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

Zhongxin Wu, Xiumei Zhang, Charlotte R. Dromard, James Tweedley, Neil Loneragan

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ABSTRACT

The partitioning of food resources among three abundant co-occurring reef-associated scorpionfish, *Hexagrammos agrammus*, *Hexagrammos otakii* and *Sebastes schlegelii*, was determined on an artificial reef zone in nearshore and offshore coastal waters of northern China, using stomach content and stable isotope analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). The three species consumed similar prey items, mainly a variety of crustaceans, teleosts, polychaetes and macroalgae, but the proportions of the items differed among species. The dietary composition of all three scorpionfish differed significantly in nearshore waters, but not between *H. otakii* and *S. schlegelii* in offshore waters where both species fed predominantly on carideans, penaeids and brachyurans. The $\delta^{13}\text{C}$ values varied significantly among the three scorpionfish in nearshore waters, suggesting that they partition food resources. No significant differences in isotopic signatures were detected between *H. otakii* and *S. schlegelii* in offshore waters. Bayesian mixing models further confirmed that all fish were carnivorous and that the main food sources were assimilated in different proportions. In the nearshore waters, resource partitioning occurs among the three scorpionfish, reducing the potential for competition and the feeding ecology implies that they have dietary plasticity, which facilitates local food web stability and their coexistence.

Keywords: artificial reef, competition, *Hexagrammos*, *Sebastes*, stable isotope analyses, stomach contents analyses.

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 m³ 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 japonicas* 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,

these three scorpionfish are particularly important for recreational and commercial fisheries in northern China, Korea and Japan (e.g., Masuda et al., 1984; Yoon, 2002; Lei, 2005).

The large-scale deployment of artificial reefs in Lidao (Fig. 1) has been assumed to alleviate habitat as a constraining factor to fishery production. Instead, inadequate food resources could intensify inter- and intra-specific competition and become the limiting factor. Although the diets of the three species have been described previously in a number of regions (Kwak et al., 2005; Seo & Hong, 2007; Tong & Guo, 2009; Zhang et al., 2014; Ji et al., 2015), they have not been studied where the species occur sympatrically to enable comparisons of the diet simultaneously.

Analysis of stomach content is widely used in dietary studies to provide a “snapshot” of the recent diet of an individual, but does not reflect the sources of carbon and nitrogen assimilated by a species. Such analyses also under-represent soft-bodied animals, which can be digested rapidly, and over-represent prey with hard parts (Fry, 1988; Hobson et al., 1995; Cresson et al., 2014b). Some of these biases can be overcome by using stable isotope analysis (SIA) to complement the stomach content analyses as SIA provides an indication of the primary source of carbon ($\delta^{13}\text{C}$) and their trophic level in the food web ($\delta^{15}\text{N}$). Stable isotope signatures integrate dietary information over a longer-term period, i.e. several months for muscle tissue (Peterson & Fry, 1987; Lorrain et al., 2002; Guelinckx et al., 2007) and thus provide an indication of trophic flows and position in the food web over longer time scales than stomach content analyses. The recent development of mixing models for stable isotope data within a Bayesian framework e.g. Stable Isotope Analysis in R (SIAR), allows the relative percentage contribution of potential food sources to the consumers diet to be estimated quantitatively (Parnell *et al.*, 2010). This provides a powerful tool for understanding food source partitioning among these fish.

In the current study, the diets of three scorpionfishes, i.e. *H. agrammus*, *H. otakii* and *S. schlegelii* were investigated using stomach content analyses, coupled

with stable isotope analyses of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, to investigate the food resource partitioning among the three species, and also identify the trophic flows of C and N in this system. Samples were collected at nearshore (~10 m deep and <100 m from shore) and offshore sites (20-30 m deep and ~2 km from shore) in the coastal waters of the Lidao artificial reef zone, in the Yellow Sea (northern China), to understand the pattern differences of food partitioning between the two types of habitat.

MATERIAL AND METHODS

STUDY SITE

This study was carried out at two locations in the coastal zone of Rongcheng Bay (37°13'N, 122°36'E) Yellow Sea, Shandong Peninsula, PR China (Fig. 1). The nearshore site was located in the subtidal zone of the rocky shore and characterized by natural rocky reef and scattered gravel, with a mean water depth of 9-11 m. The offshore site is situated in deeper waters (20-30 m) about 2 km from the coast, in an area zoned for floating kelp (*Laminaria japonica*) and scallop (*Chlamys farreri*) culture where intensive culture is practised (Wu et al., 2016). Kelp cultivation covers an area of 5,000 ha and yielded a harvest of 1,200 t wet weight km^{-2} . The sediments of these waters are characterised by their low profile and presence of a soft muddy layer, 5-15 cm deep on the surface of the substratum (Tu et al., 2007). Between 2006 and 2008, more than 7.5 million stone reefs, with a volume of 1 million m^3 , 13,015 concrete reefs (650,000 m^3) and 60 derelict vessels (4,300 m^3) were deployed in the nearshore and offshore waters to construct the artificial reef zone, consisting of 12 artificial reef groups (Wu et al., 2016). The artificial reefs deployed at the offshore site, however, have submerged some distance into the soft substratum and so do not provide the same extent of habitat as similar structures in the nearshore waters (Wu, unpublished data).

COLLECTION OF BIOTA

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

schlegelii, and samples of a range of their potential food sources as well as primary producers were collected in July 2013 (summer). At this time, mean water temperatures and salinities were 21.5°C and 31.5, respectively. Scorpionfish were collected using fish traps deployed on the benthos for about 24 h. After the traps were retrieved, fish were placed in labelled plastic bags on ice, transported to the laboratory and frozen (-20°C) prior to dissection. Each fish was measured (standard length in mm) and weighed (wet weight in g). Approximately 2 g of dorsal white muscle was collected from each fish and frozen (-20 °C) for stable isotope analyses and its stomach was dissected, labelled and frozen for diet analysis.

In nearshore waters, food items (including the amphipods; *Ampeliscidae* sp., *Amphithoe japonica*, *Caprella* sp., *Corophium chinensis*, *Gammarus* sp., *Melita palmate* and *Monoculodes* sp., isopods; *Cymodoce japonica* and *Synidotea laevidorsalis*, polychaetes; Nereididae and polychaete spp., carideans & penaeids; *Latreutes planirostris* and *Leptochela gracilis*) were collected from the stomachs of the three species of scorpionfish. The teleost *Enedrias fangi* and Brachyuran *Charybdis japonica* were caught using fishing traps. Two species of macroalgae, *Gracilaria lemaneiformis* and *Sargassum thunbergii*, and two seagrass species, *Phyllospadix watensis* and *Zostera marina*, were collected by SCUBA diving. All samples of plant tissue were washed with deionized water and kept frozen until analysis.

In offshore waters, samples of teleosts; *Enedrias fangi*, Brachyurans; *Charybdis japonica* and *Carcinoplax vestita*, Stomatopods; *Oratosquilla oratoria*, carideans & penaeids; *Trachypenaeus curvirostris*, *Palaemon gravieri*, *Alpheus distinguendus* and *Alpheus japonicas*, were collected from fish traps. Five macroalgal species (*Gracilaria lemaneiformis*, *Undaria pinnatifida*, *Grateloupia turuturu*, *Ulva pertusa* and *Enteromorpha intestinalis*) were collected by hand from the kelp culture facility.

STOMACH CONTENT ANALYSES

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

nearshore and offshore waters, respectively (Appendix). Prey items were identified to the lowest taxonomic level possible and quantified by calculating the frequency of occurrence (%F), numerical percentage (%N), and the wet weight percentage (%W) of prey, using the following equations:

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

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

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

Where A_i is the number of teleosts with prey items i in the stomach, N_i is the number of prey items i in the stomach, N_{total} is the total number of items in the stomach (excluding individuals with empty stomachs) and W_i is the wet weight of the prey item i . Individual fragments of macroalgae and seagrass were counted to estimate N_i . Note that carideans and penaeids were not separated and are treated as one dietary category carideans & penaeids.

Preliminary multivariate analyses of dietary composition

The replicate %W data for all of the identifiable prey items, were standardised, square-root transformed and used to construct a Bray-Curtis resemblance matrix. This matrix was used in a preliminary analysis to determine the major sources of diet: data were subjected to a 3-way crossed ANOSIM (Analysis of Similarities; Clarke and Green, 1988) to determine whether the diet (%W) differed among Species (3 levels; *H. agrammus*, *H. otakii* and *S. schlegelii*), Length class (2 levels; < 150 mm TL and > 150 mm TL) and Site (2 levels; nearshore and offshore). The relative magnitudes of the overall factors (subsuming both main and interaction effects) were assessed via the universally scaled ANOSIM $\bar{R}$ statistic which ranges from ~0, when the average similarity among and within groups (factors) do not differ, to 1, when all samples within each group are more similar to each other than to any of the samples from other groups (Clarke et al., 2014a). Three-way crossed ANOSIM demonstrated that significant differences were detected among Species ($\bar{R} = 0.300$; $P = 0.1\%$) and Sites

($\bar{R} = 0.263$; $P = 0.1\%$), however, there was no evidence to support a Length class difference ($\bar{R} = 0.046$; $P = 20.4\%$). As a result, Length class was removed as a factor from subsequent analyses.

As the stomachs of individual fish may contain only a small number of the 14 dietary categories, two samples of the same species, collected at the same time, may differ markedly in their dietary composition. This variability can mask subtle, but 'true', trends in diet and thus the stomachs of the samples for each species at each site were randomly sorted into groups of 2 to 4, depending on the total number of fish in the samples (Lek et al., 2011). The percentage composition of the different dietary categories based on weight, for each group of replicates (*i.e.* 2-4 fish from the same species at the same site), were averaged. The resultant data were then square-root transformed to down-weight the contributions of dietary categories with consistently high values and avoid any tendency for those dietary components to be excessively dominant. This averaging approach overcomes any potential bias created by pooled dietary data obtained from markedly unequal numbers of individual guts, *i.e.* a 'species' accumulation effect (Lek et al., 2011). While, this approach did result in a slightly unequal number of replicates across the species and sites, such unbalanced statistical designs are able to be analysed effectively using contemporary multivariate techniques such as PERMANOVA and ANOSIM (Anderson et al., 2008; Clarke & Gorley, 2015).

Intraspecific differences in dietary composition

One-way ANOSIM tests were employed to determine whether the dietary composition of *H. otakii* and *S. schlegelii* differed among these sites. It was not possible to do this for *H. agrammus* as only a single fish was caught in the offshore waters and its stomach was empty. Separate Bray-Curtis resemblance matrices were constructed for each species from the averaged replicates and subjected to ANOSIM. Non-metric multidimensional scaling (nMDS) ordination plots were used to visually demonstrate

the differences and similarities between factors. In those cases where ANOSIM detected a significant difference, Similarity Percentages analysis (SIMPER; Clarke & Gorley, 2015) and shade plots (Clarke et al., 2014b) were employed to identify those dietary categories that typified and distinguished the dietary composition of each *a priori* group (SIMPER) and those dietary categories that contributed the greatest proportion by weight to the difference (shade plot).

Interspecific differences in dietary composition

Separate one-way ANOSIM tests were employed to determine whether the dietary composition differed significantly (i) among *H. agrammus*, *H. otakii* and *S. schlegelii* in the nearshore waters and (ii) between *H. otakii* and *S. schlegelii* in the offshore waters. As with the above intraspecific analyses, nMDS ordinations, SIMPER and shade plots and were also employed to visualise and determine the cause of any interspecific differences in dietary composition.

STABLE ISOTOPE ANALYSES

All samples collected were stored at -20°C in the laboratory, freeze-dried at -80°C for 48 h and then ground to fine, homogeneous powder with a glass mortar and pestle. Fish muscle tissues were defatted in a solution of chloroform and methanol (volume ratio = 2:1). To remove any inorganic carbonates of samples, 1M HCl was added slowly to the sample until it stopped producing CO₂. Samples were then centrifuged five times at 3,000 rpm for 10 min, and the supernatant was then tested with pH paper before removal. When the pH of the supernatant was close to that of deionized water, the sample was freeze-dried and stored at -80°C, otherwise, samples were rinsed with deionized water until they reached the pH of deionized water (Jacob et al., 2005).

Samples were analysed for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at the Qingdao Institute of Marine Geology, using a Flash EA 1112 Elemental analyser coupled with a Thermo Finnigan MAT 253 stable isotope ratio mass Spectrometers (EA-IRMS), via a Thermo Finnigan

Conflo III interface. The stable isotope ratios are expressed in delta (δ) unit notation as deviations from the international standards (Vienna Pee Dee Belemnite for carbon and atmospheric nitrogen for nitrogen) according to the following formula:

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

Shapiro-Wilk tests showed that the stable isotope data were not normally distributed. Consequently, non-parametric Kruskal-Wallis tests were used to test whether the isotopic signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) differed significantly among the three scorpionfish species and the potential food sources.

Stable isotope mixing model

Bayesian stable isotope mixing models SIAR v4.0 (Stable Isotope Analysis in R) were run in R (Parnell et al., 2010) to evaluate the relative contribution of potential food sources to the diet of the three scorpionfish species in nearshore waters. Mixing models consider stable isotopic values ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of consumers, multiple potential food sources, and discrimination factors with uncertainty of these values within the model (Parnell et al., 2010). A total enrichment fractionation (TEF) of 1.3‰ was selected for ^{13}C , based on the results of a study by Cai et al. (1999) in Laoshan Bay, which is adjacent to the Lido Bay and contains similar nearshore rocky reefs. The TEF chosen for ^{15}N of 3.4‰ was selected from Minagawa and Wada (1984). Credibility intervals (CI) of 0.95, 0.75 and 0.25 were computed and displayed on figures (Parnell et al., 2010; Lebreton et al., 2012).

RESULTS

DIETARY COMPOSITION

All except one of the fish collected from offshore waters and 54% of those from the nearshore areas had identifiable items in their stomachs (Appendix). The diets of the three scorpionfish were characterized by teleosts, crustaceans, molluscs, cephalopods, polychaetes, teleost eggs, macroalgae and seagrass (Table 1). The quantitative diet

indices of %W, %N and %F all show that teleosts, crustaceans, polychaetes and macroalgae were the dominant prey items in nearshore waters. *Hexagrammos agrammus* and *H. otakii* fed on a more diverse range of prey than *S. schlegelii*. *Hexagrammos agrammus* preyed principally on polychaetes, representing 33.7% of diet by weight (%W) and 66.7% by frequency of occurrence (%F, Table 1). Crustaceans and macroalgae were also important in the diet of *H. agrammus*, accounting for 29.5% and 23.73% of the total prey items by weight, respectively. However, macroalgae presented a higher %N (80.4%) and %F (79.4%) than crustaceans (5.85% and 64.1%, respectively), due to the method of quantifying macroalgae, which was done by counting the number of digested macroalgae segments. In the nearshore waters, *Hexagrammos otakii* preyed more on crustaceans, as demonstrated by the %W (82.2%) and %F of 72.2%. Brachyurans were the dominant crustaceans in the stomach contents (%W = 67.45%), followed by carideans & penaeids (%W = 10.5%). Although macroalgae and polychaetes were present in the stomachs of *H. otaki*, the %W and %F values were much lower than those for *H. agrammus*. Teleosts, comprised the majority of the diet of *S. schlegelii*, accounting for 79.7%, 21.4% and 65.5% for %W, %N and %F, respectively, and brachyurans (F% = 20.7%).

The number of fish caught in offshore waters (23) was lower than in nearshore waters (158) and only one *H. agrammas* was caught in the offshore and this individual did not have items in the stomach (Appendix). In offshore waters, *H. otakii* and *S. schlegelii* consumed mainly crustaceans, especially Carideans & Penaeids (88.8% and 62.9% by %W, respectively), while *S. schlegelii* also consumed significant weights of stomatopods (22.4%) and brachyurans (13.7%). The stomachs of *H. otakii* contained some macroalgal material, which came from the cultivated kelp.

MULTIVARIATE ANALYSES OF DIET

Intraspecific differences in dietary composition

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

between nearshore and offshore waters ($P= 4.6\%$; $R = 0.369$), with samples from fish in offshore waters lying to the right on the nMDS plot, and typically well separated from fish in nearshore waters (Fig. 2a). Carideans & penaeids were identified by SIMPER as typifying the diet of fish from offshore waters, whereas *H. otakii* in nearshore waters consumed a more varied diet, comprising polychaetes, macroalgae, brachyurans and teleosts, in addition to carideans & penaeids (Fig. 3a).

ANOSIM detected a significant and very strong difference between the dietary composition of *S. schlegelii* in the nearshore and offshore waters ($P= 0.1\%$; $R = 0.935$), as evidenced by their complete separation on the nMDS plot (Fig. 2b). SIMPER analysis indicated that teleosts typified the diet of *S. schlegelii* in nearshore waters, while carideans & penaeids and brachyurans were found in consistently larger volumes in samples from offshore waters. This is also shown on the shade plot (Fig. 3b), which shows that polychaetes, made a substantial contribution to the prey consumed in nearshore waters, together with brachyurans and carideans & penaeids, albeit to a lesser extent than in deeper waters.

Interspecific differences in dietary composition

ANOSIM showed that the overall dietary composition of the three scorpionfish in nearshore waters differed significantly ($P = 0.1\%$; $R = 0.579$) and that each pairwise comparison was also significant ($P = 0.1\%$). The greatest difference in diet in nearshore waters was between *H. agrammus* and *S. schlegelii* ($R = 0.921$), followed by *H. otakii* and *S. schlegelii* ($R = 0.447$) and lastly between the two *Hexagrammus* species ($R = 0.347$). These trends are illustrated on the nMDS plot where the samples representing *H. agrammus* and *S. schlegelii* form a broad group that do not overlap, whereas the samples for *H. otakii* are interspersed to some extent with samples from the other two species (Fig. 4a).

The pattern of dietary composition among the three species exhibits a clear trend along the diagonal of the shade plot with macroalgae, polychaetes and amphipods

making consistently larger contributions to the stomach contents of *H. agrammus* (Fig. 5a). Although both macroalgae and polychaetes were consumed by *H. otakii*, their %Ws were lower and that of carideans & penaeids and, in some cases, teleosts, brachyurans and gastropods were higher than those of the other species. The diet of *S. schlegelii* in the nearshore was the most distinct of the three species, comprising mainly teleosts, with smaller contributions of brachyurans and carideans & penaeids (Fig. 5a).

Although ANOSIM detected a significant difference in the dietary composition of *H. otakii* and *S. schlegelii* in the offshore waters ($P = 0.1\%$), the R value for this difference was small (Global $R = 0.238$). Two of the five samples of *S. schlegelii* on the nMDS plot lie with the group of samples representing *H. otakii* (Fig. 4b). Carideans & penaeids dominated the diets of both species in the offshore waters, albeit to a greater extent in *H. otakii* (Fig. 5b).

STABLE ISOTOPES ANALYSES

In nearshore waters, the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of the three scorpionfish, and those of their potential prey, except amphipods and isopods, differed significantly (Kruskal-Wallis tests; both $df = 2$; $p = <0.001$). Thus, both of these crustacean taxa were combined, as one potential group of prey in the subsequent SIAR analysis. The mean $\delta^{13}\text{C}$ ratios (± 1 SD) of primary benthic producers in the nearshore waters varied from $-21.3 \pm 0.6\text{‰}$ for the red alga *Gracilaria lemaneiformis* to $-12.3 \pm 1.1\text{‰}$ for seagrass (Fig. 6a). The mean $\delta^{15}\text{N}$ values ranged from $6.6 \pm 0.5\text{‰}$ for the brown alga *Sargassum thunbergii* to $15.3 \pm 0.3\text{‰}$ for *H. otakii*. The stable isotope signatures of brachyurans, polychaetes and carideans & penaeids were very similar and difficult to distinguish on the bi-plot (Fig. 6a).

In offshore waters, the stable isotope signatures of *H. otakii* and *S. schlegelii* did not differ significantly ($df = 2$; $p = > 0.05$, see also Table 2). However, the main potential food taxa exhibited significant difference in their $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ratios

(Kruskal-Wallis test, $df = 9$; $p < 0.001$). Mean $\delta^{13}\text{C}$ ratios varied from $-21.1 \pm 0.6 \text{ ‰}$ for the macroalgae *G. lemaeiformis* to $-15.8 \pm 0.4 \text{ ‰}$ for the teleost *Enedrias fangi*. The mean $\delta^{15}\text{N}$ ratios ranged from the minimum value of $8.0 \pm 0.5 \text{ ‰}$ for macroalgae to $14.4 \pm 0.1 \text{ ‰}$ for *S. schlegelii* (Fig. 6b).

Interspecific comparison of stable isotopes ratios

The stable isotope ratios of species found in both nearshore and offshore waters were compared to test for spatial differences. These species included: teleosts (*H. otakii*, *S. schlegelii* and *E. fangi*), brachyuran (*C. japonica*), carideans & penaeids (*L. planirostris* and *L. gracilis* [nearshore] and *T. curvirostris*, *A. distinguendus*, *P. gravieri* and *A. japonicus* [offshore]) and macroalgae (*S. thunbergii* [nearshore] and *G. lemaeiformis*, *U. pinnatifida*, *G. turuturu*, *U. pertusa* and *E. intestinalis* [offshore]; Table 2). *Hexagrammus otakii* and *S. schlegelii* had greater $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in nearshore than offshore waters. However, the $\delta^{15}\text{N}$ of the macroalgae, brachyuran *C. japonica* and carideans & penaeids, were enriched in offshore waters. The stable isotopic signatures of the teleost *E. fangi* did not differ significantly between nearshore and offshore waters (Table 2).

Results from mixing models

The Bayesian stable isotope mixing models estimated that teleosts, carideans & penaeids, brachyurans and polychaetes were the principal contributors to the stable isotopic ratios of the three scorpionfishes in nearshore waters (Fig. 7). Carideans & penaeids were the predominant prey of *H. agrammus*, accounting for an estimated 40.6% of their diet. Other important items were brachyurans (14.9%), polychaetes (12.1%) and macroalgae (11.8%). Teleosts (22.9%), carideans & penaeids (23.0%), brachyurans (22.0%) and polychaetes (17.5%) were also estimated to be important for *H. otakii*. Carideans & penaeids were also identified as the most important potential contributor to the C and N assimilated by *Sebastes schlegelii* (31.1%) followed by

brachyurans (19.6%), polychaetes and teleosts (14.8%; Fig. 7). Generally, crustaceans (including carideans & penaeids and brachyurans) were estimated to be the major prey for the three species of scorpionfish, contributing over 50% to the isotopic ratios for each species.

DISCUSSION

This study investigated the potential partitioning of food resources of three species of scorpionfishes in the Lido coastal zone where extensive artificial reefs are deployed and mariculture of kelp and scallops is practised in the deeper, offshore waters. The combination of stomach content and stable isotopes analyses indicated the three species of scorpionfish, i.e. *Hexagrammos agrammus*, *H. otakii* and *Sebastes schlegelii*, partitioned their food resources in the nearshore waters but that this was not evident for *H. otakii* and *S. schlegelii* in the offshore. Thus, although all each species were predominantly carnivorous, feeding on differing amounts of crustaceans (brachyurans and carideans & penaeids), polychaetes, teleosts, they also fed on microalgae, albeit to a lesser extent.

Multivariate analysis of the percentage contribution by weight of the various prey categories in stomachs confirmed that they were partitioning the food resources among the three species in the nearshore waters. *Hexagrammos agrammus* and *H. otakii* preyed on a more diverse range of prey than *S. schlegelii*, which specialised on teleosts in the nearshore. The first of these species fed on relatively low trophic level food items like polychaetes, amphipods, polyplacophorans and macroalgae. These results parallel those of previous studies on the diet of *H. agrammus* (Kwak et al., 2005; Wang et al., 2012; Ji et al., 2015), confirming the significance of reef associated benthos in its diet and that it is a benthic omnivore. In spite of the occurrence of macroalgae and polychaetes in the diet of *H. otakii*, this species consumed a much higher proportion of crustaceans (predominantly brachyurans; almost 70% of diet by weight) and teleosts than *H. agrammus*. Similar observations

were reported in Gouqi Island (Wang *et al.*, 2012), in the nearshore marine waters of Qingdao, China (Ye, 2012), Northern Primorye, Russia (Balanov *et al.*, 2001; Kolpakov *et al.*, 2005) and on the Jangbong tidal flat, Incheon, Korea (Seo & Hong, 2007). These findings across broad geographic regions and those from the current study all confirm that *H.otakii* is a zoobenthivore, with preference for crustacean prey.

The diets of the two *Hexagrammos* species differed in the nearshore waters with *H. otakii* primarily preying on crustaceans and fish, while *H. agrammus* fed on smaller-sized benthic prey such as polychaetes, macroalgae, amphipods and polyplacophorans. These dietary differences reflect their differences in microhabitat use that lead to differences in prey availability (Kwak *et al.*, 2005). For example, *H. agrammus* lives among algae growing on rock, while the co-existing *H. otakii* is widely distributed on, or under rocks, and over sandy substratum between rocks (Kanamoto, 1979).

The diet of *Sebastes schlegelii* in the nearshore contrasted with that of the two species of *Hexagrammos* and was characterized by a higher order carnivorous diet, consuming greater quantities of teleosts and large crustaceans (brachyurans and carideans & penaeids). These findings are consistent with those for *S. schlegelii* by Zhang *et al.* (2014) in the Bohai Sea and Seo and Hong (2007) in the Jangbong tidal flat, Incheon, Korea. Despite *Hexagrammos otakii* and *S. schlegelii* feeding on similar prey, the latter species consumed greater proportions of fish. *Sebastes schlegelii* was identified as a potential keystone species in the system in an Ecopath food web model of the Lidao ecosystem, indicating it had a greater impact on trophic flows in the system than predicted based on its biomass alone, probably because of its consumption of higher order trophic groups (Wu *et al.*, 2016).

The differences of carbon isotopic ratios among the three scorpionfish in the nearshore waters indicate, like the stomach content analyses, trophic niche segregation among the species, which facilitates reduced competition for food resources (Mablouké *et al.*, 2013). In the nearshore waters, the $\delta^{15}\text{N}$ values also

implied differences in trophic position of the three teleosts: with *Hexagrammos agrammus* having a lower mean $\delta^{15}\text{N}$ value (14.5 ‰) than *H. otakii* (15.3 ‰) and *S. schlegelii* (15.0 ‰), indicating that the latter two species occupied a slightly higher trophic level than *H. agrammus*. Stomach contents possibly explained the cause of isotopic discrepancy existing between these fishes, with *H. agrammus* feeding primarily on polychaetes and macroalgae, which tend to have lower $\delta^{15}\text{N}$ values. In contrast, *H. otakii* and *S. schlegelii* consumed mainly crustaceans and small benthic fish, which had higher $\delta^{15}\text{N}$ values. The order for trophic levels indicated by the $\delta^{15}\text{N}$ values from the current study are consistent with the estimated mean trophic levels for these species documented in FishBase, which assigns a mean trophic level (± 1 SE) of 3.3 ± 0.5 to *H. agrammus*, 3.8 ± 0.3 to *H. otakii* and 3.7 ± 0.6 to *S. schlegelii* (Froese & Pauly, 2014).

The results produced by the mixing model in SIAR complemented those of stomach content analyses, with the macroalgae estimated to make a greater contribution to the diet of *H. agrammus* than the other two species. This result is consistent with the amount of macroalgae found in the stomach contents of *H. agrammus* (23.7 % by wet weight). Although seagrass, which is enriched in ^{13}C compared to macroalgae, was also found in the stomach contents, the stable isotope ratios of the fish indicated that little seagrass is being assimilated by fish. Thus, it is possible that fish did not eat seagrass intentionally or that seagrass were ingested as a by-product of feeding and only a small component assimilated in the tissue. Mixing models results also estimated a greater contribution of crustaceans (brachyurans) and teleosts to the diets of *H. otakii* and *S. schlegelii* than *H. agrammus*.

In contrast, the importance of some dietary components differed between the two methods used in this study. For example, SIAR estimated that carideans & penaeids were the most important prey items assimilated by *H. agrammus* (40.6%) in nearshore waters. However, stomach contents, indicated that this species consumed principally polychaetes and macroalgae. The discrepancies between stomach contents

and stable isotope analyses are due to differences in “time scales” of the data: stomach contents give dietary information on “the last meal” at a more or less instantaneous point in time, while stable isotope signatures result from feeding and assimilation of nutrients over two to three months, i.e. not only what was ingested immediately prior to capture, but also what was incorporated in the tissues after ingestion and assimilation (MacNeil et al., 2006).

From the more limited data available for the offshore water, the dietary compositions of *H. otakii* and *S. schlegelii* were more similar than in nearshore waters, with both species consuming primarily carideans & penaeids, followed by brachyurans and stomatopods. This is consistent with the high abundance of carideans & penaeids, brachyurans and stomatopods in the benthic faunal community of the offshore (Wu et al., 2012). These results from the nearshore and offshore waters confirm that *H. otakii* and *S. schlegelii* are opportunistic predators. Their similarity in stomach contents is consistent with the similar $\delta^{15}\text{N}$ signatures for *H. otakii* and *S. schlegelii* in offshore waters and is probably due to the relatively homogeneous environment in offshore waters (i.e. low profile, soft sediments) and consequently lower numbers and diversity of niches and available prey. The large production of kelp in offshore waters and its dominance as a primary producer over other potential food sources, is also likely to be an important factor as this simplifies the food web compared with nearshore waters. An Ecosim model for the study area predicted that kelp cultivation favours benthic, rather than water column production, thus the kelp culture actually supports the benthic trophic flows to benthos, including the scorpionfish (Wu et al. 2016).

SPATIAL VARIATION IN STABLE ISOTOPIC RATIOS

The isotopic signatures of selected taxa differed significantly between the nearshore and offshore waters. The $\delta^{13}\text{C}$ signatures of fish (*H. otakii* and *S. schlegelii*), brachyurans and macroalgae in nearshore waters were enriched compared with the offshore waters. This gradient pattern is consistent with the results of Newsome *et al*

(2007). The offshore isotopic ratios of C are more depleted than those along the coast indicating a greater uptake of plankton carbon in the food web than benthic sources. The mean nitrogen ratios of the teleosts were 0.4 to 1.2 ‰ higher in the nearshore site than the offshore, but not for macroalgae, carideans & penaeids and the brachyuran *Charybdis japonica*, which were higher offshore. Enriched nitrogen signatures in food webs may indicate greater anthropogenic inputs of nitrogen to the nearshore than offshore (Costanzo et al., 2001; Lepoint et al., 2004; Michener & Lajtha, 2008; Dromard et al., 2013) and the uptake of nutrients by kelp in the mariculture area of the offshore waters. During the early period of kelp growth (~November each year), large quantities of synthetic fertilizers (i.e. Urea; $\delta^{15}\text{N}$ = 1 to 2.6‰; Heaton, 1986) are released gradually into waters to stimulate kelp growth. This practice changes the concentration of dissolved inorganic nitrogen in the surround water. As a consequence, enriched ^{15}N might be assimilated into the tissue of macroalgae and phytoplankton and further transferred through the food web.

The $\delta^{15}\text{N}$ values for the fish in the current study were higher than those for the same species in Japan (Hoshika et al., 2006), South Korea (Kang et al., 2008) and Russia (Kiyashko et al., 2011), as well as Gouqi Island, in China (Table 3), particularly for *H. agrammus* and *H. otakii*. For these two species, the values in the Lidao reef system were ~3 ‰ higher than those recorded from the other regions. This suggests that anthropogenic coastal activities have increased the $\delta^{15}\text{N}$ values of the water and as a consequence increased the ^{15}N levels throughout the nearshore food web, as has been found in other regions (Costanzo et al., 2001; Lin & Fong, 2008; Letourneur et al., 2013).

Carideans & penaeids and brachyurans from the offshore waters presented higher $\delta^{15}\text{N}$ values than the values for these taxa in the nearshore waters, probably because of the higher $\delta^{15}\text{N}$ of the algal detritus in the sediments, which is then consumed by the zoobenthos. Indeed, the brachyuran *C. japonica* and carideans & penaeids are known to be omnivorous in soft mud environment and to take sediment organic matter as food source (Quan et al., 2010).

The more enriched carbon stable isotopic signatures of scorpionfish from nearshore waters may be a result of the trophic flow through a relatively long and complex food chain compared with those in offshore waters. The food web of the two waters depends on a variety of carbon sources with a range of trophic pathways. In the more complex nearshore environment, a number of primary producers may contribute to the diets of primary consumers (Alfaro et al., 2006; Franca et al., 2011), while in the offshore waters, kelp cultivation dominates the benthic primary production.

CONCLUSION

The present study has provided evidence of the partitioning of food resources among three co-occurring scorpionfish around the artificial reef in the nearshore waters of Rongcheng Bay of the northern Yellow Sea, which would reduce the potential competition for resources. These carnivorous fishes predate on a similar range of prey, including crustaceans, teleosts, polychaetes and macroalgae. The dietary composition of these three scorpionfish in nearshore waters differs, implying that they partition their resources to reduce competition. In the offshore water, the dietary data from fewer individuals indicate that *H. otakii* and *S. schlegelii* had relatively narrow prey options, mainly predated on carideans & penaeids and brachyurans living in the muddy substrate. In the offshore, our results suggested no major differences in diet between these two species which may be due to the abundance of kelp in the offshore and a simplified food web.

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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	<i>Hexagrammos agrammus</i>			<i>Hexagrammos otakii</i>			<i>Sebastes schlegelii</i>			<i>Hexagrammos otakii</i>			<i>Sebastes schlegelii</i>		
N	55 (39)			23 (18)			80 (29)			7 (7)			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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (± 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 $\delta^{15}\text{N}$ ($\pm$ 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 $\delta^{15}\text{N}$ value. — = no length range specified.

Species and Length(mm)	Rank	$\delta^{15}\text{N}$	Habitat type	Location	References
<i>Hexagrammos agrammus</i>					
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
<i>Hexagrammos otakii</i>					
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
<i>Sebastes schlegelii</i>					
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) *Hexagrammus 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 *Hexagrammus agrammus* (●) *Hexagrammus otakii* (●) and *Sebastes schlegelii* (○) caught in (a) nearshore and (b) offshore waters of Lido, 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values (± 1 SD) of the three *Hexagrammos agrammus*, *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)
<i>Hexagrammos agrammus</i>	Nearshore	55 (39)	133 (88-178)	56 (14-135)
	Offshore	1 (0)	186	159
<i>Hexagrammos otakii</i>	Nearshore	23 (18)	129 (97-196)	47 (17-159)
	Offshore	7 (7)	155 (135-169)	81 (52-109)
<i>Sebastes schlegelii</i>	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)		