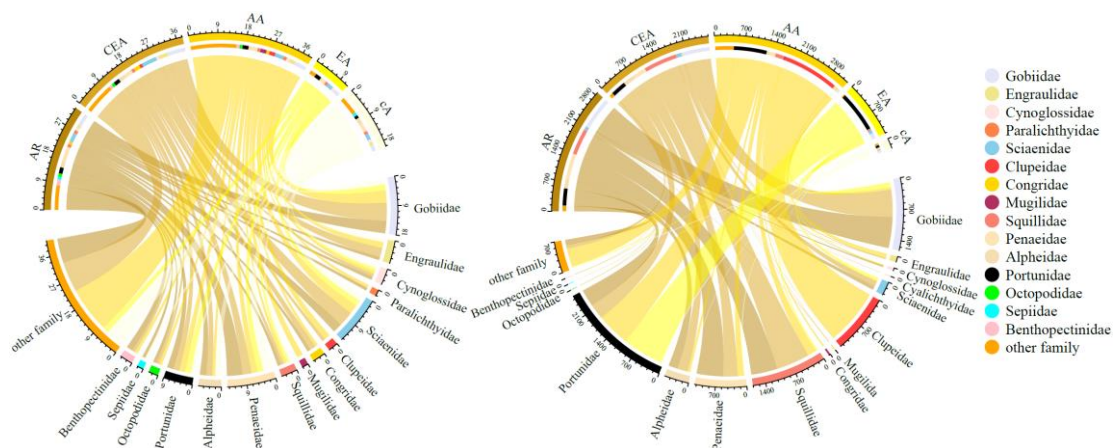
165 The predominant species in AR were Gobiidae (4 species, 12.1%, represented by
 166 *Chaeturichthys stigmatis*), followed by Penaeidae (5 species, 15.1%, represented by
 167 *Trachypenaeus curvirostris*). The most dominant species of AA was Clupeidae (2
 168 species, 5.1%), represented by the species *Sardinella zunas*. The species in NA were
 169 mainly composed of Gobiidae (2 species, 10%), Portunidae (1 species, 5%) and
 170 Penaeidae (2 species, 15%), and those in EA were composed of Portunidae (2 species,
 171 16.7%), *Portunus trituberculatus* and *Charybdis japonica*. The dominant family of
 172 CEA was Penaeidae (1 species, 2.6%). In terms of the number of individuals, Gobiidae

173 was the dominant family of RA, accounting for 26.6% of all species, followed by
 174 Penaeidae, which accounted for 21.8% of all species. Clupeidae was the main family
 175 in AA, accounting for approximately 41.6%, and Gobiidae was the main family in NA,
 176 accounting for 25.6%. The most important family in EA was Portunidae, accounting
 177 for 77.6%. *Oratosquilla oratoria* of Penaeidae was the dominant species in CEA,
 178 accounting for approximately 30% (Figure 4, Appendix 1).

179 The results of one-way ANOVA showed that the mean numbers of individuals of
 180 fish, shrimp, crab, shellfish, and cephalopods were significantly different between NA
 181 and AR and between CEA and AA ($P < 0.05$). The mean number of individuals in AR,
 182 CEA and AA was significantly higher than that in NA ($P < 0.05$).



183
 184 Figure 3. Species and mean numbers of individuals of fish, shrimp, crab, shellfish, and cephalopods in
 185 the five habitats



186
 187 Figure 4. Species and mean numbers of individuals of different families in the five habitats

188 3.3 Nekton assemblage and utilization of different habitats

189 Nearly all species were widely distributed in the five habitats, with the majority
 190 using two, three, or four habitats at the same time, but only Gobiidae, Squillidae,
 191 Cynoglossidae, Sciaenidae, Penaeidae and Portunidae were found living in all five

192 habitats, among which *C. Stigmatias*, *O. Oratoria* and *P. Trituberculatus* had the largest
 193 numbers (Appendix 1). There were 18 specific species that appeared to use a single
 194 habitat, including 10 species of Platycephalidae and Pholidae in AR, Apogonidae in NA
 195 and Mugilidae and Paguridae in AA.

196 Univariate PERMANOVA results showed that the species abundances of the five
 197 marine habitats were significantly different ($P < 0.05$) (Table 1). Analysis of each habitat
 198 separately revealed that there were significant differences in species abundance
 199 between the two groups of five habitats ($P < 0.05$) (Table 2). The community structure
 200 of the five habitats was inconsistent not only at the overall level but also among each
 201 of the habitats.

202 Table 1. Univariate PERMANOVA results for the overall interaction of species abundances of species
 203 in the five habitats

Group	Df	Sums of squares	Mean squares	F. Model	Variation (R^2)	P (>F)
site	4	5.5804	1.3951	8.5139	0.6300	0.001
Residuals	20	3.2772	0.1639		0.3700	
Total	24	8.8577			1	

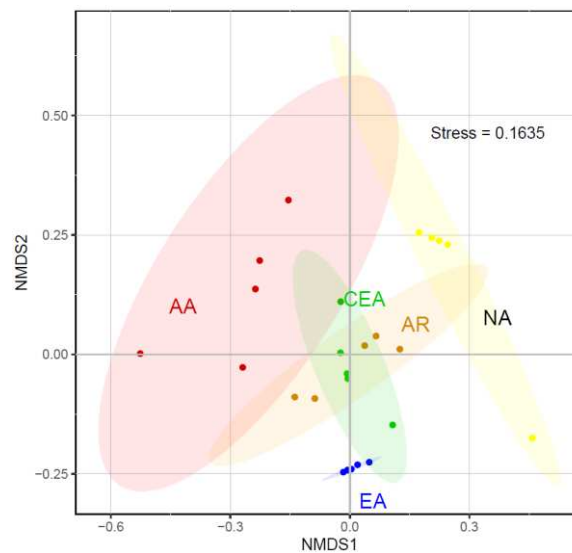
204 Note: R^2 is the variance contribution; the higher the R^2 value is, the higher the explanation degree of
 205 abundance between different habitats. The same below in Table 2.

206 Table 2. Univariate PERMANOVA results of pound-by-pair interactions of the species abundances of
 207 species in the five habitats

Group	Df	Sums of squares	Mean squares	F. Model	Variation (R^2)	Pr (>F)
AR/NA	1	1.1685	1.1685	5.5307	0.4088	0.008
AR/CEA	1	0.9411	0.9411	5.7191	0.4169	0.009
AR/AA	1	1.1030	1.1030	4.43213	0.3565	0.009
AR/EA	1	1.2615	1.2615	10.4299	0.5659	0.01
NA/CEA	1	1.5579	1.5579	10.2601	0.5619	0.007
NA/AA	1	1.3624	1.3624	5.7695	0.4190	0.012
NA/EA	1	1.8698	1.8698	17.2758	0.6834	0.008
CEA/AA	1	1.4096	1.4096	7.4413	0.4819	0.009
CEA/EA	1	1.6588	1.6588	26.9704	0.7712	0.007
AA/EA	1	1.6184	1.6184	11.0992	0.5811	0.01

208 The results of clustering analysis were visualized by nonmetric multidimensional
 209 scale analysis (Figure 5). According to NMDS, habitat arrangement in the multivariate
 210 space clearly revealed a separation along the NMDS 1 axis, which clearly showed that
 211 AA and NA were located on the left and right sides of the central axis, respectively. AA
 212 has the largest shaded area, and EA has the smallest, suggesting that the possible range

213 of species abundance changes in the five habitats was AA>AR>NA>CEA>EA (Figure
214 5). The shaded range of CEA overlaps with those of AR, EA and AA but not that of NA,
215 which proves that CEA was a transitional area among several other habitats. However,
216 the shaded range of NA overlaps with that of AA and AR, indicating that there was a
217 potential relationship between species composition among these three habitats. EA,
218 similar to NA, hardly overlaps with other habitats except for CEA, indicating that the
219 marine nekton assemblages in these two habitats form an isolated group with little
220 overlap with other habitats. NA overlaps with habitats other than EA, which may
221 explain why nekton assemblages in natural waters exist in other habitats.



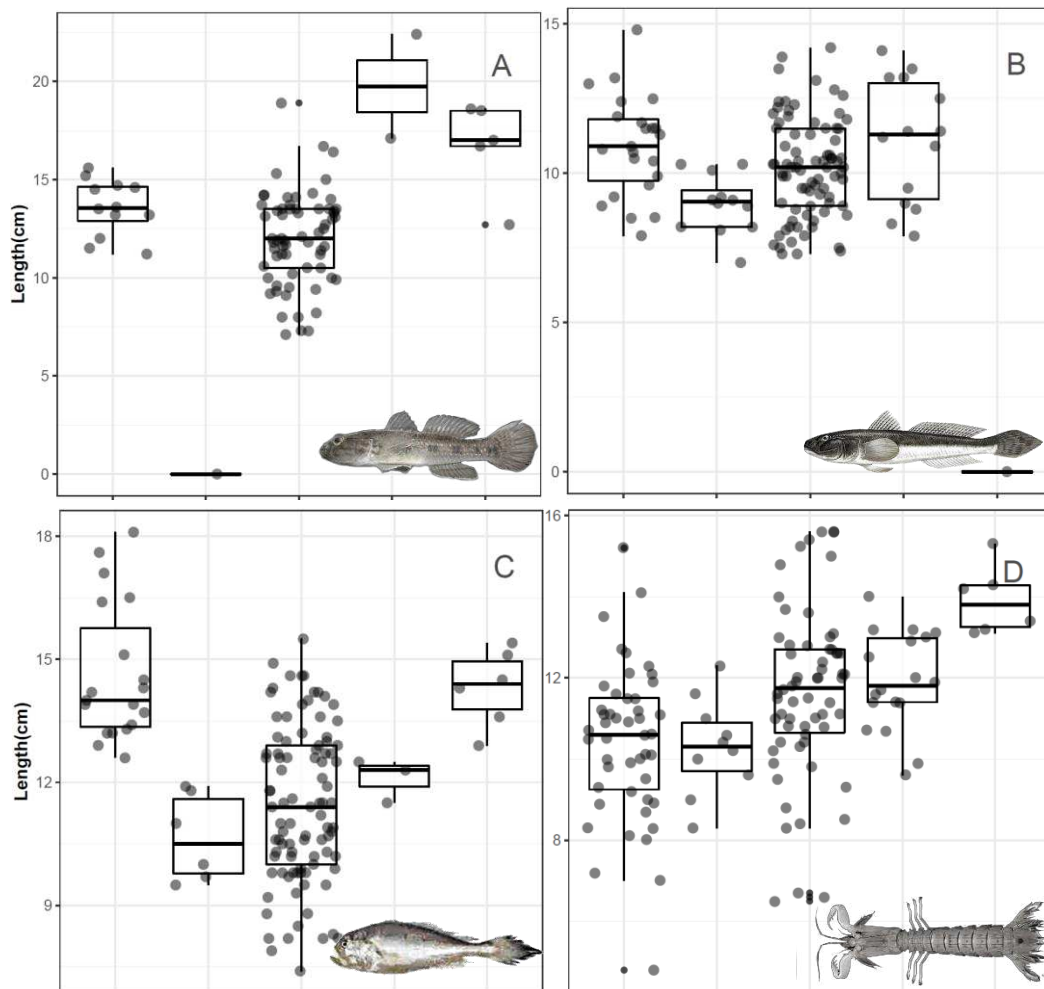
222
223 Figure 5. Visualization of the nonmetric multidimensional scale (NMDS) results for the composition of
224 the nekton assemblages sampled by trawling in the different marine habitat types of Haizhou Bay. The
225 stress function value ($0.1635 < 0.2$) shows the rationality of the ranking model. Different colored shaded
226 areas represent the different habitat types, and the points indicate each sampling site. The size of the
227 shaded area reflects the species abundance, and the degree of overlap of shaded areas represents the
228 degree of difference in species abundance between habitats.

229 3.4 Length and size classes of nekton

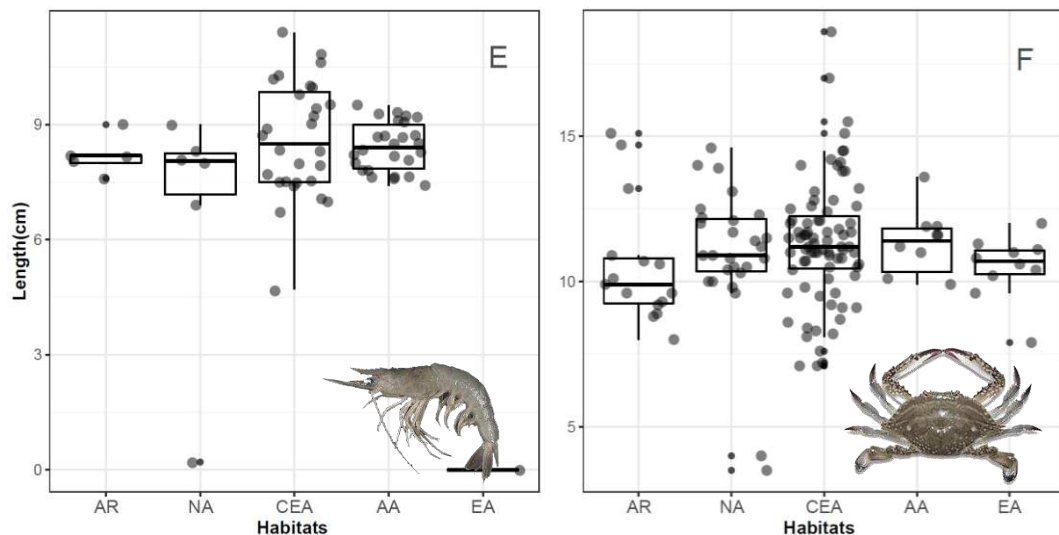
230 According to the KDEs of nekton with different body lengths, the three fish with
231 the highest abundance (A. *C. stigmatias*, B. *Amblychaeturichthys hexanema*, and C.
232 *Collichthys lucidus*), and the top three most abundant shrimp and crabs (D. *O. oratoria*,
233 E. *T. curvirostris*, and F. *P. trituberculatus*) were selected, and their body length
234 distribution in different habitats is described in Figure 6. There were significant
235 differences in the body length distributions of the six species in the five habitats

236 ($P<0.05$). The maximum and minimum lengths of *C. stigmatias* appeared in AA (22.4
 237 cm) and CEA (7.1 cm), and those of *A. hexanema* appeared in NA (7.0 cm) and AR
 238 (14.8 cm), respectively. The mean lengths of *C. lucidus* were 14.63 ± 1.64 cm,
 239 10.65 ± 0.94 cm, 11.28 ± 1.85 cm, 12.1 ± 0.43 cm and 14.3 ± 0.85 cm in AR, NA, CEA, AA,
 240 and EA, respectively. These results indicated that *C. lucidus* has a strong ability to shift
 241 among habitats and a wide distribution in different habitats. The body length of *C.*
 242 *lucidus* was larger in EA and AR than in the other habitats, possibly because the cross-
 243 habitat shifts of *C. lucidus* led to some potential connectivity between RA and EA. The
 244 maximum sizes of *O. oratoria* and *P. trituberculatus* were 15.6 cm and 18.6 cm in CEA,
 245 and the minimum sizes were 4.8 cm and 3.5 cm in AR and NA. The maximum and
 246 minimum sizes of *T. curvirostris* were 11.4 cm and 4.7 cm in CEA. These results could
 247 indicate some possible individual shifts between habitats.

248



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254
255

Figure 6. Kernel density analysis for the different size classes of six abundant nekton species with multihabitat associations in Haizhou Bay. The upper and lower limits of the box indicate the 1st and 3rd quantiles, respectively, and the inner line of the box indicates the average of the data distribution.
(A. *C. stigmatias*, B. *A. hexanema*, C. *C. lucidus*, D. *O. oratoria*, E. *T. curvirostris*, F. *P. trituberculatus*)

256 4 Discussion

257 Understanding the relationships between habitats for baseline establishment is an
258 important part of ecosystem approaches in fishery management, biodiversity
259 conservation and spatial planning in coastal areas (Olds et al. 2016; Liao et al. 2017).
260 In the past, the study of nekton resources in Haizhou Bay focused only on artificial reef
261 areas and contrasting areas (Zhang et al. 2006; Sun et al. 2006; Su et al. 2015), which
262 is not scientific or reasonable. The contrasting areas include CEA, AA, NA and other
263 habitats, and AA is subdivided into a shellfish culturing area, an algae culturing area
264 and so on. Therefore, the contrasting areas previously surveyed may represent
265 combinations of any of these habitats, thereby greatly affecting the evaluation of the
266 effect of artificial reefs on MPAs and the results of fishery resource analysis. In addition,
267 comparing the distribution of nekton resources in a single habitat cannot explain the
268 intrinsic connection of habitats (Diana et al. 2018). Therefore, based on the principle of
269 ecological connectivity, the study area of Haizhou Bay is clearly divided into five
270 habitats through sampling data over the years, which is conducive to the study of the
271 potential relationship between the characteristics of nekton resources and the
272 interaction of population structure in the artificial reefs and adjacent waters.

273 EA is a major source of nutrient transport to the ocean and represents an important
 274 transition region in which energy from land and sea mixes, and this area is dominated
 275 by a variety of nekton communities (Pasquaud et al. 2008; Emily et al. 2017). However,
 276 we did not observe a high level of species abundance in EA, which may be related to
 277 EA being subjected to the strongest interference by humans (Li et al. 2011).
 278 Furthermore, the water level of EA fluctuates due to the intermittent influence of tides,
 279 which may lead to different nekton assemblages (Reis et al. 2019). AA presents a unique
 280 nekton assemblage with a variety of benthic shellfish and snails that other habitats do
 281 not include, which could explain the remarkable effect of the high benthic abundance
 282 in AA. The current of Haizhou Bay includes a branch of the Yellow Sea Warm Current
 283 and the coastal current, which flows from northeast to southwest (Sun et al. 2003).
 284 Therefore, the high species abundance in CEA is expected as it is a gathering place of
 285 AA, RA and NA habitats. Artificial reefs are an effective way to increase habitats and
 286 populations of fish species (Folpp et al. 2020); thus, we assume that AR is a mosaic that
 287 connects other habitats. Since artificial reefs were placed in habitats, the abundance of
 288 nekton has showed an increasing trend over time (Wang et al. 2017), similar to results
 289 from most temperate seas (Lowry et al. 2014; Smith et al. 2016; Folpp et al. 2020). The
 290 mean abundance of nekton in AR was higher than that in the other habitats except for
 291 CEA, and it featured a relatively unique nekton assemblage (Figure 2). In addition, AR
 292 includes almost all species present in the other habitats except for species specific to
 293 EA, AA, and NA, especially benthic mollusks of AA, and this pattern reflects the good
 294 effect produced by artificial reefs over many years. In the present study, 14.3%-29% of
 295 families and 26.1%-50.3% of individual species were found in at least three habitats,
 296 and very few species utilized a single habitat (Table 3). Most nekton use a variety of
 297 habitats, suggesting that they may migrate and emigrate between habitats and
 298 demonstrating connectivity between the nekton assemblages of artificial reefs and the
 299 nekton assemblages in adjacent waters.

300 Table 3. Percentage contribution of all family members and individuals of each habitat group

Habitat group	All Family/%	Individuals/%
AR	4.8	0.02
AA	23.8	1.1

NA	2.4	0.03
EA	0	0
AR/AA/EA	19	49.3
AR/EA/NA	14.3	38.3
AR/AA/NA	16.7	26.1
AA/EA/NA	19	41.5
AR/AA/EA/NA	14.3	50.3

301 In the present study, strong links were also identified between the size-related
302 nekton assemblages and the distribution of habitats in artificial reefs and adjacent
303 waters. Little fluctuation in the body length of Gobiidae was found between habitats,
304 which may be related to its weak swimming ability and settlement behavior after
305 reaching adulthood (Han et al. 2013). The body size of *P. trituberculatus*, with strong
306 swimming ability, also showed little variation in this study. The reason is that *P.*
307 *trituberculatus* is active in coastal waters but not in offshore regions, such as AR and
308 NA (Song et al. 1989). Moreover, EA is also the discharge area of *P. trituberculatus* in
309 Haizhou Bay, which verifies the view in this study. In total, the body length of each
310 nekton gradually increases from EA to RA, not only indicating that EA is a breeding
311 ground for most fish, shrimp and crabs but also reflecting the ontogenesis of some
312 marine nekton in terms of the use of different habitats. The body length gradient of *C.*
313 *lucidus* and *O. oratoria* in different habitats indicates strong cross-habitat shifts, and a
314 larger length of *C. lucidus* appears in both AR and EA, which suggests that artificial
315 reefs increase fish abundance by attracting fish (Lowry et al. 2014). Our findings
316 support the conclusion that in this coastal region, many nekton, such as *C. lucidus* and
317 *O. oratoria*, undergo migration and diffusion from estuarine habitats to artificial reefs
318 in Haizhou Bay with increasing size during their life history. This finding reveals the
319 connectivity between artificial reefs and adjacent waters.

320 According to the characteristics of the species and environment in temperate seas,
321 it is worth considering selecting the appropriate netting gear during the survey. The
322 coastal waters of the Yellow Sea where Haizhou Bay is located are mainly dominated
323 by muddy and sandy habitats, in which the underwater visibility is low, making it
324 difficult to study the nekton communities by means of diving or snorkeling
325 (Dorenbosch et al. 2007; Wang et al. 2011). Moreover, the current velocity is relatively

326 high, and the methods of underwater visual surveys, such as baited remote underwater
327 video systems (BRUVs), are very limited (Reis et al. 2019). Therefore, trawl and gill
328 nets are more commonly used for sampling in this region. However, trawling is the
329 fishing method with the greatest harm to fisheries and marine environments (Yang
330 1997). In the future, habitat connectivity could be assessed by combining trawling and
331 gill net methods. In addition, it remains unclear whether some migratory species use a
332 variety of habitats to complete their life cycle at different stages of their life histories
333 or whether they simply exhibit cross-habitat behavior. It may be necessary to combine
334 otolith microelement (Thais et al. 2020) or environmental DNA (eDNA) (Yamanaka et
335 al., 2016) techniques for further in-depth analysis.

336 5 Conclusion

337 The connectivity of artificial reefs and adjacent waters in temperate seas is largely
338 unknown. In this study, a mesoscale survey of nekton communities associated with
339 artificial reefs and adjacent waters in Haizhou Bay was conducted for the first time and
340 describes the characteristics of the rich diversity of nekton in the coastal ecosystem of
341 Haizhou Bay. We found evidence to support habitat connectivity between artificial reefs
342 and adjacent waters in Haizhou Bay. In addition to describing some nekton assemblages
343 and multihabitat associations, the results also emphasize the ontogenetic variation in
344 some nekton and the influence of specific habitats on their life history. We can infer that
345 artificial reefs and adjacent habitats are connected via the movement of organisms and
346 individual shifts. In the past, research on the relationship between nekton communities
347 and habitats in Haizhou Bay was not deep enough. In the future, the principles of
348 connectivity should be taken into account, and artificial reefs should be considered a
349 key component of broader coastal habitat mosaics when designing MPAs and MPA
350 networks that can be applied at different stages of implementation and in different
351 combinations of scenarios.

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357 Data Availability statement

358 The data that support the findings of this study are available from the
359 corresponding author [Shuo Zhang], upon reasonable request.

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491

492 Appendix 1. The number of individuals in the artificial reef area (AR), comprehensive effect area
493 (CEA), aquaculture area (AA), estuary area (EA) and natural area (NA) in Haizhou Bay

	Species	Family	Number of Individuals				
			AR	CEA	AA	EA	NA
Fish	<i>Amblychaeturichthys hexanema</i>	Gobiidae	35	0	2	112	65
	<i>Coilia mystus</i>	Engraulidae	0	0	1	0	0
	<i>Lateolabrax japonicus</i>	Percichthyidae	4	0	0	0	0
	<i>Largehead hairtail</i>	Trichiuridae	0	7	0	0	2
	<i>Larimichthys polyactis</i>	Sciaenidae	33	1	1	35	10
	<i>Konosirus punctatus</i>	Clupeidae	0	0	3	0	2
	<i>Conger myriaster</i>	Congridae	4	0	1	0	3
	<i>Enedrias fangi</i>	Pholidae	1	0	0	0	0
	<i>Engraulis japonicus</i>	Engraulidae	0	0	0	0	1
	<i>Leiognathus rivulatus</i>	Leiognathidae	0	0	0	0	2
	<i>Collichthys lucidus</i>	Sciaenidae	32	6	3	38	93
	<i>Syngnathussp</i>	Syngnathidae	0	0	5	0	0
	<i>Cynoglossus joyneri</i>	Cynoglossidae	31	2	7	13	12
	<i>Odontamblyopus rubicundus</i>	Gobiidae	9	1	10	0	87
	<i>Pennahia argentata</i>	Sciaenidae	16	0	5	6	17
	<i>Rhynchoconger ectenurus</i>	Congridae	0	0	0	0	3
	<i>Johnius belangerii</i>	Sciaenidae	6	0	0	0	8
	<i>Symechogobius hasta</i>	Gobiidae	0	0	1	0	138
	<i>Chaeturichthys stigmatias</i>	Gobiidae	453	57	41	0	345
	<i>Apogon lineatus</i>	Apogonidae	0	3	0	0	0
	<i>Scomberomorus niphonius</i>	Scombrida	0	0	0	0	1
	<i>Paralichthys olivaceus</i>	Paralichthyidae	1	0	1	0	0
	<i>Thryssa kammalensis</i>	Engraulidae	0	0	102	0	17
	<i>Pampus argenteus</i>	Stromateidae	0	1	1	0	0
	<i>Cryptocentrus filifer</i>	Gobiidae	0	0	0	0	2
	<i>Sardinella zunas</i>	Clupeidae	0	0	1340	0	0
	<i>Repomucenus olidus</i>	Callionymidae	13	1	0	0	0
	<i>Tridentiger barbatus</i>	Gobiidae	239	0	0	10	11
	<i>Platycephalus indicus</i>	Platycephalidae	1	0	0	0	0
	<i>Mugilsoiuy Basilewsky</i>	Mugilidae	0	0	20	0	0
	<i>Mugil cephalus</i>	Mugilidae	0	0	18	0	0

	<i>Ilisha elongata</i>	Pristigasteridae	0	0	310	0	22
	<i>Nibea albiflora</i>	Sciaenidae	0	0	0	0	1
	<i>Setipinna tenuifilis</i>	Engraulidae	3	1	0	0	2
	<i>Harpadon nehereus</i>	Synodontidae	0	0	0	0	1
Shrimp	<i>Oratosquilla oratoria</i>	Squillidae	621	10	213	44	798
	<i>Trachypenaeus curvirostris</i>	Penaeidae	509	16	151	0	308
	<i>Alpheus japonicus</i>	Alpheidae	50	12	64	0	189
	<i>Parapenaepsis tenella</i>	Penaeidae	100	16	2	0	36
	<i>Penaeus penicillatus</i>	Penaeidae	2	0	0	2	1
	<i>Fenneropenaeus chinensis</i>	Penaeidae	2	2	0	0	0
	<i>Parapenaepsis hardwickii</i>	Penaeidae	3	0	0	0	0
	<i>Alpheus distinguendus</i>	Alpheidae	166	5	0	0	61
	<i>Exopalaemon carinicauda</i>	Palaemonidae	30	9	10	15	0
	<i>Metapenaepsis dalei</i>	Penaeidae	10	0	0	0	0
	<i>Penaeus semisulcatus</i>	Penaeidae	0	0	0	1	0
Crab	<i>Charybdis japonica</i>	Portunidae	265	0	179	14	24
	<i>Portunus trituberculatus</i>	Portunidae	142	55	610	966	343
	<i>Heikea japonica</i>	Dorippidae	3	0	4	7	2
	<i>Matuta planipes</i>	Calappidae	0	0	0	0	1
	<i>Eucrate crenata</i>	Goneplacoidea	0	0	2	0	0
	<i>Paguridae</i>	Paguridae	0	0	50	0	0
	<i>Pyrhila pisum</i>	Leucosiidae	0	0	2	0	0
Cephalopods	<i>Octopus variabilis</i>	Octopodidae	1	0	0	0	4
	<i>Octopusocellatus</i>	Octopodidae	0	0	1	0	0
	<i>Loligo beka</i>	Loliginidae	76	15	0	0	49
	<i>Sepiella maindroni</i>	Sepiidae	1	0	0	0	0
	<i>cuttlefish</i>	Sepiidae	0	7	0	0	0
Shellfish	<i>Rapana venosa</i>	Muricidae	2	0	4	0	0
	<i>Mactra antiquata</i>	Mactridae	0	0	0	0	1
	<i>Scapharca subcrenata</i>	Arcidae	0	0	27	0	1
	<i>Solen grandis</i>	Solenidae	0	0	1	0	0
	<i>Mytilus coruscus</i>	Mytilidae	0	0	1	0	0
	<i>Glossaulax didyma</i>	Naticidae	0	0	11	0	0
	<i>ostrea gigas tnunb</i>	Ostreidae	0	0	1	0	0
Echinoderms	<i>Asterias amurensi</i>	Benthopectinidae	2	0	0	0	1
	<i>Tamaria sp</i>	Benthopectinidae	1	0	3	0	0
	<i>Echinoidea</i>	Echinoidea	0	0	5	0	0

Figures

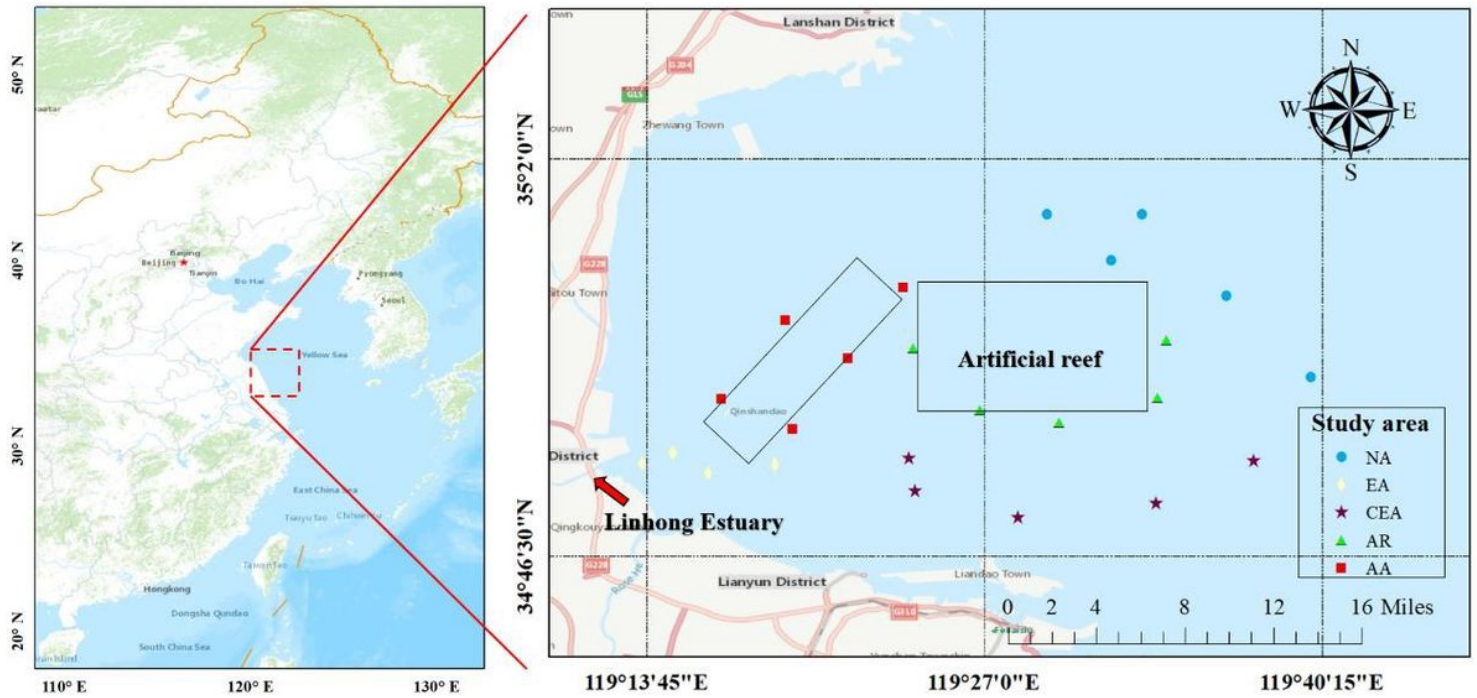


Figure 1

Study area and sampling site 110 AR, CEA, AA, EA and NA represent the artificial reef area, comprehensive effect area, aquaculture 111 area, estuary area and natural area, respectively. Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

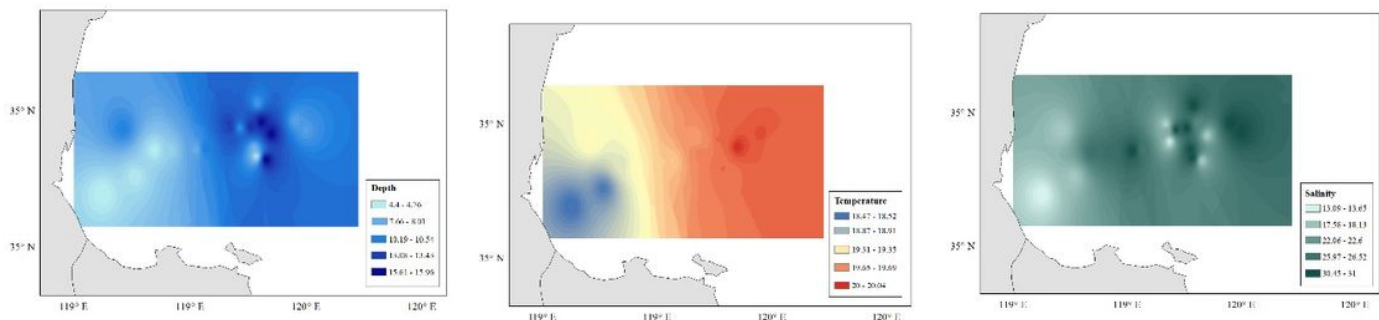


Figure 2

The depth, temperature and salinity distribution of the study area in Haizhou Bay 156 3.2 Nekton community characteristics

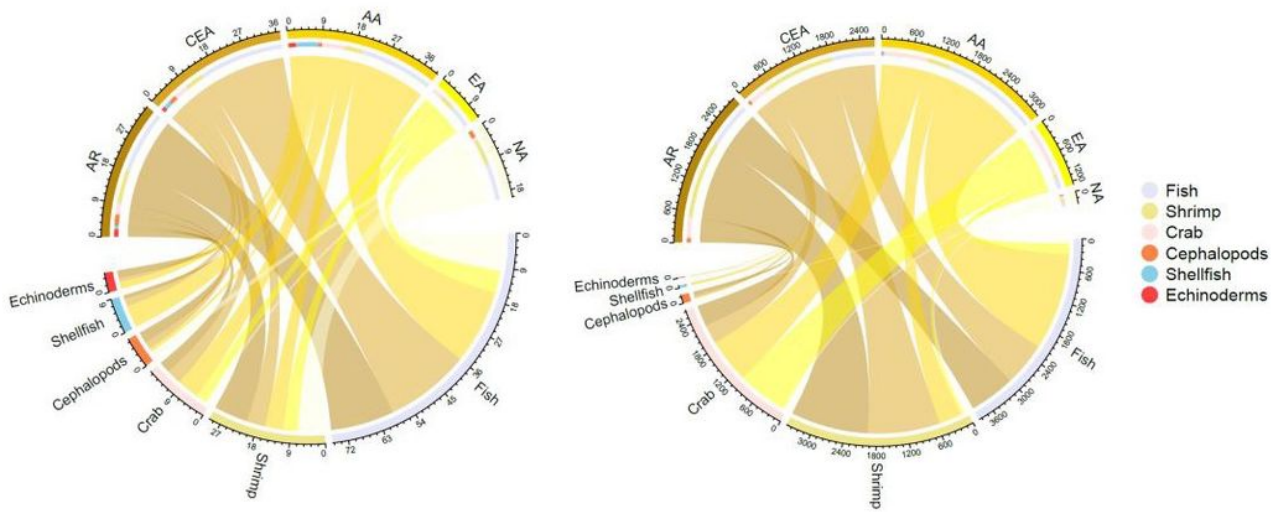


Figure 3

Species and mean numbers of individuals of fish, shrimp, crab, shellfish, and cephalopods in 184 the five habitats

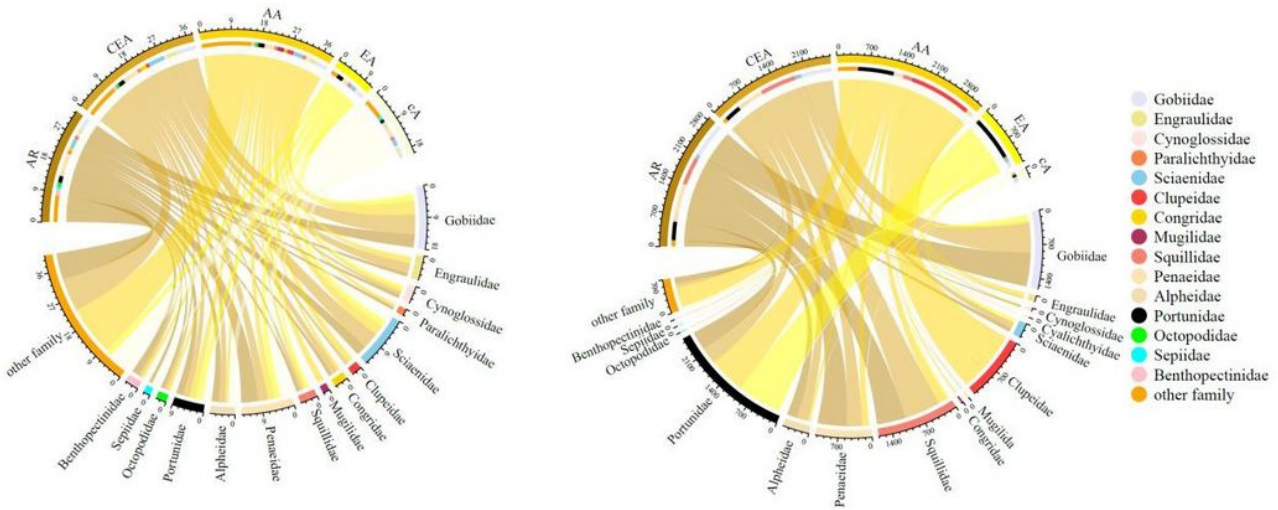


Figure 4

Species and mean numbers of individuals of different families in the five habitats

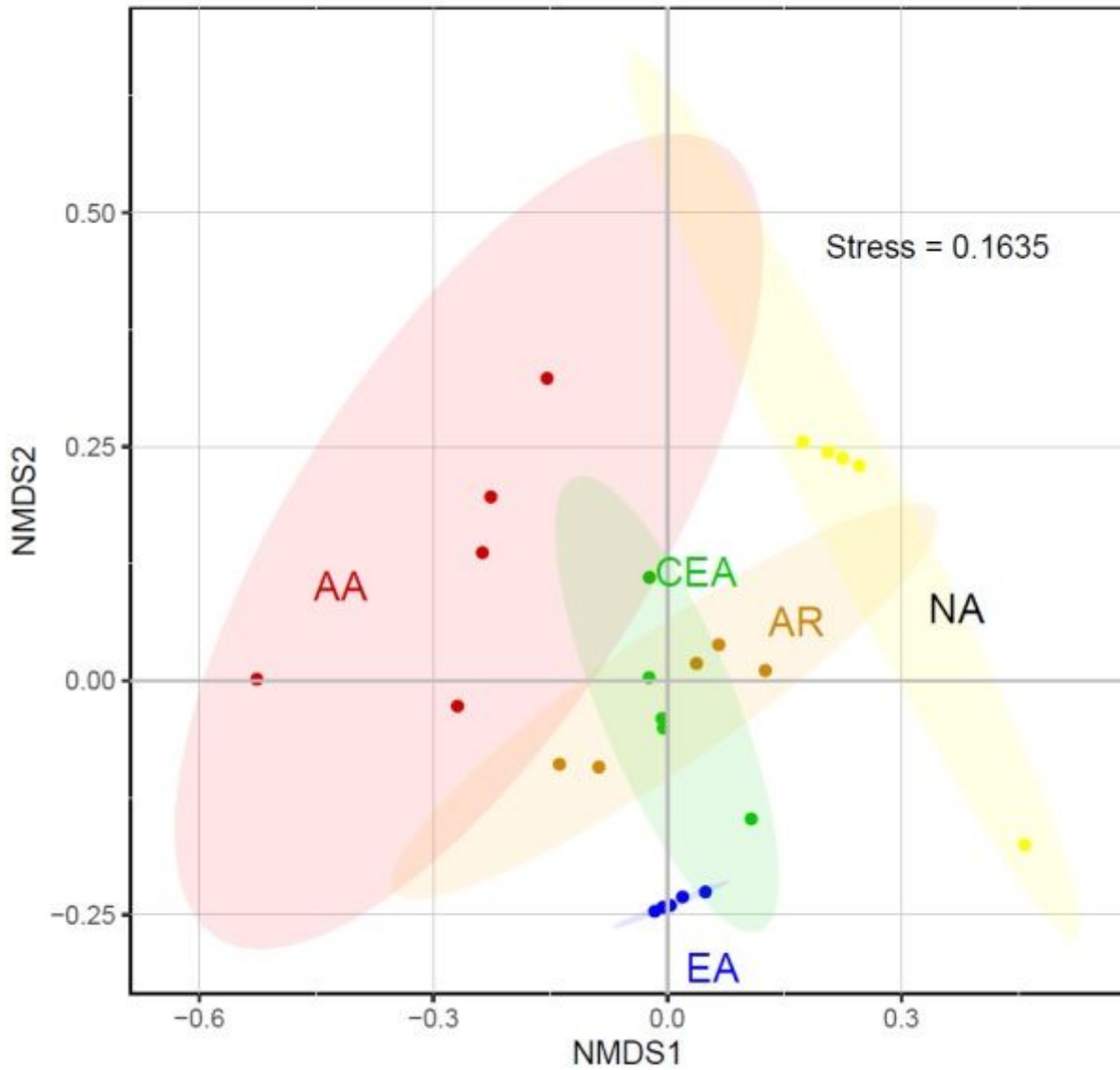


Figure 5

Visualization of the nonmetric multidimensional scale (NMDS) results for the composition of 223 the nekton assemblages sampled by trawling in the different marine habitat types of Haizhou Bay. The 224 stress function value ($0.1635 < 0.2$) shows the rationality of the ranking model. Different colored shaded 225 areas represent the different habitat types, and the points indicate each sampling site. The size of the 226 shaded area reflects the species abundance, and the degree of overlap of shaded areas represents the 227 degree of difference in species abundance between habitats.

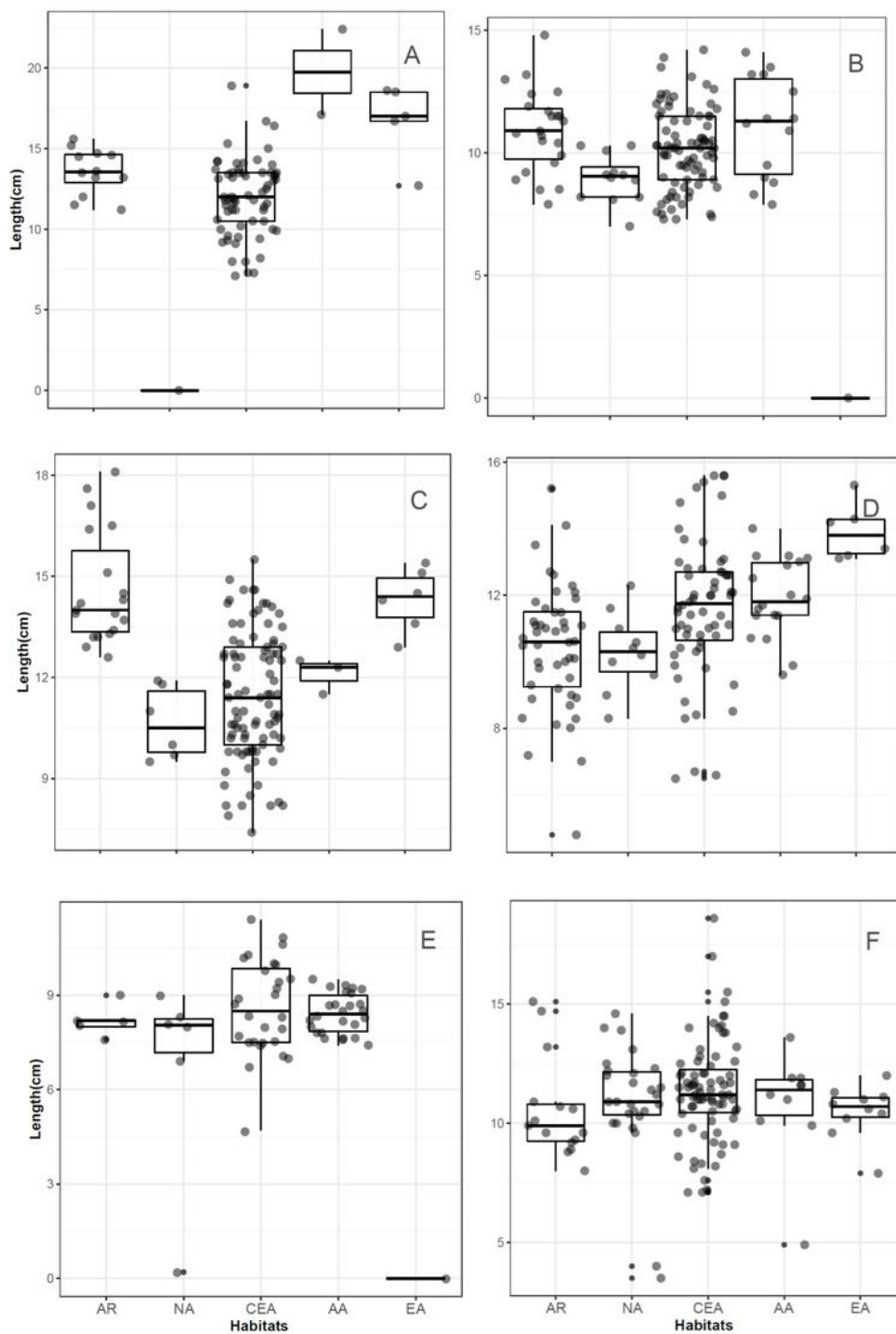


Figure 6

Kernel density analysis for the different size classes of six abundant nekton species with 251 multihabitat associations in Haizhou Bay. The upper and lower limits of the box indicate the 1st and 252 3rd quantiles, respectively, and the inner line of the box indicates the average of the data distribution. 253 (A. *C. stigmatias*, B. *A. hexanema*, C. *C. lucidus*, D. *O. oratoria*, E. *T. curvirostris*, F. *P. 254 trituberculatus*)

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