Partitioning of food resources among three sympatric scorpionfish (Scorpaeniformes) in coastal waters of the northern Yellow Sea

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2 **(Scorpaeniformes) in coastal waters of the northern Yellow Sea**

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21

22   **ABSTRACT**

23   The partitioning of food resources among three abundant co-occurring reef-associated

24   scorpionfish, *Hexagrammos agrammus*, *Hexagrammos otakii* and *Sebastes schlegelii*,

25   was determined on an artificial reef zone in nearshore and offshore coastal waters of

26   northern China, using stomach content and stable isotope analyses (δ^{13}C and δ^{15}N).

27   The three species consumed similar prey items, mainly a variety of crustaceans,

28   teleosts, polychaetes and macroalgae, but the proportions of the items differed among

29   species. The dietary composition of all three scorpionfish differed significantly in

30   nearshore waters, but not between *H. otakii* and *S. schlegelii* in offshore waters where

31   both species fed predominantly on carideans, penaeids and brachyurans. The δ^{13}C

32   values varied significantly among the three scorpionfish in nearshore waters,

33   suggesting that they partition food resources. No significant differences in isotopic

34   signatures were detected between *H. otakii* and *S. schlegelii* in offshore waters.

35   Bayesian mixing models further confirmed that all fish were carnivorous and that the

36   main food sources were assimilated in different proportions. In the nearshore waters,

37   resource partitioning occurs among the three scorpionfish, reducing the potential for

38   competition and the feeding ecology implies that they have dietary plasticity, which

39   facilitates local food web stability and their coexistence.

40

41   **Keywords:** artificial reef, competition, *Hexagrammos*, *Sebastes*, stable isotope

42   analyses, stomach contents analyses.

43

INTRODUCTION

The coexistence of similar fish species in the same habitat is often facilitated by the partitioning of resources in different dimensions such as food, habitat and time (Pianka, 1973; Schoener, 1974; Ross, 1986). Of these, food partitioning is regarded as the most important among temperate, and possibly also tropical marine reef fish assemblages, as it reduces the potential for competition among the species within a fish assemblage (Helfman, 1978; Schoener, 1983; Ross, 1986; Linke et al., 2001; Lek et al., 2011). Understanding the trophic relationship or food partitioning mechanism among fishes, provides insights into the interactions between species and the ability to predict responses to environmental change and anthropogenic perturbations that are likely to results in changes to marine food webs.

Artificial reefs are being used increasingly to provide habitat and enhance fishery resources around the world (Clark & Edwards, 1999; Santos & Monteiro, 2007; Cresson et al., 2014a), particularly in the coastal seas of China. For example, in the Shandong Peninsula (northern China), ~10 million m^3 of artificial reefs of various types, covering 15,000 ha, had been deployed at 170 sites by 2013 (Wu et al., 2016). These reefs were deployed to restore the degraded coastal environment and provide habitat for the aquaculture-based enhancement of cultured juveniles of the economically important sea cucumber *Apostichopus japonica*s and abalone *Haliotis discus hannai* (Zhang et al., 2009). The complex and multi-cavity designed artificial reef structures deployed in different marine ecosystems also provide spatial heterogeneity for reef-associated fish, including recreationally targeted species such as the Spottybelly Greenling *Hexagrammos agrammus*, Fat Greenling *Hexagrammos otakii* and Korean Rockfish *Sebastes schlegelii,* and thus attract and aggregate individuals of these species (García-Charton et al., 2004). These three species of Scorpaeniformes dominate the demersal fish community and contributed more than 80% of the wet weight of the annual catch composition in long-trap net surveys in the area (Wu et al., 2012). Due to their abundance in coastal algal beds, and natural and artificial reefs,

72　these three scorpionfish are particularly important for recreational and commercial

73　fisheries in northern China, Korea and Japan (e.g., Masuda et al., 1984; Yoon, 2002; Lei,

74　2005).

75　　　　The large-scale deployment of artificial reefs in Lidao (Fig. 1) has been assumed

76　to alleviate habitat as a constraining factor to fishery production. Instead, inadequate

77　food resources could intensify inter- and intra-specific competition and become the

78　limiting factor. Although the diets of the three species have been described previously

79　in a number of regions (Kwak et al., 2005; Seo & Hong, 2007; Tong & Guo, 2009; Zhang

80　et al., 2014; Ji et al., 2015), they have not been studied where the species occur

81　sympatrically to enable comparisons of the diet simultaneously.

82　　　　Analysis of stomach content is widely used in dietary studies to provide a

83　"snapshot" of the recent diet of an individual, but does not reflect the sources of

84　carbon and nitrogen assimilated by a species. Such analyses also under-represent soft-

85　bodied animals, which can be digested rapidly, and over-represent prey with hard

86　parts (Fry, 1988; Hobson et al., 1995; Cresson et al., 2014b). Some of these biases can

87　be overcome by using stable isotope analysis (SIA) to complement the stomach

88　content analyses as SIA provides an indication of the primary source of carbon (δ^{13}C)

89　and their trophic level in the food web (δ^{15}N). Stable isotope signatures integrate

90　dietary information over a longer-term period, i.e. several months for muscle tissue

91　(Peterson & Fry, 1987; Lorrain et al., 2002; Guelinckx et al., 2007) and thus provide an

92　indication of trophic flows and position in the food web over longer time scales than

93　stomach content analyses. The recent development of mixing models for stable

94　isotope data within a Bayesian framework e.g. Stable Isotope Analysis in R (SIAR),

95　allows the relative percentage contribution of potential food sources to the consumers

96　diet to be estimated quantitatively (Parnell *et al.*, 2010). This provides a powerful tool

97　for understanding food source partitioning among these fish.

98　　　　In the current study, the diets of three scorpionfishes, i.e. *H. agrammus,*

99　*H. otakii* and *S. schlegelii* were investigated using stomach content analyses, coupled

100   with stable isotope analyses of $\delta^{13}C$ and $\delta^{15}N$, to investigate the food resource

101   partitioning among the three species, and also identify the trophic flows of C and N in

102   this system. Samples were collected at nearshore (~10 m deep and <100 m from shore)

103   and offshore sites (20-30 m deep and ~2 km from shore) in the coastal waters of the

104   Lidao artificial reef zone, in the Yellow Sea (northern China), to understand the pattern

105   differences of food partitioning between the two types of habitat.

106

107   **MATERIAL AND METHODS**

108   *STUDY SITE*

109   This study was carried out at two locations in the coastal zone of Rongcheng Bay

110   (37°13'N, 122°36'E) Yellow Sea, Shandong Peninsula, PR China (Fig. 1). The nearshore

111   site was located in the subtidal zone of the rocky shore and characterized by natural

112   rocky reef and scattered gravel, with a mean water depth of 9-11 m. The offshore site

113   is situated in deeper waters (20-30 m) about 2 km from the coast, in an area zoned for

114   floating kelp (*Laminaria japonica)* and scallop *(Chlamys farreri)* culture where intensive

115   culture is practised (Wu et al., 2016). Kelp cultivation covers an area of 5,000 ha and

116   yielded a harvest of 1,200 t wet weight km^{-2}. The sediments of these waters are

117   characterised by their low profile and presence of a soft muddy layer, 5-15 cm deep

118   on the surface of the substratum (Tu et al., 2007). Between 2006 and 2008, more than

119   7.5 million stone reefs, with a volume of 1 million m^3, 13,015 concrete reefs (650,000

120   m^3) and 60 derelict vessels (4,300 m^3) were deployed in the nearshore and offshore

121   waters to construct the artificial reef zone, consisting of 12 artificial reef groups (Wu

122   et al., 2016). The artificial reefs deployed at the offshore site, however, have

123   submerged some distance into the soft substratum and so do not provide the same

124   extent of habitat as similar structures in the nearshore waters (Wu, unpublished data).

125

126   *COLLECTION OF BIOTA*

127   Individuals of the three species of scorpionfish, i.e. *H. agrammus, H. otakii* and *S.*

128 *schlegelii,* and samples of a range of their potential food sources as well as primary

129 producers were collected in July 2013 (summer). At this time, mean water

130 temperatures and salinities were 21.5°C and 31.5, respectively. Scorpionfish were

131 collected using fish traps deployed on the benthos for about 24 h. After the traps were

132 retrieved, fish were placed in labelled plastic bags on ice, transported to the laboratory

133 and frozen (-20°C) prior to dissection. Each fish was measured (standard length in mm)

134 and weighed (wet weight in g). Approximately 2 g of dorsal white muscle was collected

135 from each fish and frozen (-20 °C) for stable isotope analyses and its stomach was

136 dissected, labelled and frozen for diet analysis.

137 In nearshore waters, food items (including the amphipods; *Ampeliscidae* sp.,

138 *Amphithoe japonica*, *Caprella* sp., *Corophium chinensis*, *Gammarus* sp., *Melita*

139 *palmate and Monoculodes* sp., isopods; *Cymodoce japonica* and *Synidotea*

140 *laevidorsalis*, polychaetes; Nereididae and polychaete spp., carideans & penaeids;

141 *Latreutes planirostris* and *Leptochela gracilis*) were collected from the stomachs of the

142 three species of scorpionfish. The teleost *Enedrias fangi* and Brachyuran *Charybdis*

143 *japonica* were caught using fishing traps. Two species of macroalgae, *Gracilaria*

144 *lemaeiformis* and *Sargassum thunbergii,* and two seagrass species, *Phyllospadixi*

145 *watensis* and *Zostera marina*, were collected by SCUBA diving. All samples of plant

146 tissue were washed with deionized water and kept frozen until analysis.

147 In offshore waters, samples of teleosts; *Enedrias fangi*, Brachyurans; *Charybdis*

148 *japonica* and *Carcinoplax vestita*, Stomatopods; *Oratosquilla oratoria*, carideans &

149 penaeids; *Trachypenaeus curvirostris, Palaemon gravieri, Alpheus distinguendus* and

150 *Alpheus japonicas*, were collected from fish traps. Five macroalgal species

151 (*Gracilaria lemaeiformis, Undaria pinnatifida, Grateloupia turuturu, Ulva pertusa* and

152 *Enteromorpha intestinalis*) were collected by hand from the kelp culture facility.

153

154 *STOMACH CONTENT ANALYSES*

155 A total of 158 and 23 individuals of the three scorpionfishes were collected from

156   nearshore and offshore waters, respectively (Appendix). Prey items were identified to

157   the lowest taxonomic level possible and quantified by calculating the frequency of

158   occurrence (%F), numerical percentage (%N), and the wet weight percentage (%W) of

159   prey, using the following equations:

160          $\%F = A_i/N \times 100$

161          $\%N = N_i/N_{total} \times 100$

162          $\%W = W_i/W_{total} \times 100$

163   Where A_i is the number of teleosts with prey items i in the stomach, N_i is the

164   number of prey items i in the stomach, N_{total} is the total number of items in the

165   stomach (excluding individuals with empty stomachs) and W_i is the wet weight of the

166   prey item i. Individual fragments of macroalgae and seagrass were counted to estimate

167   N_i. Note that carideans and penaeids were not separated and are treated as one

168   dietary category carideans & penaeids.

169

170   *Preliminary multivariate analyses of dietary composition*

171   The replicate %W data for all of the identifiable prey items, were standardised,

172   square-root transformed and used to construct a Bray-Curtis resemblance matrix. This

173   matrix was used in a preliminary analysis to determine the major sources of diet: data

174   were subjected to a 3-way crossed ANOSIM (Analysis of Similarities; Clarke and Green,

175   1988) to determine whether the diet (%W) differed among Species (3 levels;

176   *H. agrammus*, *H. otakii* and *S. schlegelii*), Length class (2 levels; < 150 mm TL and > 150

177   mm TL) and Site (2 levels; nearshore and offshore). The relative magnitudes of the

178   overall factors (subsuming both main and interaction effects) were assessed via the

179   universally scaled ANOSIM $\bar{R}$ statistic which ranges from ~0, when the average

180   similarity among and within groups (factors) do not differ, to 1, when all samples within

181   each group are more similar to each other than to any of the samples from other

182   groups (Clarke et al., 2014a). Three-way crossed ANOSIM demonstrated that

183   significant differences were detected among Species ($\bar{R}$ = 0.300; *P* = 0.1%) and Sites

184   ($\bar{R}$ = 0.263; P = 0.1%), however, there was no evidence to support a Length class

185   difference ($\bar{R}$ = 0.046; P = 20.4%). As a result, Length class was removed as a factor

186   from subsequent analyses.

187   As the stomachs of individual fish may contain only a small number of the 14

188   dietary categories, two samples of the same species, collected at the same time, may

189   differ markedly in their dietary composition. This variability can mask subtle, but 'true',

190   trends in diet and thus the stomachs of the samples for each species at each site were

191   randomly sorted into groups of 2 to 4, depending on the total number of fish in the

192   samples (Lek et al., 2011). The percentage composition of the different dietary

193   categories based on weight, for each group of replicates (*i.e.* 2-4 fish from the same

194   species at the same site), were averaged. The resultant data were then square-root

195   transformed to down-weight the contributions of dietary categories with consistently

196   high values and avoid any tendency for those dietary components to be excessively

197   dominant. This averaging approach overcomes any potential bias created by pooled

198   dietary data obtained from markedly unequal numbers of individual guts, *i.e.* a 'species'

199   accumulation effect (Lek et al., 2011). While, this approach did result in a slightly

200   unequal number of replicates across the species and sites, such unbalanced statistical

201   designs are able to be analysed effectively using contemporary multivariate

202   techniques such as PERMANOVA and ANOSIM (Anderson et al., 2008; Clarke & Gorley,

203   2015).

204

205   *Intraspecific differences in dietary composition*

206   One-way ANOSIM tests were employed to determine whether the dietary composition

207   of *H. otakii* and *S. schlegelii* differed among these sites. It was not possible to do this

208   for *H. agrammus* as only a single fish was caught in the offshore waters and its stomach

209   was empty. Separate Bray-Curtis resemblance matrices were constructed for each

210   species from the averaged replicates and subjected to ANOSIM. Non-metric

211   multidimensional scaling (nMDS) ordination plots were used to visually demonstrate

212 the differences and similarities between factors. In those cases where ANOSIM

213 detected a significant difference, Similarity Percentages analysis (SIMPER; Clarke &

214 Gorley, 2015) and shade plots (Clarke et al., 2014b) were employed to identify those

215 dietary categories that typified and distinguished the dietary composition of each *a*

216 *priori* group (SIMPER) and those dietary categories that contributed the greatest

217 proportion by weight to the difference (shade plot).

218

219 *Interspecific differences in dietary composition*

220   Separate one-way ANOSIM tests were employed to determine whether the

221 dietary composition differed significantly (i) among *H.* agrammus, *H. otakii* and

222 *S. schlegelii* in the nearshore waters and (ii) between *H. otakii* and *S. schlegelii* in the

223 offshore waters. As with the above intraspecific analyses, nMDS ordinations, SIMPER

224 and shade plots and were also employed to visualise and determine the cause of any

225 interspecific differences in dietary composition.

226

227 *STABLE ISOTOPE ANALYSES*

228 All samples collected were stored at -20°C in the laboratory, freeze-dried at -80°C for

229 48 h and then ground to fine, homogeneous powder with a glass mortar and pestle.

230 Fish muscle tissues were defatted in a solution of chloroform and methanol (volume

231 ratio = 2:1). To remove any inorganic carbonates of samples, 1M HCl was added slowly

232 to the sample until it stopped producing CO_2. Samples were then centrifuged five times

233 at 3,000 rpm for 10 min, and the supernatant was then tested with pH paper before

234 removal. When the pH of the supernatant was close to that of deionized water, the

235 sample was freeze-dried and stored at -80°C, otherwise, samples were rinsed with

236 deionized water until they reached the pH of deionized water (Jacob et al., 2005).

237   Samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ at the Qingdao Institute of Marine

238 Geology, using a Flash EA 1112 Elemental analyser coupled with a Thermo Finnigan

239 MAT 253 stable isotope ratio mass Spectrometers (EA-IRMS), via a Thermo Finnigan

240 Conflo III interface. The stable isotope ratios are expressed in delta (δ) unit notation as

241 deviations from the international standards (Vienna Pee Dee Belemnite for carbon and

242 atmospheric nitrogen for nitrogen) according to the following formula:

243     $\delta^{13}C$ or $\delta^{15}N$ ‰=[(R_{sample}/$R_{standard}$)-1]*1000, where R = ^{13}C/^{12}C or ^{15}N/^{14}N.

244   Shapiro-Wilk tests showed that the stable isotope data were not normally

245 distributed. Consequently, non-parametric Kruskal-Wallis tests were used to test

246 whether the isotopic signatures ($\delta^{13}C$ and $\delta^{15}N$) differed significantly among the three

247 scorpionfish species and the potential food sources.

248

249 *Stable isotope mixing model*

250 Bayesian stable isotope mixing models SIAR v4.0 (Stable Isotope Analysis in R) were

251 run in R (Parnell et al., 2010) to evaluate the relative contribution of potential food

252 sources to the diet of the three scorpionfish species in nearshore waters. Mixing

253 models consider stable isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of consumers, multiple

254 potential food sources, and discrimination factors with uncertainty of these values

255 within the model (Parnell et al., 2010). A total enrichment fractionation (TEF) of 1.3‰

256 was selected for ^{13}C, based on the results of a study by Cai et al. (1999) in Laoshan Bay,

257 which is adjacent to the Lidao Bay and contains similar nearshore rocky reefs. The TEF

258 chosen for ^{15}N of 3.4‰ was selected from Minagawa and Wada (1984). Credibility

259 intervals (CI) of 0.95, 0.75 and 0.25 were computed and displayed on figures (Parnell

260 et al., 2010; Lebreton et al., 2012).

261

262 **RESULTS**

263 *DIETARY COMPOSITION*

264 All except one of the fish collected from offshore waters and 54% of those from the

265 nearshore areas had identifiable items in their stomachs (Appendix). The diets of the

266 three scorpionfish were characterized by teleosts, crustaceans, molluscs, cephalopods,

267 polychaetes, teleost eggs, macroalgae and seagrass (Table 1). The quantitative diet

268   indices of %W, %N and %F all show that teleosts, crustaceans, polychaetes and

269   macroalgae were the dominant prey items in nearshore waters. *Hexagrammos*

270   *agrammus* and *H. otakii* fed on a more diverse range of prey than *S. schlegelii.*

271   *Hexagrammos agrammus* preyed principally on polychaetes, representing 33.7% of

272   diet by weight (%W) and 66.7% by frequency of occurrence (%F, Table 1). Crustaceans

273   and macroalgae were also important in the diet of *H. agrammus*, accounting for 29.5%

274   and 23.73% of the total prey items by weight, respectively. However, macroalgae

275   presented a higher %N (80.4%) and %F (79.4%) than crustaceans (5.85% and 64.1%,

276   respectively), due to the method of quantifying macroalgae, which was done by

277   counting the number of digested macroalgae segments. In the nearshore waters,

278   *Hexagrammos otakii* preyed more on crustaceans, as demonstrated by the %W (82.2%)

279   and %F of 72.2%. Brachyurans were the dominant crustaceans in the stomach contents

280   (%W = 67.45%), followed by carideans & penaeids (%W = 10.5%). Although macroalgae

281   and polychaetes were present in the stomachs of *H. otaki*, the %W and %F values were

282   much lower than those for *H. agrammus*. Teleosts, comprised the majority of the diet

283   of *S. schlegelii*, accounting for 79.7%, 21.4% and 65.5% for %W, %N and %F,

284   respectively, and brachyurans (F% = 20.7%).

285   The number of fish caught in offshore waters (23) was lower than in nearshore

286   waters (158) and only one *H. agrammas* was caught in the offshore and this individual

287   did not have items in the stomach (Appendix). In offshore waters, *H. otakii* and

288   *S. schlegelii* consumed mainly crustaceans, especially Carideans & Penaeids (88.8%

289   and 62.9% by %W, respectively), while *S. schlegelii* also consumed significant weights

290   of stomatopods (22.4%) and brachyurans (13.7%). The stomachs of *H. otakii* contained

291   some macroalgal material, which came from the cultivated kelp.

292

293   *MULTIVARIATE ANALYSES OF DIET*

294   *Intraspecific differences in dietary composition*

295   ANOSIM showed that the dietary composition of *H. otakii* differed significantly

296   between nearshore and offshore waters (P= 4.6%; R = 0.369), with samples from fish

297   in offshore waters lying to the right on the nMDS plot, and typically well separated

298   from fish in nearshore waters (Fig. 2a). Carideans & penaeids were identified by

299   SIMPER as typifying the diet of fish from offshore waters, whereas *H. otakii* in

300   nearshore waters consumed a more varied diet, comprising polychaetes, macroalgae,

301   brachyurans and teleosts, in addition to carideans & penaeids (Fig. 3a).

302   ANOSIM detected a significant and very strong difference between the dietary

303   composition of *S. schlegelii* in the nearshore and offshore waters (P= 0.1%; R = 0.935),

304   as evidenced by their complete separation on the nMDS plot (Fig. 2b). SIMPER analysis

305   indicated that teleosts typified the diet of *S. schlegelii* in nearshore waters, while

306   carideans & penaeids and brachyurans were found in consistently larger volumes in

307   samples from offshore waters. This is also shown on the shade plot (Fig. 3b), which

308   shows that polychaetes, made a substantial contribution to the prey consumed in

309   nearshore waters, together with brachyurans and carideans & penaeids, albeit to a

310   lesser extent than in deeper waters.

311

312   *Interspecific differences in dietary composition*

313   ANOSIM showed that the overall dietary composition of the three scorpionfish in

314   nearshore waters differed significantly (P = 0.1%; R = 0.579) and that each pairwise

315   comparison was also significant (P = 0.1%). The greatest difference in diet in nearshore

316   waters was between *H. agrammus* and *S. schlegelii* (R = 0.921), followed by *H. otakii*

317   and *S. schlegelii* (R = 0.447) and lastly between the two *Hexagrammus* species

318   (R = 0.347). These trends are illustrated on the nMDS plot where the samples

319   representing *H. agrammus* and *S. schlegelii* form a broad group that do not overlap,

320   whereas the samples for *H. otakii* are interspersed to some extent with samples from

321   the other two species (Fig. 4a).

322   The pattern of dietary composition among the three species exhibits a clear trend

323   along the diagonal of the shade plot with macroalgae, polychaetes and amphipods

324 making consistently larger contributions to the stomach contents of *H. agrammus*

325 (Fig. 5a). Although both macroalgae and polychaetes were consumed by *H. otakii,*

326 their %Ws were lower and that of carideans & penaeids and, in some cases, teleosts,

327 brachyurans and gastropods were higher than those of the other species. The diet of

328 *S. schlegelii* in the nearshore was the most distinct of the three species, comprising

329 mainly teleosts, with smaller contributions of brachyurans and carideans & penaeids

330 (Fig. 5a).

331 Although ANOSIM detected a significant difference in the dietary composition

332 of *H. otakii* and *S. schlegelii* in the offshore waters (P = 0.1%), the R value for this

333 difference was small (Global R = 0.238). Two of the five samples of *S. schlegelii* on the

334 nMDS plot lie with the group of samples representing *H. otakii* (Fig. 4b). Carideans &

335 penaeids dominated the diets of both species in the offshore waters, albeit to a greater

336 extent in *H. otakii* (Fig. 5b).

337

338 *STABLE ISOTOPES ANALYSES*

339 In nearshore waters, the δ^{13}C and δ^{15}N values of the three scorpionfish, and those of

340 their potential prey, except amphipods and isopods, differed significantly (Kruskal-

341 Wallis tests; both df = 2; p = <0.001). Thus, both of these crustacean taxa were

342 combined, as one potential group of prey in the subsequent SIAR analysis. The mean

343 δ^{13}C ratios (± 1 SD) of primary benthic producers in the nearshore waters varied from

344 -21.3 ± 0.6‰ for the red alga *Gracilaria lemaeiformis* to -12.3 ± 1.1‰ for seagrass

345 (Fig. 6a). The mean δ^{15}N values ranged from 6.6 ± 0.5 ‰ for the brown alga *Sargassum*

346 *thunbergii* to 15.3 ± 0.3 ‰ for *H. otakii*. The stable isotope signatures of brachyurans,

347 polychaetes and carideans & penaeids were very similar and difficult to distinguish on

348 the bi-plot (Fig. 6a).

349 In offshore waters, the stable isotope signatures of *H. otakii* and *S. schlegelii*

350 did not differ significantly (df = 2; p = > 0.05, see also Table 2). However, the main

351 potential food taxa exhibited significant difference in their δ^{13}C and δ^{15}N ratios

352 (Kruskal-Wallis test, df = 9; *p* < 0.001). Mean δ^{13}C ratios varied from -21.1 ± 0.6 ‰ for

353 the macroalgae *G. lemaeiformis* to -15.8 ± 0.4 ‰ for the teleost *Enedrias fangi*. The

354 mean δ^{15}N ratios ranged from the minimum value of 8.0 ± 0.5‰ for macroalgae to

355 14.4 ± 0.1‰ for *S. schlegelii* (Fig. 6b).

356

357 *Interspecific comparison of stable isotopes ratios*

358 The stable isotope ratios of species found in both nearshore and offshore waters were

359 compared to test for spatial differences. These species included: teleosts (*H. otakii*,

360 *S. schlegelii* and *E. fangi*), brachyuran (*C. japonica*), carideans & penaeids

361 (*L. planirostris* and *L. gracilis* [nearshore] and *T. curvirostris, A. distinguendus,*

362 *P. gravieri* and *A. japonicus* [offshore]) and macroalgae (*S. thunbergii* [nearshore] and

363 *G. lemaeiformis, U. pinnatifida, G. turuturu, U. pertusa* and *E. intestinalis* [offshore];

364 Table 2). *Hexagrammus otakii* and *S. schlegellii* had greater δ^{13}C and δ^{15}N values in

365 nearshore than offshore waters. However, the δ^{15}N of the macroalgae, brachyuran *C.*

366 *japonica* and carideans & penaeids, were enriched in offshore waters. The stable

367 isotopic signatures of the teleost *E. fangi* did not differ significantly between nearshore

368 and offshore waters (Table 2).

369

370 *Results from mixing models*

371 The Bayesian stable isotope mixing models estimated that teleosts, carideans &

372 penaeids, brachyurans and polychaetes were the principal contributors to the stable

373 isotopic ratios of the three scorpionfishes in nearshore waters (Fig. 7). Carideans &

374 penaeids were the predominant prey of *H. agrammus*, accounting for an estimated

375 40.6% of their diet. Other important items were brachyurans (14.9%), polychaetes

376 (12.1%) and macroalgae (11.8%). Teleosts (22.9%), carideans & penaeids (23.0%),

377 brachyurans (22.0%) and polychaetes (17.5%) were also estimated to be important for

378 *H. otakii*. Carideans & penaeids were also identified as the most important potential

379 contributor to the C and N assimilated by *Sebastes schlegelii* (31.1%) followed by

380 brachyurans (19.6%), polychaetes and teleosts (14.8%; Fig. 7). Generally, crustaceans

381 (including carideans & penaeids and brachyurans) were estimated to be the major prey

382 for the three species of scorpionfish, contributing over 50% to the isotopic ratios for

383 each species.

384

385 **DISCUSSION**

386 This study investigated the potential partitioning of food resources of three species of

387 scorpionfishes in the Lidao coastal zone where extensive artificial reefs are deployed

388 and mariculture of kelp and scallops is practised in the deeper, offshore waters. The

389 combination of stomach content and stable isotopes analyses indicated the three

390 species of scorpionfish, i.e *Hexagammos agrammus*, *H. otakii* and *Sebastes schlegelii*,

391 partitioned their food resources in the nearshore waters but that this was not evident

392 for *H. otakii* and *S. schlegelii* in the offshore. Thus, although all each species were

393 predominantly carnivorous, feeding on differing amounts of crustaceans (brachyurans

394 and carideans & penaeids), polychaetes, teleosts, they also fed on microalgae, albeit

395 to a lesser extent.

396 Multivariate analysis of the percentage contribution by weight of the various

397 prey categories in stomachs confirmed that they were partitioning the food resources

398 among the three species in the nearshore waters. *Hexagrammos agrammus* and

399 *H. otakii* preyed on a more diverse range of prey than *S. schlegelii,* which specialised

400 on telesosts in the nearshore. The first of these species fed on relatively low trophic

401 level food items like polychaetes, amphipods, polyplacophorans and macroalgae.

402 These results parallel those of previous studies on the diet of *H. agrammus* (Kwak et

403 al., 2005; Wang et al., 2012; Ji et al., 2015), confirming the significance of reef

404 associated benthos in its diet and that it is a benthic omnivore. In spite of the

405 occurrence of macroalgae and polychaetes in the diet of *H. otakii*, this species

406 consumed a much higher proportion of crustaceans (predominantly brachyurans;

407 almost 70% of diet by weight) and teleosts than *H. agrammus*. Similar observations

408   were reported in Gouqi Island (Wang *et al.*, 2012), in the nearshore marine waters of

409   Qingdao, China (Ye, 2012), Northern Primorye, Russia (Balanov et al., 2001; Kolpakov

410   et al., 2005) and on the Jangbong tidal flat, Incheon, Korea (Seo & Hong, 2007). These

411   findings across broad geographic regions and those from the current study all confirm

412   that *H.otakii* is a zoobenthivore, with preference for crustacean prey.

413   The diets of the two *Hexagrammos* species differed in the nearshore waters

414   with *H. otakii* primarily preying on crustaceans and fish, while *H. agrammus* fed on

415   smaller-sized benthic prey such as polychaetes, macroalgae, amphipods and

416   polyplacophorans. These dietary differences reflect their differences in microhabitat

417   use that lead to differences in prey availability (Kwak et al., 2005). For example,

418   *H. agrammus* lives among algae growing on rock, while the co-existing *H. otakii* is

419   widely distributed on, or under rocks, and over sandy substratum between rocks

420   (Kanamoto, 1979).

421   The diet of *Sebastes schlegelii* in the nearshore contrasted with that of the two

422   species of *Hexagrammos* and was characterized by a higher order carnivorous diet,

423   consuming greater quantities of teleosts and large crustaceans (brachyurans and

424   carideans & penaeids). These findings are consistent with those for *S. schlegelii* by

425   Zhang *et al*. (2014) in the Bohai Sea and Seo and Hong (2007) in the Jangbong tidal flat,

426   Incheon, Korea. Despite *Hexagrammos otakii* and *S. schlegelii* feeding on similar prey,

427   the latter species consumed greater proportions of fish. *Sebastes schlegelii* was

428   identified as a potential keystone species in the system in an Ecopath food web model

429   of the Lidao eocsystem, indicating it had a greater impact on trophic flows in the

430   system than predicted based on its biomass alone, probably because of its

431   consumption of higher order trophic groups (Wu et al., 2016).

432   The differences of carbon isotopic ratios among the three scorpionfish in the

433   nearshore waters indicate, like the stomach content analyses, trophic niche

434   segregation among the species, which facilitates reduced competition for food

435   resources (Mablouké *et al.*, 2013). In the nearshore waters, the δ^{15}N values also

436   implied differences in trophic position of the three teleosts: with *Hexagrammos*

437   *agrammus* having a lower mean $\delta^{15}N$ value (14.5 ‰) than *H. otakii* (15.3 ‰) and

438   *S. schlegelii* (15.0 ‰), indicating that the latter two species occupied a slightly higher

439   trophic level than *H. agrammus*. Stomach contents possibly explained the cause of

440   isotopic discrepancy existing between these fishes, with *H. agrammus* feeding

441   primarily on polychaetes and macroalgae, which tend to have lower $\delta^{15}N$ values. In

442   contrast, *H. otakii* and *S. schlegelii* consumed mainly crustaceans and small benthic

443   fish, which had higher $\delta^{15}N$ values. The order for trophic levels indicated by the $\delta^{15}N$

444   values from the current study are consistent with the estimated mean tropic levels for

445   these species documented in FishBase, which assigns a mean trophic level ($\pm$ 1 SE) of

446   3.3 $\pm$ 0.5 to *H. agrammus*, 3.8 $\pm$ 0.3 to *H. otakii* and 3.7 $\pm$ 0.6 to *S. schlegelii* (Froese &

447   Pauly, 2014).

448   The results produced by the mixing model in SIAR complemented those of

449   stomach content analyses, with the macroalgae estimated to make a greater

450   contribution to the diet of *H. agrammus* than the other two species. This result is

451   consistent with the amount of macroalgae found in the stomach contents of

452   *H. agrammus* (23.7 % by wet weight). Although seagrass, which is enriched in ^{13}C

453   compared to macroalgae, was also found in the stomach contents, the stable isotope

454   ratios of the fish indicated that little seagrass is being assimilated by fish. Thus, it is

455   possible that fish did not eat seagrass intentionally or that seagrass were ingested as

456   a by-product of feeding and only a small component assimilated in the tissue. Mixing

457   models results also estimated a greater contribution of crustaceans (brachyurans) and

458   teleosts to the diets of *H. otakii* and *S. schlegelii* than *H. agrammus*.

459   In contrast, the importance of some dietary components differed between the

460   two methods used in this study. For example, SIAR estimated   that carideans &

461   penaeids were the most important prey items assimilated by *H. agrammus* (40.6%) in

462   nearshore waters. However, stomach contents, indicated that this species consumed

463   principally polychaetes and macroalgae. The discrepancies between stomach contents

464 and stable isotope analyses are due to differences in "time scales" of the data:

465 stomach contents give dietary information on "the last meal" at a more or less

466 instantaneous point in time, while stable isotope signatures result from feeding and

467 assimilation of nutrients over two to three months, i.e. not only what was ingested

468 immediately prior to capture, but also what was incorporated in the tissues after

469 ingestion and assimilation (MacNeil et al., 2006).

470 From the more limited data available for the offshore water, the dietary

471 compositions of *H. otakii* and *S. schlegelii* were more similar than in nearshore waters,

472 with both species consuming primarily carideans & penaeids, followed by brachyurans

473 and stomatopods. This is consistent with the high abundance of carideans & penaeids,

474 brachyurans and stomatopods in the benthic faunal community of the offshore (Wu

475 et al., 2012). These results from the nearshore and offshore waters confirm that *H.*

476 *otakii* and *S. schlegelii* are opportunistic predators. Their similarity in stomach contents

477 is consistent with the similar δ^{15}N signatures for *H. otakii* and *S. schlegelii* in offshore

478 waters and is probably due to the relatively homogeneous environment in offshore

479 waters (i.e. low profile, soft sediments) and consequently lower numbers and diversity

480 of niches and available prey. The large production of kelp in offshore waters and its

481 dominance as a primary producer over other potential food sources, is also likely to be

482 an important factor as this simplifies the food web compared with nearshore waters.

483 An Ecosim model for the study area predicted that kelp cultivation favours benthic,

484 rather than water column production, thus the kelp culture actually supports the

485 benthic trophic flows to benthos, including the scorpionfish (Wu et al. 2016).

486

487 SPATIAL VARIATION IN STABLE ISOTOPIC RATIOS

488 The isotopic signatures of selected taxa differed significantly between the nearshore

489 and offshore waters. The δ^{13}C signatures of fish (*H. otakii* and *S. schlegelii*),

490 brachyurans and macroalgae in nearshore waters were enriched compared with the

491 offshore waters. This gradient pattern is consistent with the results of Newsome *et al*

492   (2007). The offshore isotopic ratios of C are more depleted than those along the coast

493   indicating a greater uptake of plankton carbon in the food web than benthic sources.

494   The mean nitrogen ratios of the teleosts were 0.4 to 1.2 ‰ higher in the nearshore

495   site than the offshore, but not for macroalgae, carideans & penaeids and the

496   brachyuran *Charybdis japonica*, which were higher offshore. Enriched nitrogen

497   signatures in food webs may indicate greater anthropogenic inputs of nitrogen to the

498   nearshore than offshore (Costanzo et al., 2001; Lepoint et al., 2004; Michener & Lajtha,

499   2008; Dromard et al., 2013) and the uptake of nutrients by kelp in the mariculture area

500   of the offshore waters. During the early period of kelp growth (~November each year),

501   large quantities of synthetic fertilizers (i.e. Urea; δ^{15}N= 1 to 2.6‰; Heaton, 1986) are

502   released gradually into waters to stimulate kelp growth. This practice changes the

503   concentration of dissolved inorganic nitrogen in the surround water. As a consequence,

504   enriched ^{15}N might be assimilated into the tissue of macroalgae and phytoplankton

505   and further transferred through the food web.

506   The δ^{15}N values for the fish in the current study were higher than those for the

507   same species in Japan (Hoshika et al., 2006), South Korea (Kang et al., 2008) and Russia

508   (Kiyashko et al., 2011), as well as Gouqi Island, in China (Table 3), particularly for

509   *H. agrammus* and *H. otakii*. For these two species, the values in the Lidao reef system

510   were ~3 ‰ higher than those recorded from the other regions. This suggests that

511   anthropogenic coastal activities have increased the δ^{15}N values of the water and as a

512   consequence increased the ^{15}N levels throughout the nearshore food web, as has been

513   found in other regions (Costanzo et al., 2001; Lin & Fong, 2008; Letourneur et al., 2013).

514   Carideans & penaeids and brachyurans from the offshore waters presented

515   higher δ^{15}N values than the values for these taxa in the nearshore waters, probably

516   because of the higher δ^{15}N of the algal detritus in the sediments, which is then

517   consumed by the zoobenthos. Indeed, the brachyuran *C. japonica* and carideans &

518   penaeids are known to be omnivorous in soft mud environment and to take sediment

519   organic matter as food source (Quan et al., 2010).

520   The more enriched carbon stable isotopic signatures of scorpionfish from

521   nearshore waters may be a result of the trophic flow through a relatively long and

522   complex food chain compared with those in offshore waters. The food web of the two

523   waters depends on a variety of carbon sources with a range of trophic pathways. In the

524   more complex nearshore environment, a number of primary producers may contribute

525   to the diets of primary consumers (Alfaro et al., 2006; Franca et al., 2011), while in the

526   offshore waters, kelp cultivation dominates the benthic primary production.

527

528   **CONCLUSION**

529   The present study has provided evidence of the partitioning of food resources among

530   three co-occurring scorpionfish around the artificial reef in the nearshore waters of

531   Rongcheng Bay of the northern Yellow Sea, which would reduce the potential

532   competition for resources. These carnivorous fishes predate on a similar range of prey,

533   including crustaceans, teleosts, polychaetes and macroalgae. The dietary composition

534   of these three scorpionfish in nearshore waters differs, implying that they partition

535   their resources to reduce competition. In the offshore water, the dietary data from

536   fewer individuals indicate that *H. otakii* and *S. schlegelii* had relatively narrow prey

537   options, mainly predating on carideans & penaeids and brachyurans living in the

538   muddy substrate. In the offshore, our results suggested no major differences in diet

539   between these two species which may be due to the abundance of kelp in the offshore

540   and a simplified food web.

541

553

554 **REFERENCES**

555 Alfaro, A.C., F. Thomas, L. Sergent & M. Duxbury, 2006. Identification of trophic
556 interactions within an estuarine food web (northern New Zealand) using fatty
557 acid biomarkers and stable isotopes. Estuarine, Coastal and Shelf Science 70:
558 271-286.

559 Anderson, M.J., R.N. Gorley & K.R. Clarke, 2008. PERMANOVA+ for PRIMER: guide to
560 software and statistical methods. PRIMER-E, Plymouth, UK.

561 Balanov, A.A., A.I. Markevich, D.V. Antonenko & K.D. Crow, 2001. The first occurrence
562 of hybrids of *Hexagrammos otakii* x *H. octogrammus* and description of *H. otakii*
563 (Hexagrammidae) from Peter the Great Bay (the Sea of Japan). Journal of
564 Ichthyology 41: 728-738.

565 Cai, D., F. Meng, Y. Han & S. Gao, 1999. Studies on ^{13}C/^{12}C ratios as a tracer for food
566 web in a marine ecosystem-the trophic relations in pelagic food webs in Laoshan
567 Bay. Oceanologia Et Limnologia Sinica 30: 671-678.

568 Clark, S. & A. Edwards, 1999. An evaluation of artificial reef structures as tools for
569 marine habitat rehabilitation in the Maldives. Aquatic Conservation 9: 5-21.

570 Clarke, K.R. & R.H.Green, 1988. Statistical design and analysis for a 'biological effects'
571 study. Marine Ecology Progress Series 46: 213-226.

572 Clarke, K.R., R.N.Gorley, P.J.Somerfield & R.M.Warwick, 2014a. Change in Marine
573 Communities: an Approach to Statistical Analysis and Interpretation, 3 ed.

574   PRIMER-E Ltd, Plymouth, UK.

575   Clarke, K.R., J.R. Tweedley & F.J. Valesini, 2014b. Simple shade plots aid better long-
576   term choices of data pre-treatment in multivariate assemblage studies. Journal
577   of the Marine Biological Association of the United Kingdom 94: 1-16.

578   Clarke, K.R. & R.N.Gorley, 2015. PRIMER v7: User Manual/Tutorial. PRIMER-E,
579   Plymouth.

580   Costanzo, S.D., M.J. O'Donohue, W.C. Dennison, N.R.Loneragan & M.Thomas, 2001. A
581   New Approach for Detecting and Mapping Sewage Impacts. Marine Pollution
582   Bulletin 42: 149-156.

583   Cresson, P., S.Ruitton & M. Harmelin-Vivien, 2014a. Artificial reefs do increase
584   secondary biomass production: mechanisms evidenced by stable isotopes.
585   Marine Ecology Progress Series 509: 15-26.

586   Cresson, P., S. Ruitton, M. Ourgaud & M. Harmelin-Vivien, 2014b. Contrasting
587   perception of fish trophic level from stomach content and stable isotope
588   analyses: a Mediterranean artificial reef experience. Journal of Experimental
589   Marine Biology and Ecology 452: 54-62.

590   Dromard, C.R., Y. Bouchon-Navaro, S. Cordonnier, M.F. Fontaine, M. Verlaque,
591   M.Harmelin-Vivien & C. Bouchon, 2013. Resource use of two damselfishes,
592   *Stegastes planifrons* and *Stegastes adustus*, on Guadeloupean reefs (Lesser
593   Antilles): Inference from stomach content and stable isotope analysis. Journal of
594   Experimental Marine Biology and Ecology 440: 116-125.

595   Franca, S., R.P. Vasconcelos, S. Tanner, C. Maguas, M.J. Costa & H.N. Cabral, 2011.
596   Assessing food web dynamics and relative importance of organic matter sources
597   for fish species in two Portuguese estuaries: a stable isotope approach. Marine
598   Environmental Research 72: 204-215.

599   Froese, R. & D. Pauly, 2014. Fishbase. World Wide Web electronic publication,
600   version(08/2014). www.fishbase.org.

601   Fry, B., 1988. Food web structure on Georges Bank from stable C, N, and S isotopic
602   compositions. Limnology & Oceanography 33: 1182-1190.

603   García-Charton, J.A., Á. Pérez-Ruzafa, P. Sánchez-Jerez, J.T. Bayle-Sempere, O. Reñones
604   & D. Moreno, 2004. Multi-scale spatial heterogeneity, habitat structure, and the
605   effect of marine reserves on Western Mediterranean rocky reef fish assemblages.
606   Marine Biology 144: 161-182.

607 Guelinckx, J., J. Maes, P. Van Den Driessche, B. Geysen, F. Dehairs & F. Ollevier, 2007.
608   Changes in δ^{13}C and δ^{15}N in different tissues of juvenile sand goby
609   *Pomatoschistus minutus*: a laboratory diet-switch experiment. Marine Ecology
610   Progress Series 341: 205-215.

611 Helfman, G., 1978. Patterns of community structure in fishes: summary and overview.
612   Environmental Biology of Fishes 3: 129-148.

613 Hobson, K.A., W.G. Ambrose & P.E. Renaud, 1995. Sources of primary production,
614   benthic-pelagic coupling, and trophic relationships within the Northeast Water
615   Polynya: Insights from δ^{13}Cand δ^{15}N analysis. Marine Ecology Progress Series 128:
616   1-10.

617 Hoshika, A., M.J. Sarker, S. Ishida, Y. Mishima & N. Takai, 2006. Food web analysis of an
618   eelgrass (*Zostera marina* L.) meadow and neighbouring sites in Mitsukuchi Bay
619   (Seto Inland Sea, Japan) using carbon and nitrogen stable isotope ratios. Aquatic
620   Botany 85: 191-197.

621 Jacob, U., K. Mintenbeck, T. Brey, R. Knust & K. Beyer, 2005. Stable isotope food web
622   studies: a case for standardized sample treatment. Marine Ecology Progress
623   Series 287: 251-253.

624 Ji, D., X. Bian, N. Song & T. Gao, 2015. Feeding ecology of *Hexagrammos agrammus* in
625   Lidao Rongcheng, China. Journal of Fishery Sciences of China(in Chinese) 22: 88-
626   98.

627 Jiang, R., S. Zhang, K. Wang, X. Zhou & J. Zhao, 2014. Stable isotope analysis of the
628   offshore food web of Gouqi Island. Chinese Journal of Ecology(in Chinese) 33:
629   930-938.

630 Kanamoto, Z., 1979. On the ecology of Hexagrammid fish V: Food items of *Agrammus*
631   *agrammus* (Temminck et Schlegel) and *Hexagrammos otakii* Jordan et Starks
632   sampled from different habitats around a small reef. Japanese Journal of
633   Ecology(in Chinese) 29: 265-271.

634 Kang, C.K., E.J. Choy, Y. Son, J.Y. Lee, J.K. Kim, Y. Kim & K. S.Lee, 2008. Food web
635   structure of a restored macroalgal bed in the eastern Korean peninsula
636   determined by C and N stable isotope analyses. Marine Biology 153: 1181-1198.

637 Kiyashko, S.I., T.A. Velivetskaya & A.V. Ignatiev, 2011. Sulfur, carbon, and nitrogen stable
638   isotope ratios in soft tissues and trophic relationships of fish from the near-shore
639   waters of the peter the great bay in the Sea of Japan. Russian Journal of Marine
640   Biology 37: 297-302.

641   Kolpakov, N., E. Barabanshchikov & Y.D. Valuev, 2005. First Findings of the Fat Greenling
642   *Hexagrammos otakii* (Hexagrammidae) in Waters off Northern Primorye. Journal
643   of Ichthyology 45: 682-684.

644   Kwak, S., G. Baeck & D. Klumpp, 2005. Comparative feeding ecology of two sympatric
645   greenling species, *Hexagrammos otakii* and *Hexagrammos agrammus* in eelgrass
646   *Zostera marina* beds. Environmental Biology of Fishes 74: 129-140.

647   Lebreton, B., P. Richard, R. Galois, G. Radenac, A. Brahmia, G. Colli, M. Grouazel, C.
648   André, G. Guillou & G.F. Blanchard, 2012. Food sources used by sediment
649   meiofauna in an intertidal *Zostera noltii* seagrass bed: a seasonal stable isotope
650   study. MarineBiology 159: 1537-1550.

651   Lei, J., 2005. Marine fish culture theory and techniques. China Agriculture Press, Beijing.

652   Lek, E., D.V. Fairclough, M.E. Platell, K.R. Clarke, J.R. Tweedley & I.C.Potter, 2011. To
653   what extent are the dietary compositions of three abundant, co-occurring labrid
654   species different and related to latitude, habitat, body size and season? Journal
655   of Fish Biology 78: 1913-1943.

656   Lepoint, G., P. Dauby & S. Gobert, 2004. Applications of C and N stable isotopes to
657   ecological and environmental studies in seagrass ecosystems. Marine Pollution
658   Bulletin 49: 887-891.

659   Letourneur, Y., T. Lison de Loma, P. Richard, M.L. Harmelin-Vivien, P. Cresson, D. Banaru,
660   M.F. Fontaine, T. Gref & S.Planes, 2013. Identifying carbon sources and trophic
661   position of coral reef fishes using diet and stable isotope (δ^{15}N and δ^{13}C) analyses
662   in two contrasted bays in Moorea, French Polynesia. Coral Reefs 32: 1091-1102.

663   Lin, D.T. & P. Fong, 2008. Macroalgal bioindicators (growth, tissue N, δ^{15}N) detect
664   nutrient enrichment from shrimp farm effluent entering Opunohu Bay, Moorea,
665   French Polynesia. Marine Pollution Bulletin 56: 245-249.

666   Linke, T., M.Platell & I. Potter, 2001. Factors influencing the partitioning of food
667   resources among six fish species in a large embayment with juxtaposing bare
668   sand and seagrass habitats. Journal of Experimental Marine Biology and Ecology
669   266: 193–217.

670   Lorrain, A., Y.M. Paulet, L. Chauvaud, N. Savoye, A. Donval & C. Saout, 2002. Differential
671   δ^{13}C and δ^{15}N signatures among scallop tissues: implications for ecology and
672   physiology. Journal of Experimental Marine Biology and Ecology 275: 47-61.

673   MacNeil, M.A., K.G.Drouillard & A.T. Fisk, 2006. Variable uptake and elimination of

674   stable nitrogen isotopes between tissues in fish. Canadian Journal of Fisheries
675   and Aquatic Sciences 63: 345-353.

676   Masuda, H., K. Amaoka, C. Arago, T. Ueno & T. Yoshino, 1984. The Fishes of the
677   Japanese Archipelago. Tokai University Press, Tokyo.

678   Michener, R. & K. Lajtha, 2008. Stable isotopes in ecology and environmental science.
679   Blackwell Publishing, Oxford, UK.

680   Minagawa, M.& E. Wada, 1984. Stepwise enrichment of 15N along food chains: further
681   evidence and the relation between δ15N and animal age. Geochimica et
682   Cosmochimica Acta 48: 1135-1140.

683   Newsome, S.D., C.M. del, Rio, S. Bearhop & D.L.Phillips, 2007. A niche for isotopic

684   ecology. Frontiers in Ecology and the Environment 5: 429–436.

685   Parnell, A.C., R. Inger, S. Bearhop & A.L. Jackson, 2010. Source partitioning using stable
686   isotopes: coping with too much variation. Plos One 5: e9672.

687   Peterson, B.J.& B. Fry, 1987. Stable isotopes in ecosystem studies. Annual review of
688   ecology and systematics 18: 293-320.

689   Pianka, E.R., 1973. The structure of lizard communities. Annual review of ecology and
690   systematics 4: 53-74.

691   Quan, W., L. Shi & Y. Chen, 2010. Stable isotopes in aquatic food web of an artificial
692   lagoon in the Hangzhou Bay, China. Chinese Journal of Oceanology and
693   Limnology 28: 489-497.

694   Ross, S.T., 1986. Resource partitioning in fish assemblages: a review of field studies.
695   Copeia, 1986: 352-388.

696   Santos, M.N. & C.C.Monteiro, 2007. A fourteen-year overview of the fish assemblages
697   and yield of the two oldest Algarve artificial reefs (southern Portugal).
698   Hydrobiologia 580: 225-231.

699   Schoener, T.W., 1974. Resource partitioning in ecological communities. Science 185:
700   27-39.

701   Schoener, T.W., 1983. Field experiments on interspecific competition. American
702   Naturalist 122: 240-285.

703   Seo, I.S. & J.S. Hong, 2007. Comparative Feeding Ecology of Sympatric Greenling

704   *Hexagrammos otakii* and Schlegel's Black Rockfish *Sebastes schlegeli* in the
705   Jnngbong Tidal Flat, Incheon, Korea. Korean Journal of Fisheries and Aquatic
706   Sciences(in Korean) 40: 84-94.

707 Tong, Y. & X. Guo, 2009. Feeding competition between two rockfish *Sebastes schlegeli*
708   and *Hexagrammos otakii*. Journal of Fishery Sciences of China(in Chinese) 16,
709   541-549.

710 Tu, Z., X. Zhang, P. Zhang & T. Gao, 2007. Artificial fish reef near Lidao Island in
711   Rongcheng—early assessment on resource and environment in sea area for
712   artificial fish reef. Marine Fisheries Information 22: 1-7.

713 Wang, K., S. Zhang, Z. Wang, J. Zhao, M. Xu & J. Xu, 2012. Dietary composition and food
714   competition of six main fish species in rocky reef habitat off Gouqi Island Chinese
715   Journal of Applied Ecology(in Chinese) 23: 536-544.

716 Wu, Z., L. Zhang, X. Zhang, P. Zhang & W. Li, 2012. Nekton community structure and its
717   relationship with the main enviornmental variables in Lidao artificial reef zones
718   of Rongcheng. Acta Ecologica Sinica(in Chinese) 32: 6737-6746.

719 Wu, Z., X. Zhang, H.M.Lozano & N.R. Loneragan, 2016. Trophic flows, kelp culture and
720   fisheries in the marine ecosystem of an artificial reef zone in the Yellow Sea.
721   Estuarine, Coastal and Shelf Science S 182: 86-97.

722 Yoon, C.H. 2002. Fishes of Korea with Pictorial Key and Systematic List. Academy
723   Publishing Company. Seoul.

724 Zhang, B., Z. Li & X. Jin, 2014. Food composition and prey selectivity of *Sebastes*
725   *schlegeli*. Journal of Fishery Sciences of China(in Chinese) 21: 134-141.

726 Zhang, X., X. Wang, Z. Tu, P. Zhang, Y. Wang, T. Wang & X. Wang, 2009. Current status
727   and prospect of fisheries resource enhancement in Shandong Province. China
728   Fisheries Economics(in Chinese) 2: 51-58.

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TABLES

Table 1. Percentage contribution of each prey item by wet weight (%W), number (%N) and frequency of occurrence (%F) in stomachs contents of *Hexagrammos agrammus, Hexagrammos otakii* and *Sebastes schlegelii* in the nearshore site and of *H.otakii* and *S. schlegelii* in the offshore site of Lidao, northern China. N is the number of studied stomachs; number of stomachs with dietary items is shown in parentheses.

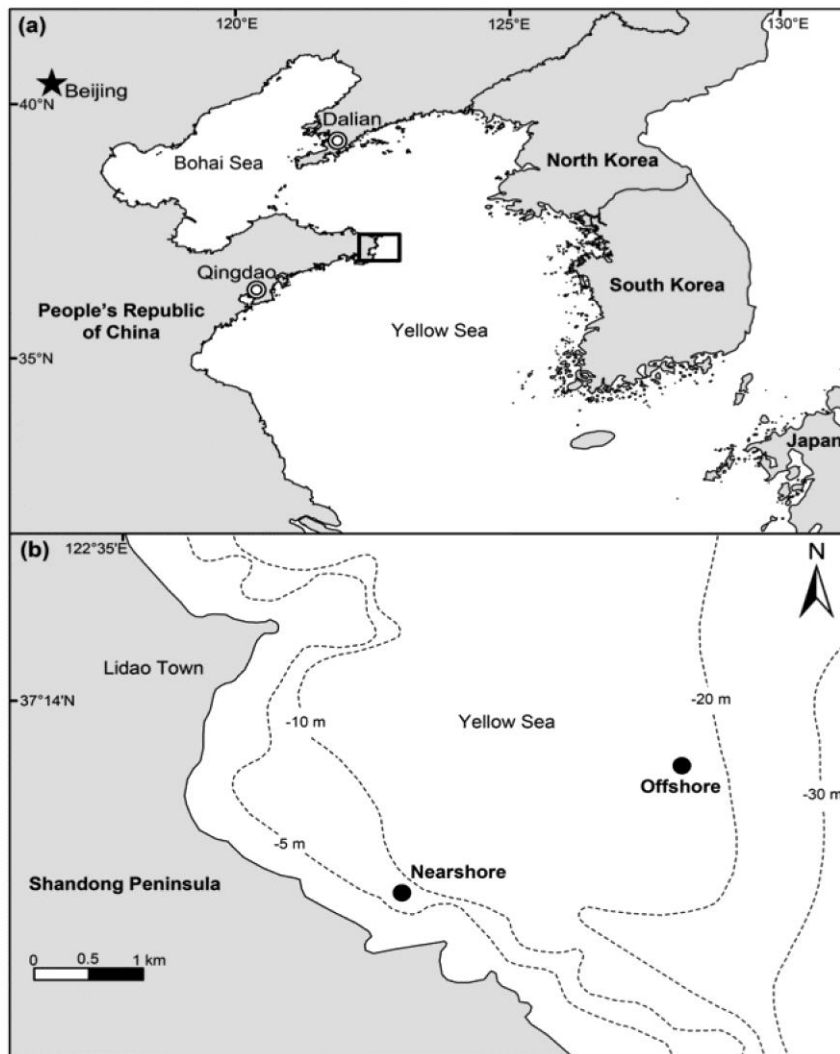| Sites	Nearshore									Offshore					
Teleost species N	*Hexagrammos agrammus* 55 (39)			*Hexagrammos otakii* 23 (18)			*Sebastes schlegelii* 80 (29)			*Hexagrammos otakii* 7 (7)			*Sebastes schlegelii* 15 (15)		
Prey items	%W	%N	%F	%W	%N	%F	%W	%N	%F	%W	%N	%F	%W	%N	%F
Teleosts	6.93	0.06	2.56	11.45	1.28	22.22	79.67	21.43	65.52				0.01	6.67	13.33
Crustaceans	29.50	5.85	64.10	82.21	5.61	72.22	20.31	64.29	51.72	97.6	50	100	99.13	86.67	93.33
Carideans & penaeids	2.84	0.19	5.13	10.47	1.79	38.89	0.65	26.53	20.69	88.75	11.9	85.71	62.94	28.89	53.33
Acetes							0.05	1.02	3.45						
Stomatopods													22.58	2.22	6.67
Amphipods	3.22	4.35	46.15	0.46	1.79	27.78	0.04	10.20	10.34	4.54	30.95	28.57	0.16	11.11	20
Mysidacea							0.00	2.04	3.45						
Isopoda	2.09	0.39	12.82	0.70	0.77	16.67	0.11	3.06	6.90	4.31	7.14	42.86	6.99	28.89	40
Leptostraca							0.04	13.27	10.34				0.01	2.22	6.67
Cumacea	0.06	0.13	5.13												
Megalopa larva	0.16	0.19	5.13												
Brachyurans	8.82	0.58	23.08	67.45	1.28	16.67	19.42	8.16	20.69				6.45	13.33	40
Unidentified	12.31	-	28.21	3.13	-	16.67	0.01	-	3.45						
Molluscs	2.81	0.58	20.51	3.06	2.04	27.78							0.02	2.22	6.67
Polyplacophora	1.98	0.45	15.38												
Gastropods	0.83	0.13	5.13	3.06	2.04	27.78							0.02	2.22	6.67
Cephalopoda													0.66	2.22	6.67
Teuthids													0.66	2.22	6.67
Polychaetes	33.70	5.59	66.67	2.24	9.44	55.56	0.01	9.18	24.14	2.19	3.57	14.29	0.18	2.22	6.67
Nereididae	33.70	5.59	66.67	2.24	9.44	55.56	0.01	9.18	24.14	2.19	3.57	14.29	0.18	2.22	6.67
Teleosteggs				0.01	8.16	5.56									
Macroalgae	23.73	80.38	79.49	0.70	66.33	55.56	0.00	2.04	3.45	0.21	46.43	28.57			
Seagrass	3.33	7.54	25.64	0.32	7.14	27.78	0.00	3.06	3.45						

Table 2. Mean stable isotope values for δ^{13}C and δ^{15}N (± 1 SE) for the three species of scorpionfish, their potential prey and primary producers. Significance values (P) of Kruskal-Wallis tests between nearshore (N) and offshore (O) values are shown for each group. P values < 0.05 are in bold. Macroalgae comparisons were conducted between *Sargassum thunbergii* (nearshore) and all macroalgae (offshore). Numbers in brackets indicate the number of individuals used for the tests.

| Species	Isotope	Mean value (± 1 SE)		P
		Nearshore	Offshore	
Teleosts				
Hexagrammos agrammus	δ^{13}C	-17.03 ± 0.58 (14)	-16.38 (1)	0.280
	δ^{15}N	14.47 ± 0.50 (14)	14.18 (1)	0.510
Hexagrammos otakii	δ^{13}C	-15.99 ± 0.49 (11)	-16.64 ± 0.16 (8)	**< 0.001**
	δ^{15}N	15.31 ± 0.30 (11)	14.11 ± 0.49 (8)	**< 0.001**
Sebastes schlegelii	δ^{13}C	-16.71 ± 0.54 (9)	-18.03 ± 0.16 (2)	**0.006**
	δ^{15}N	14.98 ± 0.59 (9)	14.44 ± 0.07 (2)	**0.006**
Enedrias fangi	δ^{13}C	-16.38 ± 1.14 (2)	-15.82 ± 0.92 (6)	0.510
	δ^{15}N	13.96 ± 0.59 (2)	13.90 ± 0.40 (6)	0.740
Invertebrates				
Charybdis japonica	δ^{13}C	-17.17 ± 0.94 (8)	-18.31 ± 0.58 (9)	**0.009**
	δ^{15}N	13.18 ± 0.37 (8)	13.69 ± 0.28 (9)	**0.006**
Carideans & penaeids	δ^{13}C	-18.81 ± 0.01 (2)	-18.20 ± 0.34 (29)	**0.030**
	δ^{15}N	12.05 ± 0.23 (2)	13.13 ± 0.71 (29)	**0.030**
Primary producers				
Macroalgae	δ^{13}C	-18.33 ± 0.72 (5)	-18.99 ± 1.50 (22)	**0.007**
	δ^{15}N	6.56 ± 0.47 (5)	8.22 ± 0.54 (22)	**0.006**

Table 3. Comparison of mean δ¹⁵N (± SE) for the three species of scorpionfish from the present study with those from other studies in the East China Sea, Japan, Korea and Russia. Values are ranked from highest to lowest **δ¹⁵N** value. — = no length range specified.

| Species and Length(mm)	Rank	δ¹⁵N	Habitat type	Location	References
Hexagrammos agrammus					
88-186	1	14.47 ± 0.50	Rocky reef	Yellow Sea	This study
—	2	14.2	Mud substrate	Yellow Sea	This study
—	3	11.6 ± 0.6	Natural macroalgae bed	East coast of Korea	Kang et al,2008
—	4	10.8 ± 0.2	Barren ground	East coast of Korea	Kang et al,2008
—	5	10.8 ± 0.4	Restored macroalgae bed	East coast of Korea	Kang et al,2008
—	6	8.4±1.0	Natural macroalgae bed	Gouqi Island, East China Sea	Jiang,2015
Hexagrammos otakii					
97-196	1	15.3 ± 0.30	Rocky reef	Yellow Sea	This study
135-169	2	14.1 ± 0.49	Mud substrate	Yellow Sea	This study
—	3	11.6 ± 0.4	Restored macroalgae bed	East coast of Korea	Kang et al,2008
—	4	11.3 ± 0.9	Natural macroalgae bed	East coast of Korea	Kang et al,2008
—	5	10.6 ± 0.4	Barren ground	East coast of Korea	Kang et al,2008
—	6	7.5±0.4	Natural macroalgae bed	Gouqi Island, East China Sea	Jiang,2014
Sebastes schlegelii					
63-174	1	15.0 ± 0.59	Rocky reef	Yellow Sea	This study
138–178	2	14.5±0.4	Seagrass bed	Mitsukuchi Bay, Seto Inland Sea	Akira Hoshika et al,2007
102-166	3	14.4 ± 0.07	Mud substrate	Yellow Sea	This study
135–146	4	13.4±0.4	Seagrass bed	Mitsukuchi Bay, Seto Inland Sea	Akira Hoshika et al,2006
—	5	12.6 ± 0.3	Unknown	Peter the Great Bay, Sea of Japan	Kiyashko,2011
—	6	12.3 ± 0.7	Natural macroalgae bed	East coast of Korea	Kang et al,2008
—	7	11.5 ± 0.1	Barren ground	East coast of Korea	Kang et al,2008
—	8	11.3 ± 0.6	Restored macroalgae bed	East coast of Korea	Kang et al,2008

FIGURES

Fig. 1. Map showing the location of (a) Shandong Peninsula in China and (b) the nearshore and offshore sampling sites in Rongcheng Bay, in the Yellow Sea.
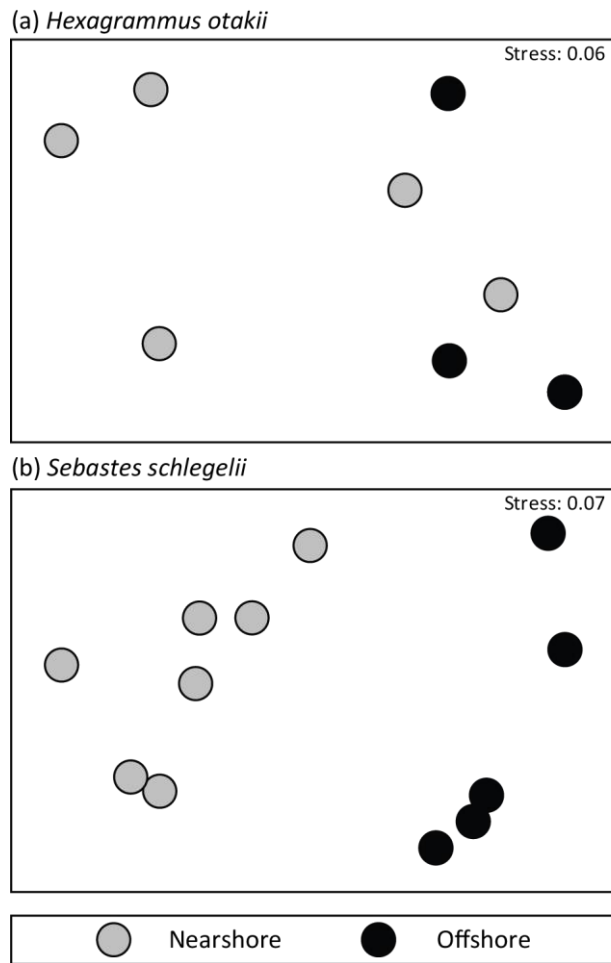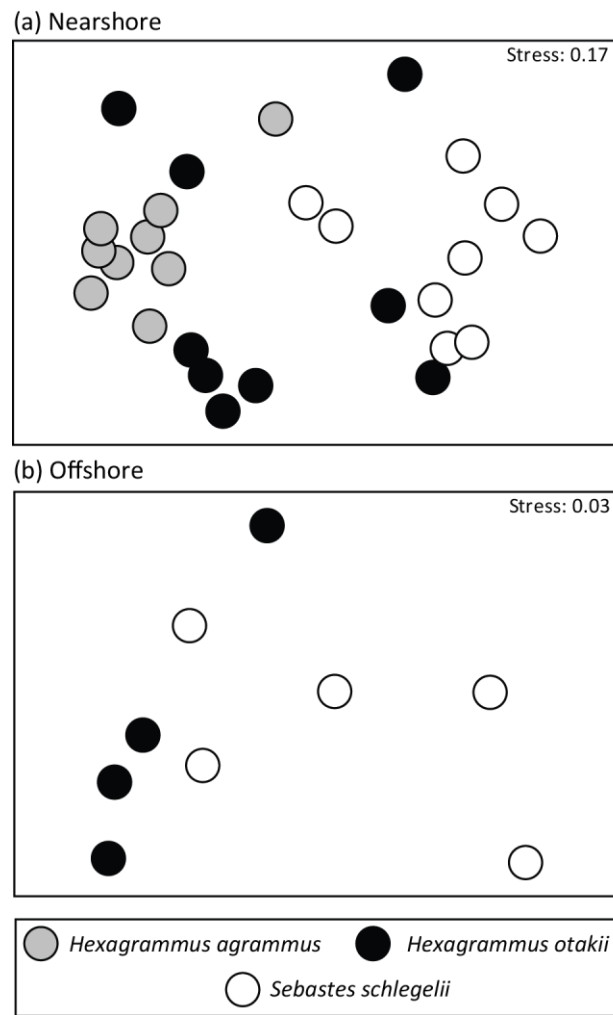
Fig. 2. nMDS ordination plots, derived from Bray-Curtis resemblances matrices constructed from percentage volumetric contributions by wet weight (%W) of each prey category to the stomach contents of (a) *Hexagrammos otakii* and (b) *Sebastes schlegelii* caught in nearshore (●) and offshore waters (●) of Lidao, northern China.
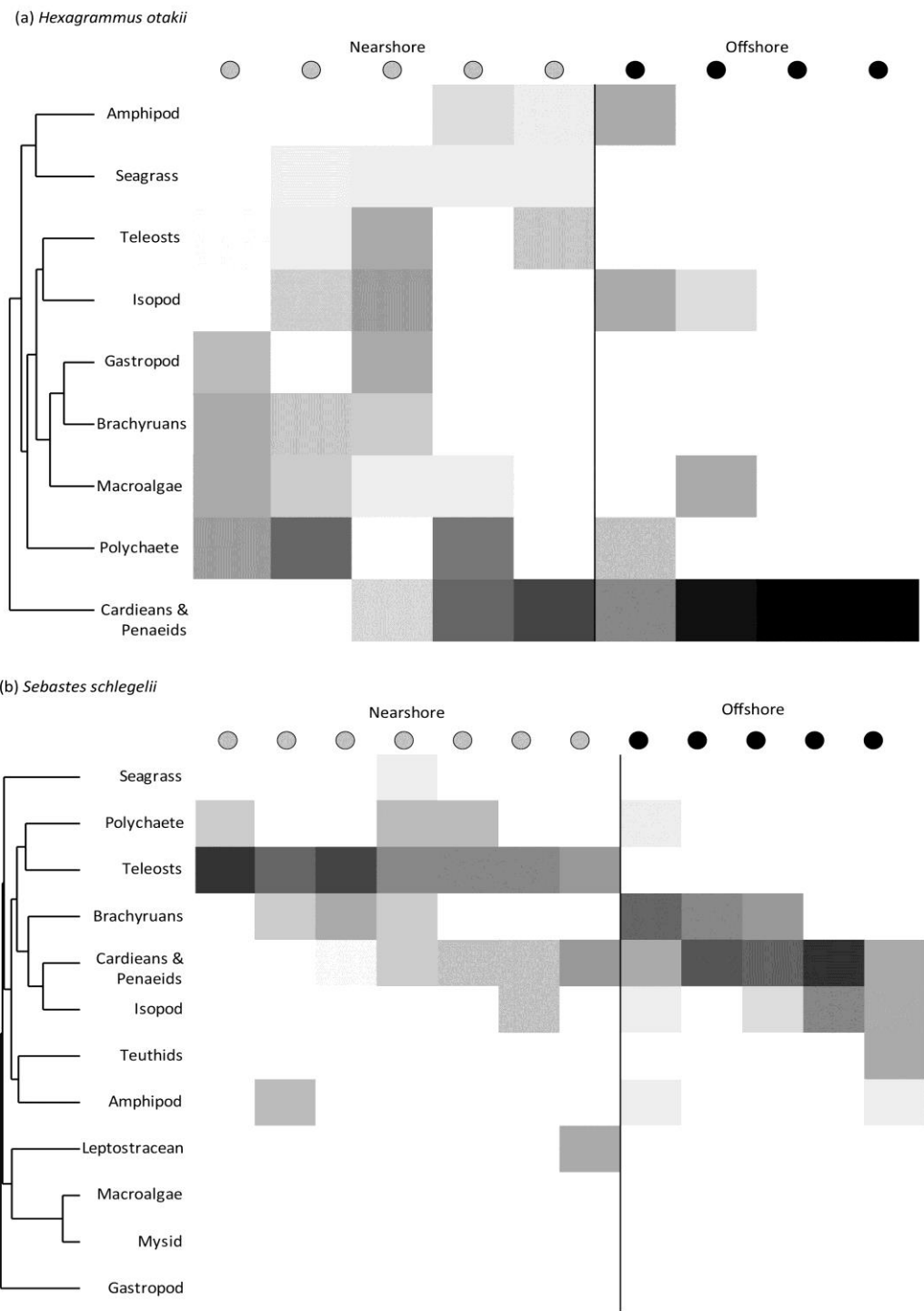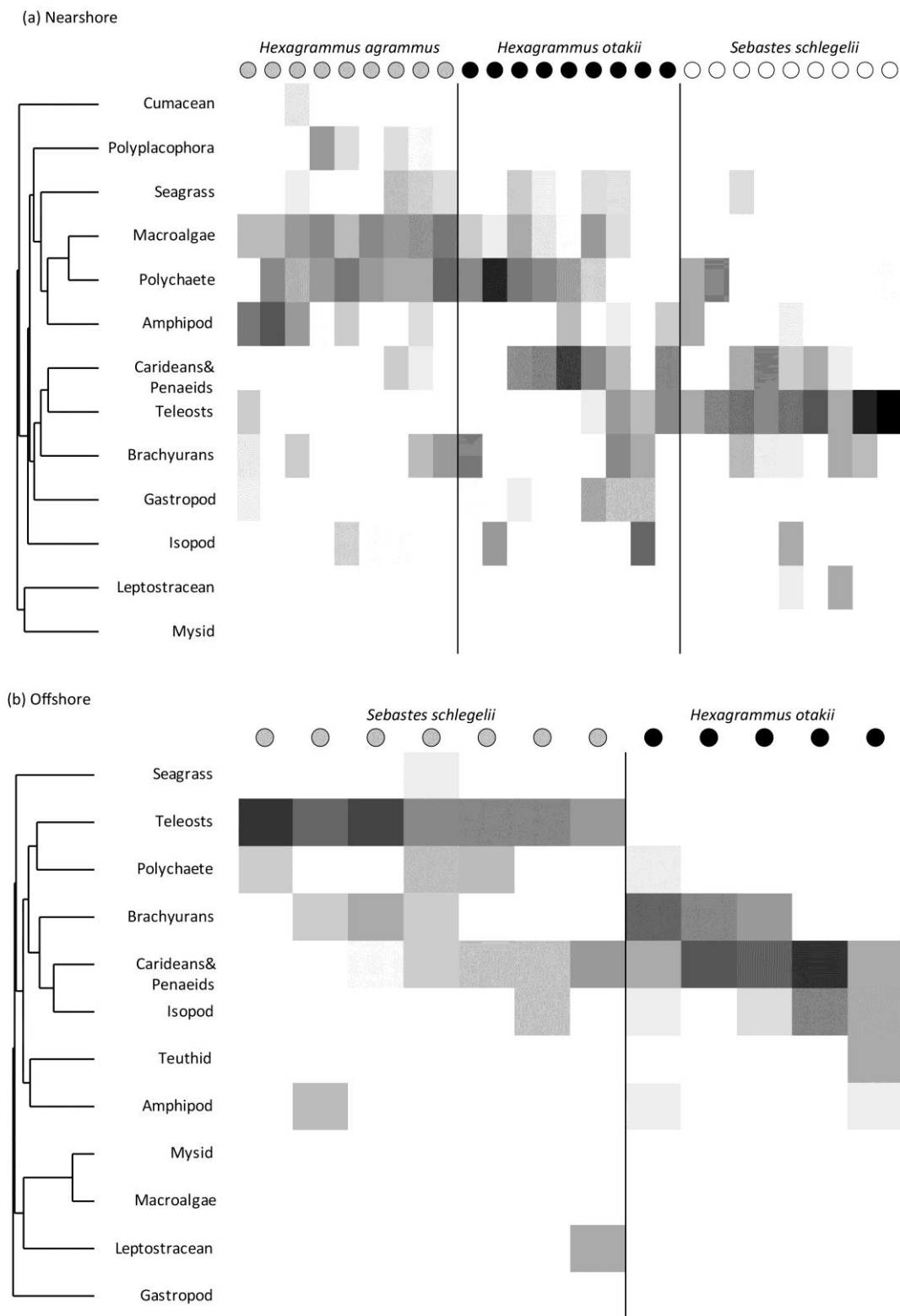
Fig. 3. Shade plots, showing, by depth of shading, the mean percentage volumetric contributions by wet weight (%W) of each prey category to the stomach contents of (a) *Hexagrammos otakii* and (b) *Sebastes schlegelii* caught in nearshore (⬤) and offshore waters (⬤) of Lidao, northern China.

Fig. 4. nMDS ordination plots, derived from Bray-Curtis resemblances matrices constructed from percentage contributions by wet weight (%W) of each prey category to the stomach contents of *Hexagrammos agrammus*(⬤) *Hexagrammos otakii* (●) and *Sebastes schlegelii*(◯) caught in (a)nearshore and (b) offshore waters of Lidao, northern China.

Fig. 5. Shade plots, showing, by depth of shading, the mean percentage contributions by wet weight (%W) of each prey category to the stomach contents of *Hexagrammos agrammus* (⬤) *Hexagrammos otakii* (●) and *Sebastes schlegelii* (○) caught in (a) nearshore and (b) offshore waters of Lidao, northern China.
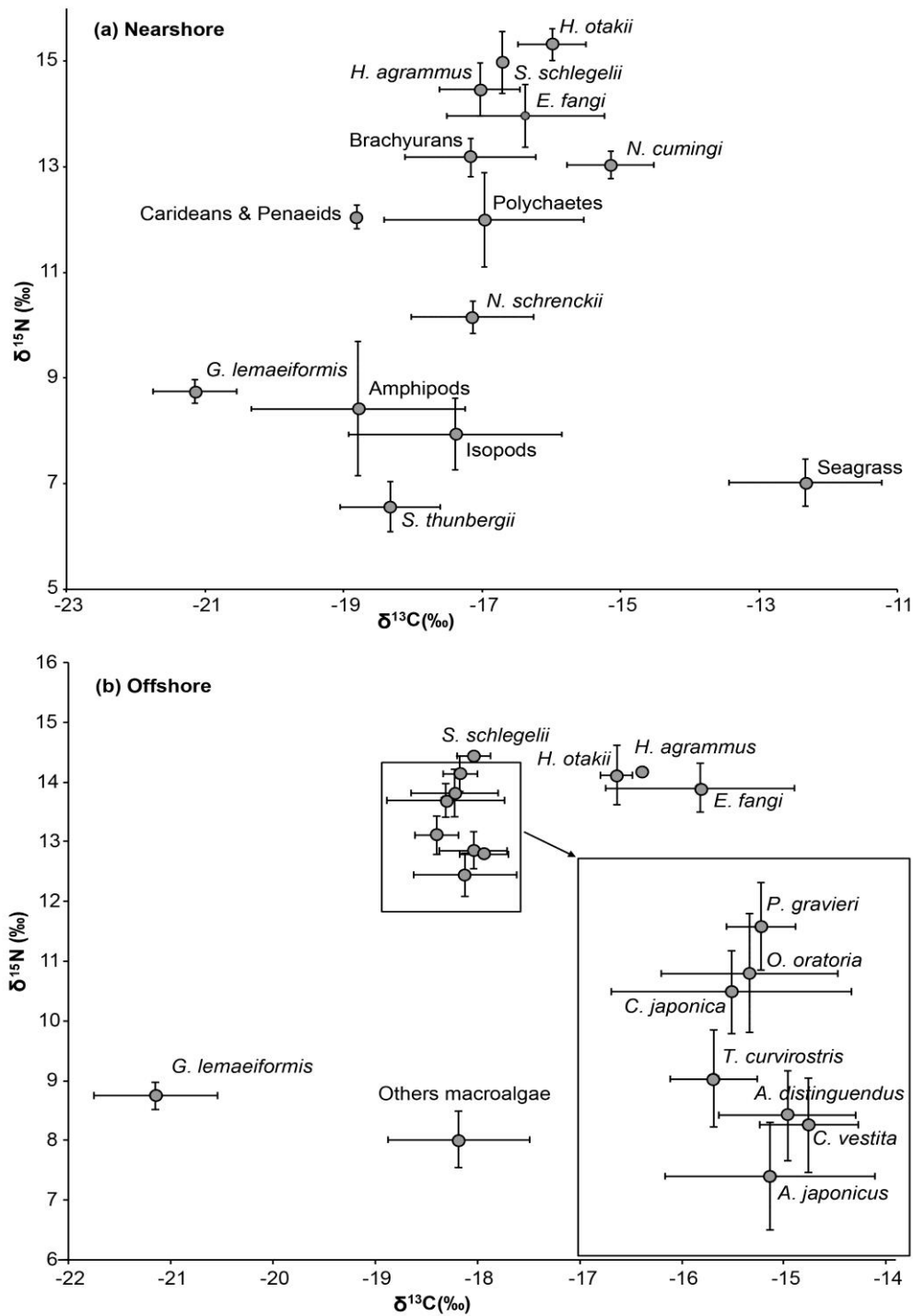
Fig. 6. Bi-plot showing the mean δ¹³C and δ¹⁵N values (± 1 SD) of the three *Hexagrammos agrammos*, *H. otakii* and *Sebastes schlegelii* and their potential food sources collected in the (a) nearshore and (b) offshore waters of Lidao, northern China. Potential food sources were: Brachyurans(*Charybdis japonica*), Carideans & penaeids (*Latreutes planirostris* and *Leptochela gracilis*), polychaetes (*Nereididae* and Polychaete spp.), amphipods (Ampeliscidae sp., *Amphithoe japonica*, *Caprella* sp., *Corophium chinensi*, *Gammarus* sp., *Melita palmate* and *Monoculodes* sp.), isopods (*Cymodoce japonica* and *Synidotea laevidorsalis*), seagrass (*Phyllospadix iwatensis* and *Zostera marina*) and macroalgae (*Undaria pinnatifida, Grateloupia turuturu, Ulva pertusa* and *Enteromorpha stinalis*).
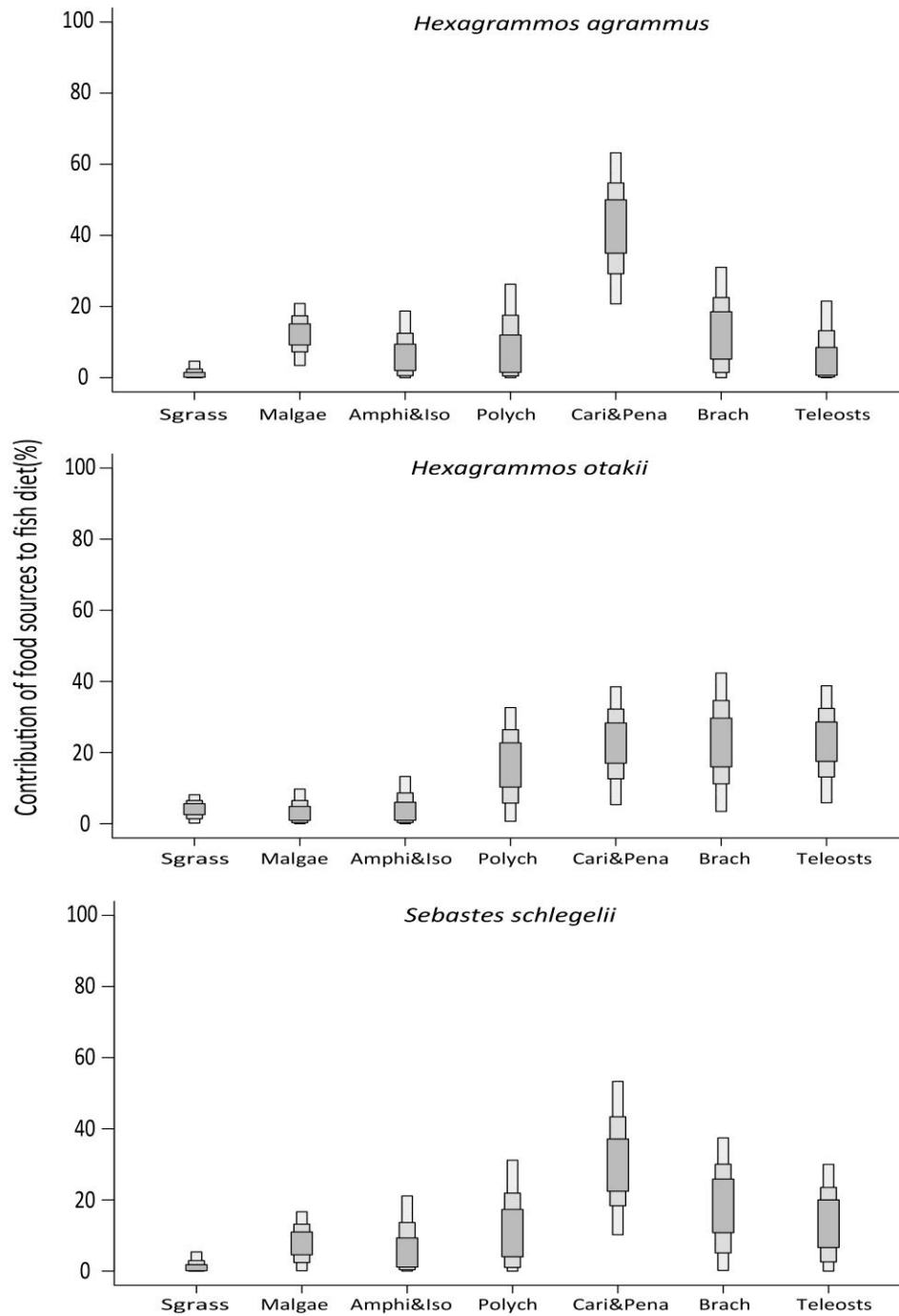
Fig. 7. Mean contribution of potential food sources (%) to the diet of *Hexagrammos otakii,*
H. agrammus and *Sebastes schlegelii,* calculated with Bayesian mixing models in SIAR (with a
credibility interval of 50%, 75% and 95%, respectively) at the nearshore site. Sgrass = seagrass,
Malgae = macroalgae, Amphi&Iso = amphipods & isopods, Polych = polychaetes, Cari&Pena =
carideans & penaeids and Brach = brachyurans.

Appendix. Total number of stomachs examined (N), mean standard length (mm) and mean wet weight (g) of the three scorpionfish species collected in the nearshore and offshore waters of Lidao, northern China. The number of stomachs with dietary items, and range of lengths and weights are in parentheses.

Species	Site	N	Length (mm)	Wet weight (g)
Hexagrammos agrammus	Nearshore	55 (39)	133 (88-178)	56 (14-135)
	Offshore	1 (0)	186	159
Hexagrammos otakii	Nearshore	23 (18)	129 (97-196)	47 (17-159)
	Offshore	7 (7)	155 (135-169)	81 (52-109)
Sebastes schlegelii	Nearshore	80 (29)	131(63-174)	69 (6.5-162)
	Offshore	15 (15)	132 (102-166)	79 (34-146)
Total	**Nearshore**	**158 (86)**		
	Offshore	**23 (22)**		