OPEN ACCESS Science International

ISSN 2305-1884
DOI: 10.17311/sciintl.2016.94.100

Research Article
Diet Patterns of the Marbled Flounder, *Pseudopleuronectes yokohamae*, in the Mid-Western Coast of Korea

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Abstract
Background and Objective: Stomach contents of *Pseudopleuronectes yokohamae* collected from mid-Western coast of Korea were analysed to determine dietary composition as a function of fish life stage, water temperature and time. This study was to provide needed data on feeding behavior to aid in conservation and management for this ecologically and economically important species of fish. **Materials and Methods:** Prey items were identified to the lowest possible taxa with a dissecting microscope. Distance-based linear modelling (DISTLM) using total length, maturity, sampling month, water temperature and sex as predictors was used to assess diet composition. Logistic regression on stomach fullness (0: empty, 1: with content) using the same predictors as above was used to assess feeding intensity. **Results:** Fish Total Length (TL) ranged between 8.6 and 26.8 cm. Polychaetes dominated the diet of both smaller and larger fishes. Ophiuroids and bivalves were secondary and more frequently consumed by larger specimens. Distance-based linear modelling showed diet composition of *P. yokohamae* varying significantly with maturity and Total Length (TL) but not with sampling month, water temperature, nor gender. **Conclusion:** Data showed *P. yokohamae* select for polychaetes, foraging near the substrate. Mature individuals consumed more ophiuroids than did juveniles, indicating intra-specific competition avoidance during feeding for this species.

Key words: Fisheries, fisheries management, flatfishes, trophodynamics, *Pseudopleuronectes*, marbled flounder, polychaetes

Received: October 24, 2016 Accepted: November 07, 2016 Published: November 15, 2016

Citation: Joo Myun Park, Seok Nam Kwak, Hee Chan Choi, Laith A. Jawad and Ralf Riedel, 2016. Diet patterns of the marbled flounder, *Pseudopleuronectes yokohamae*, in the mid-Western coast of Korea. Sci. Int., CC: CC-CC.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The marbled flounder, *Pseudopleuronectes yokohamae* is a righteyed, benthic flounder (Pleuronectiformes; Pleuronectidae) widely distributed throughout temperate waters in the Western Pacific. This species inhabits sandy and muddy bottoms in coastal habitats[1,2]. Pleuronectidae comprise of 103 species in 40 genera distributed worldwide[3] with more than 26 species in Korean waters[4]. All of the species of this family are demersal. Fishes of this family have a flattened body, a pale underside and ventrally located eyes, which make them well adapted to the benthic environment.

Righteye flounders are commercially important in Korea[2], where they are primarily caught in gillnets and trawls[5]. In the Western sea of Korea, commercial landings of righteye flounders comprised mainly of *Kareius bicoloratus*, *Pleuronichthys cornutus* and the marbled flounder, this latter the most abundant in weight[6-8]. The *P. yokohamae* is also ecologically important, due to its high abundance throughout its habitats[8,9].

Due to the commercial and ecological importance of the marbled flounder, many studies about its fisheries and ecology have been conducted throughout its range. Results of studies on dietary habits of juvenile[10] and adult[11], early life history[12,13], growth[14] and reproduction[15,16] have been used in conservation and management of this species. Righteyed flounders are considered benthic invertebrate feeders that occupy intermediate trophic levels[3]. Studies on the feeding ecology of *P. yokohamae* may shed more light on the functional role of this species in marine ecosystems[17,18], providing additional insight for management and conservation[19,20]. More specifically, studies on dietary changes with fish size and studies on diet patterns with time may further provide management with information on ecological linkages and feeding behaviour for this species.

The objectives of this study were to determine dietary composition of the marbled flounder and to identify diet patterns with fish size, time of year, gender and water temperature. The results from this study will add to management and conservation of this species in Korean waters.

MATERIALS AND METHODS

Study area and sampling: Fish were collected off the West Korean coast (Fig. 1)[21] in the Yellow Sea, a semi-enclosed shallow basin with depths below 100 m, home of many commercially important benthic fishes[2].

Sampling was conducted monthly from April-July, 2008. Fish were collected at depths of 20-30 m using a 20 m long, 4 m wide bottom trawl, with wing and body stretched mesh of 3 cm and a codend mesh liner of 1 cm. Trawling was done during daylight at neap tides. Immediately after capture, fish were placed in ice and transported to laboratory at Pukyong National University, South Korea. Total Length (mm, TL) and wet weight (g) were taken at the laboratory. Similarly, stomachs were removed and the contents preserved in 5% formalin for at least 24 h, prior to long-term storage in 70% isopropanol.

Diet composition: Prey items were identified to the lowest possible taxa with a dissecting microscope. Prey fragments were taken to be parts of a whole organism and counted as such. No evidence of regurgitation was observed. The numbers and wet weights of each prey were recorded.

Prey composition patterns according to time and fish maturity were inferred. Fish maturity was estimated according to size, following Seo *et al*[6]. The four sampling months were taken to assess temporal patterns in feeding. Maturity groups were 14.0-19.9 cm TL (immature, n = 59) and 20.0-40.6 cm TL, (mature, n = 149), months were April (n = 42), May (n = 59), June (n = 43) and July (n = 64).

Diet was described as frequency of occurrence ($F\% = 100 \times A_i \times N^{-1}$), as a numerical percentage ($N\% = 100 \times N_i \times N_T^{-1}$) and as a mass percentage ($W\% = 100 \times W_i \times W_T^{-1}$), where A_i was the number of fish preying on species i, N the total number of fish examined (excluding those with empty stomachs), N_i (W_i) the number (weight in mg) of prey individuals i and N_T (W_T) total number (weight in mg) of prey individuals. The index of relative importance (IRI)[22] was calculated for each prey item as $(N\%+W\%) \times F\%$ and also expressed as a percentage (IRI%).

The asymptote of the curve relating the cumulative number of prey against the number of stomachs examined was determined to demonstrate sample size (number of stomachs) adequacy. The order of stomachs to construct the cumulative prey curve was randomized 100 times to assess variability in the asymptote[23]. The cumulative prey curve was constructed by plotting the mean numbers of all prey taxa against the number of stomachs analysed. The asymptote was defined as in Huveneers *et al*.[24].

Diet patterns: Because data from each fish yielded multiple prey taxa, we opted to analyse patterns of prey composition using multivariate statistical methods. Prey biomass was examined using distance-based linear modeling (DISTLM)[25,26], using total length, maturity stage, sampling month, water

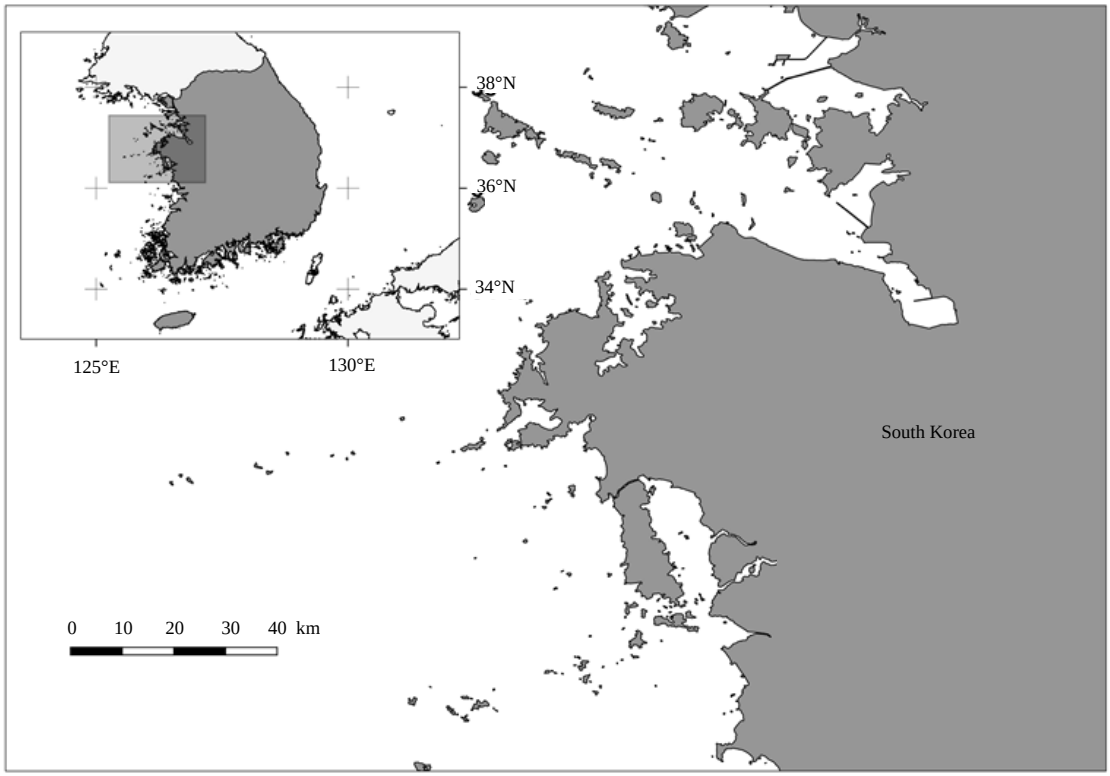

Fig. 1: Study location indicating sampling area (shaded square in inset) for feeding patterns of *Pseudopleuroneces yokohamae*

temperature and sex as predictors. Fish with empty stomachs were not included in analysis for prey composition. Distance-based linear modelling was chosen due to relaxation of data homoschedasticity as an underlying assumption of analysis. The Bray-Curtis distance matrix was used as the basis for estimating effect size and significance of factors.

A DISTLM conditional test was performed using a forward selection of the predictors, with permutation tests on the R^2 criterion[25]. The most parsimonious model was identified, using the Akaike Information Criterion. A similarity percentage (SIMPER) analysis was used to estimate the contribution of each prey taxa to the statistically significant predictors from the DISTLM.

Patterns of fish feeding activity were estimated using empty stomachs as a surrogate for no feeding. Activity was examined using binomial logistic regression. Fish were coded as either 0 (empty stomach) or 1 (content present). Codes were taken as the response variable. The independent variables were as above for the DISTLM analysis. The logit link function for the regression was used. Data were split to test for model fitting. The training set comprised of 85% of the data and the remainder of data was assigned to the testing set. Deviance decline following the regression analysis was used to assess the effect of the independent variables in estimating the response.

RESULTS

Diet composition: Two hundred and eight marbled flounder specimens ranging between 14.0 and 40.6 cm TL (Mean±SD = 23.5±5.3 cm) were collected. Of the 208 stomachs, 13 were empty. Cumulative prey curves for overall diet (number of prey taxa found) reached an asymptote after 146 stomachs (Fig. 2), less than the total stomachs examined.

Eleven prey taxa were identified in the 195 stomachs with content (Table 1). Polychaetes were the most common prey item, consisting of 96.4% by occurrence, 63.6% by number, 76.8% by weight and 88.2% in IRI. Amphipods were the second-largest dietary component, totalling 44.6% by occurrence, 19.0% by number, 0.6% by weight and 5.4% in IRI. Both ophiuroids and bivalves made up each of 2.6% in IRI and all of remaining prey taxa constituted less than 1.0% in IRI.

Diet patterns: Polychaetes were the most common prey item observed (Table 1). The contribution of polychaetes in dietary composition, however, were lower in mature fish (Fig. 3). The highest contribution of prey for mature fish was for ophiuroids, followed by crustaceans. Temporally,

polychaetes were evenly distributed in diets during all months, with bivalves and ophiuroids becoming more pronounced in June (Fig. 3).

The multivariate DISTLM test indicated that the diet of marbled flounder was related with total length and maturity only. The DISTLM conditional test indicated diet to be related with total length and water temperature (Table 2). The most parsimonious model for diet composition included total length, month and water temperature. Although the effects of two predictors were significant ($p<0.05$), the conditional test model explained only 7.6% of the data variability.

The SIMPER test showed that polychaetes were present in relatively greater contributions in the diets of smaller marbled flounder, whereas the opposite trend was true for ophiuroids and bivalves. Among prey taxa, ophiuroids and bivalves contributed mostly to the dissimilarity between immature and mature fish.

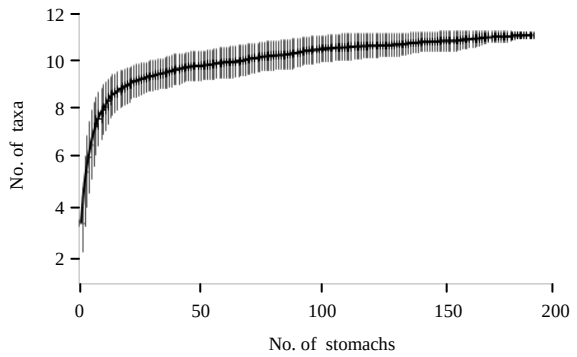

Fig. 2: Cumulative prey curve (prey taxa per stomach) for *Pseudopleuronectes yokohamae* collected in the mid-Western coast of South Korea. Error bars represent standard deviations

Table 1: Stomach contents *Pseudopleuronectes yokohamae* showing frequency of occurrence (F), number (N), weight (W, mg) and index of relative importance (IRI)

Taxa	F	N	W	IRI
Polychaeta	188	1,606	301,161	13,531.3
Amphipoda	87	481	1,541	866.8
Bivalvia	67	97	30,550	399.6
Ophiuroidea	53	166	31,555	397.2
Brachyura	30	36	9,288	58.4
Gastropoda	24	40	11,888	56.8
Isopoda	29	56	3,750	47.2
Cumacea	27	33	306	19.2
Caridea	10	9	1,502	3.8
Stomatopoda	1	1	589	0.1
Sipunculida	2	2	20	0.1
Totals	518.00	2,527.00	392,150	15,380.3

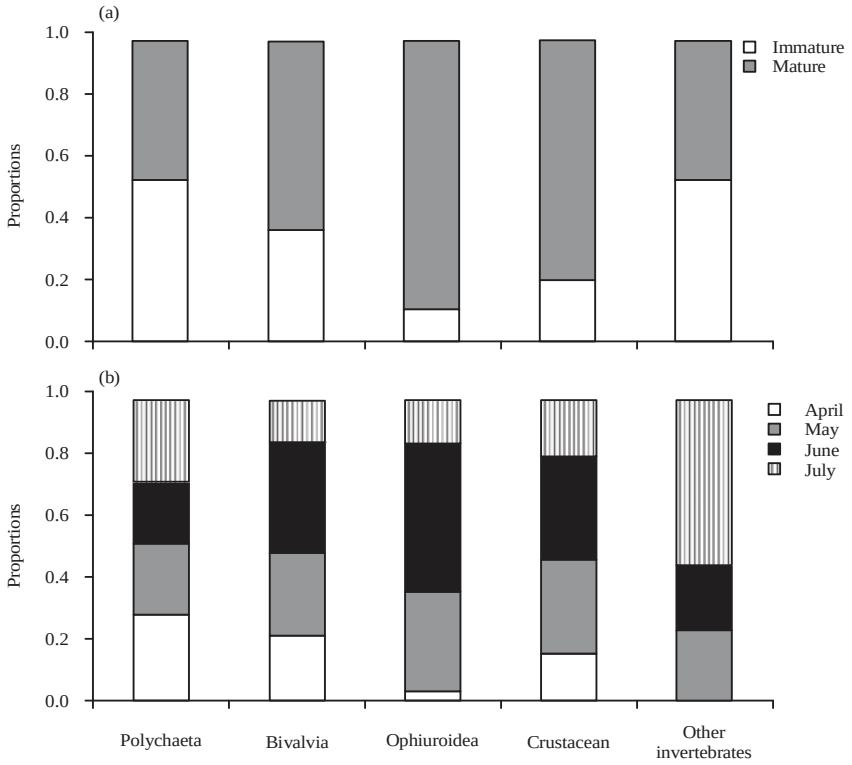

Fig. 3(a-b): Percentage mass contributions of prey groups for (a) Immature and mature and (b) Four months for *Pseudopleuronectes yokohamae* in coastal waters of mid-Western South Korea

Table 2: Distance-based linear modeling analysis relating *Pseudopleuronectes yokohamae* diet with total length, maturity, month, water temperature and sex, individually (marginal test) and sequentially (conditional test), for a population from the mid-Western coast of South Korea

Predictor variables	Pseudo-F	R^2	p-value	Prop
Marginal test				
Month	1.503		0.199	0.008
Temperature	1.527		0.214	0.008
Total length	6.536		0.001	0.033
Maturity	5.685		0.001	0.029
Sex	1.251		0.292	0.006
Conditional test				
Total length	6.536	0.033	0.001	0.033
Month	2.215	0.044	0.084	0.011
Temperature	6.693	0.076	0.001	0.032

Prop: Proportion of variance explained by each variable

No evidence for feeding intensity with fish size, maturity, sampling month or water temperature was found, as measured by frequency of empty fish stomachs.

DISCUSSION

The data showed strong evidence of the benthic nature of marbled flounders. The greatest contributors in the diets of marbled flounder were polychaetes, which tend to dwell at the surface of the substrate. This suggests that marbled flounder forage close or near the surface, without much burrowing activity during feeding. Diets of marbled flounders reported here are comparable to those reported for other species of righteyed and lefteyed flatfishes, where polychaetes were a major food item[27-29]. In the Bohai sea, the dominance of polychaetes in the diets of flatfishes has been shown relatively low, despite their presence on the substrate, indicating flounder preference for other benthic invertebrates and teletosts[28]. Polychaetes, however are considered to be an important food resource for some righteye flounders[27,29,30] and also some other benthic fishes[31,32].

There have been observations of region-specific differences in food resources for marbled flounder in Korean waters. Marbled flounders from the coastal waters off Tongyeong (Southeastern Korea) fed mainly on bivalves and secondly on polychaetes[11], whereas relatively smaller fishes inhabiting eelgrass bed on the Southern coast consumed mainly polychaetes and amphipods[10]. In the Sendai Bay of Japan, polychaetes were major components in the diet of marbled flounders, with bivalve and sea anemones contributing moderately to the diets of that species[33]. Those studies also indicated that marbled flounder preferred polychaetes, with bivalves only abundant in the diets of its Southeastern population of South Korea[11]. Generally, fish target the most abundant locally available prey, which is indicative of the substrate's faunistic composition.

This study showed an unusual feeding behavior for marbled flounders, based on the frequent consumption of ophiuroids. Ophiuroids are not commonly major food items for bottom-feeding fish[28,34,35], with only a few species specializing in that prey group[36-39]. Ophiuroids are relatively indigestible, offering a low energy return, which begs the question as to why some fish select this prey as food. Ohmura *et al.*[38] suggested that selection for ophiuroids reduces intra and interspecific competition between co-occurring fish species. However, this hypothesis may not apply to marbled flounders, since competition for food is not a major factor for this species. Further research examining the physiological importance of ophiuroids is called for.

Diet changes according to fish size are common in species and are usually related to maximizing energy intake[40]. The diets of many species are segregated by life history stage, keeping intra-specific competition low[41,42]. The stomach content analysis herein showed that dietary composition of marbled flounder changed as predator size increased. Although, polychaetes were always a major dietary component of both immature and mature groups, mature individuals targeted proportionately more ophiuroids and bivalves. This may be partly explained by the higher ability of mature marbled flounders to handle these prey, as larger fish more easily crush hard-bodied prey[43].

Diet of marbled flounders in this study did not significantly vary between sexes. Although, some flatfishes show differences in diet with sex[44], most species from this group do not[45,46]. Similarly, feeding intensity did not show any pattern with sex, fish size, maturity stage or sampling month. This is evidence that feeding behavior for this species is relatively constant with ontogenetic stage and during the spring and summer (temporal range of this study). True seasonal changes were not assessed in this study due to the limited range of months sampled. Seasonal changes in fish feeding habits are associated with changes in food availability caused by environmental factors and physiological variation[17]. In this study, marbled flounders mainly selected polychaetes during all months, with some increase in consumption of ophiuroids and bivalves during May and June. Studies covering at least one year of sampling may shed more light into feeding behaviour for marbled flounder with time.

CONCLUSION

In conclusion, this study offers a baseline investigation on diets of the marbled flounder, an important species for the fisheries in the mid-Western coast of Korea. Stomach contents indicated polychaetes to be the major food item, regardless

of size, sex or sampling month. Some temporal changes in diets were also observed, however small in effect. Dietary studies for marbled flounder covering a wider region and sampling fish throughout at least one year might resolve some of the questions left unanswered in this study. Such follow up studies are critical to supplement understanding of the feeding dynamics for this species, offering much needed advice for management and conservation of this commercially and ecologically important species for Korea.

ACKNOWLEDGMENTS

This research was funded by the Korea Inter-University of Ocean Science at Pukyong National University, South Korea, under the approval of the Research and Training Fishery, Ministry of Ocean and Fisheries, South Korea.

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