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Size-related and Temporal Dietary Variations of *Hexagrammos otakii* in the Mid-western Coast of Korea

By Hee Chan Choi, Sung-Hoi Huh[1] and Joo Myun Park[2,]*

Oceanic Climate & Ecology Research Division, National Institute of Fisheries Science, Busan 46083, Republic of Korea
[1]Department of Oceanography, Pukyong National University, Busan 48280, Republic of Korea
[2]Department of Biological Sciences, Macquarie University, Sydney, NSW 2109, Australia

ABSTRACT **Stomach contents of *Hexagrammos otakii* collected from mid-western coast of Korea were analysed to determine dietary composition, and ontogenetic and temporal dietary variations. Fish total length (TL) ranged between 5.6 and 32.4 cm. *H. otakii* primarily consumed amphipods, carid shrimps and teleosts, but also ate various benthic crustaceans and other invertebrates with small amounts. Carid shrimps and teleosts dominated the diet of both immature and mature fishes. Amphipods and polychaetes were secondary, and the former more frequently consumed by smaller specimens, while the latter was more abundant in the diets of larger fishes. DISTLM (distance-based linear modelling) showed diet composition of *H. otakii* varying significantly with TL, season and water temperature, but did not with maturity.**

Key words: Stomach contents, dietary variations, *Hexagrammos otakii*, mid-western waters of Korea

INTRODUCTION

Hexagrammos otakii is a demersal greenling (Scorpaeniformes; Hexagrammidae) that widely distributed throughout temperate waters in the northwest Pacific including waters off Japan and southern Korea. This species inhabits shallow rocky shores and/or seagrass beds with sandy-mud sediment in coastal habitats (Yamada *et al*., 1986; Kim *et al*., 2004). Hexagrammidae fishes constitute 12 species within 5 genera distributed worldwide (Froese and Pauly, 2017), with 5 species in Korean waters (Kim *et al*., 2005). All of the species of this family are demersal.

Greenlings, including *H. otakii* and *H. agrammus*, are commercially important fishes in Korean waters (Kim *et al*., 2004), where they are primarily caught in gillnets and trawls (KOSTAT, 1990∼2016). *H. otakii* is also ecologically important, due to its high abundance throughout its habitats (Park *et al*., 2013; Jeong *et al*., 2014), and predation on various benthic invertebrates and fishes (Seo and Hong, 2007). Due to the commercial and ecological importance of *H. otakii*, many studies about its ecology and biology have been conducted. Results of studies on dietary habits (Kwak *et al*., 2005), early life history (Hamai and Kyushin, 1966), distribution (Fukuhara and Fushimi, 1983) and reproduction (Choi *et al*., 1997) have been used in conservation and management of this species. *H. otakii* is considered benthic invertebrate feeders that occupy intermediate trophic levels (Froese and Pauly, 2017). Studies on the feeding ecology of *H. otakii* may shed light on the functional role of this species in marine ecosystems (Wootton, 1990; Brodeur and Pearcy, 1992), providing additional insight for management and conservation (Micheli and Halpern, 2005; Greenstreet and Rogers, 2006).

Dietary variations in relation to various factors were common trends for many fish species. These were often related to fish size (e.g. Choi *et al*., 2016), maturity (e.g. Lopez-Lopez *et al*., 2011), gender (e.g. Cortés, 2000), habitat (e.g. Szedlmayer and Lee, 2004) and season (e.g. Park and Huh, 2017), but some fish species did not show

*Corresponding author: Joo Myun Park Tel: 61-2-9850-8234, Fax: 61-2-9850-8245, E-mail: joomyun@gmail.com

these trends. To analyse dietary patterns of prey composition, we have to opt various multivariate statistical methods, and also consider which factor is the most important to explain these changes, because dietary data from each individual of fish species yielded multiple prey taxa, and multiple factors were involved together in the diet changes. More specifically, studies on dietary patterns may further provide management with information on ecological linkages and data on stock structures for this species.

The objectives of this study were to determine dietary composition of *H. otakii* and to identify dietary variations with respect to fish size, maturity, season and water temperature. The results would be highly beneficial both for management purposes and future trophic community analyses in Korean waters.

MATERIALS AND METHODS

1. Study area and sampling

Fish were collected off the western Korean waters (36°25′N∼37°00′N, 126°00′E∼126°20′E; see also Choi *et al*., 2016) in the Yellow Sea. Samples were collected as by-catch from research trawls on board in spring (April∼May), Summer (June∼July) and Autumn (November) of 2008. Water temperature in the study area ranged from 6.9 to 22.5°C, with a peak in July and reaching a minimum during spring. 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 cod end 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 where total length (mm, TL) and wet weight (g) were measured. The stomachs were removed and the contents preserved in 5% formalin for at least 24 hours, prior to long-term storage in 70% isopropanol.

2. Stomach content analysis

Prey items were identified to the lowest possible taxa with a dissecting microscope. The numbers and wet weights of each prey were recorded. Prey composition patterns according to time, fish size (TL) and maturity were inferred. Fish maturity was estimated according to size, following Choi *et al*. (1997). The three sampling seasons were taken to assess temporal patterns. Maturity groups were 5.6∼21.1 cm TL (immature; n=58) and 22.2∼32.4 cm TL, (mature; n=41), seasons were spring (n=32), summer (n=46) and autumn (n=21).

Diets were quantified by 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 weight 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) of prey individuals i, and N_T (W_T) total number (weight) of prey individuals. Next, the index of relative importance (IRI) (Pinkas *et al*., 1971) was calculated for each prey item as follows: $IRI = (\%N + \%W) \times \%F$, and 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 (Ferry and Cailliet, 1996). The cumulative prey curve was constructed by plotting the mean numbers of 13 prey taxa (polychaetes, gastropods, bivalves, cephalopods, isopods, amphipods, mysids, penaeid shrimps, carid shrimps, hermit crabs, crabs, stomatopods and teleosts) against the number of stomachs analysed. When as at least ten previous values of the total number of prey taxa were in the range of the asymptotic number of prey ±0.5 (Huveneers *et al*., 2007).

3. Dietary variation analysis

To examine the relative extents to which potential predictors explained most of the variability in dietary composition, distance-based linear modeling (DISTLM) was employed (Anderson *et al*., 2008; Dunn, 2009). Prey biomass was standardised by expressing the weight of each prey item as a proportion of the total weight in each stomach, then square root transformed to avoid any tendency for the main dietary components to be excessively dominant (Platell and Potter, 2001), and then Bray-Curtis dissimilarity matrices were constructed. DISTLM performs multivariate multiple regression based on a given distance measure to model the relationship between multivariate data and one or more predictor variables. The potential predictors were total length, maturity, season and water temperature. Total length and water temperature were considered continuous variables, and maturity and season were considered a categorical variable. DISTLM produces both a marginal test, which assesses how much variation is explained by each predictor when taken alone (ignoring all other variables), and a conditional test, which assesses how much is explained by all of the variables (Anderson *et al*., 2008). The most parsi-

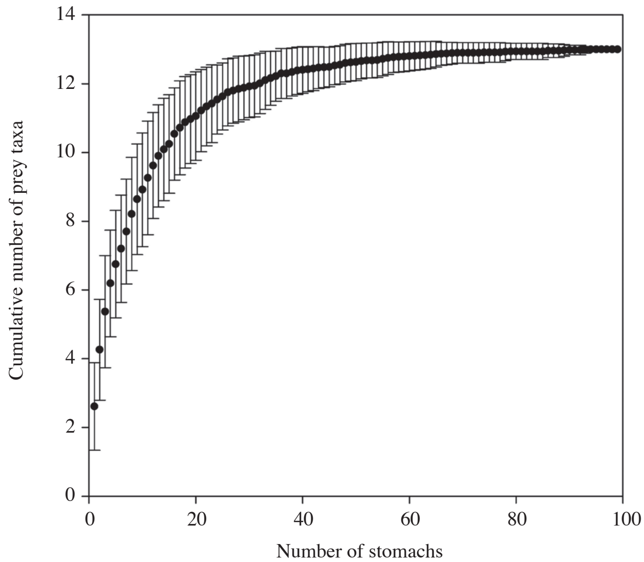

Fig. 1. Cumulative prey curve (prey taxa per stomach) for *Hexagrammos otakii* collected in the mid-western coast of Korea. Error bars represent standard deviations.

monious model was identified using the Akaike Information Criterion (AIC), because R^2 simply increases with an increasing number of predictors (Anderson *et al*., 2008). DISTLM conditional test was performed using a forward selection of the predictors, using permutation tests on the R^2 criterion (Anderson *et al*., 2008).

Two-way crossed analysis of similarities (ANOSIMs) were used to test for any significant differences in dietary compositions with respect to maturity or season, and with the magnitude of the *R*-statistic indicating the relative importance of any such differences (Clarke *et al*., 2014). SIMPER (similarity percentages) was used to estimate the contribution of each prey taxa to the statistically significance in the diets by each of categorical predictors (i.e. maturity and season) from the DISTLM and ANOSIM. All of above tests were performed using the routines in the PRIMER v7 multivariate statistics package (www.primer-e.com) and the PERMANOVA+ add-on module (Anderson *et al*., 2008).

RESULTS

1. Overall dietary composition

Ninety-nine specimens of *H. otakii* ranging from 5.6 to 32.4 cm TL (mean ± SD = 20.1 ± 7.8 cm) were collected. Cumulative prey curves for overall diet (number of prey taxa found) reached an asymptote after 64 stomachs (Fig. 1), less than the total stomachs examined.

Table 1. Stomach contents *Hexagrammos otakii* showing frequency of occurrence, number, weight, and index of relative importance (IRI)

Prey items	%F	%N	%W	%IRI
Polychaeta	28.3	5.4	5.6	3.4
Gastrapoda	5.1	0.5	0.3	<0.1
Bivalvia	5.1	0.6	0.9	0.1
Cephalopoda	5.1	0.6	10.5	0.6
Loliginidae	4.0	0.4	7.4	
Octopodidae	1.0	0.1	2.9	
Sepiolidae	1.0	0.1	0.2	
Amphipoda	63.6	55.1	0.9	38.9
Caprellidae	5.1	1.6	0.2	
Ampeliscidae	2.0	0.3	<0.1	
Jassa falcata	15.2	3.3	0.1	
Oedicerotidae	4.0	0.5	<0.1	
Pontogeneiidae	6.1	1.4	<0.1	
Unidentified	58.6	48.1	0.5	
Mysidacea	10.1	1.5	2.2	0.4
Isopoda	9.1	1.1	0.2	0.1
Anomura	10.1	1.6	1.3	0.3
Paguridea	2.0	0.2	0.4	
Galatheidea	2.0	0.2	0.4	
Porcellanidae	6.1	1.2	0.5	
Stomatopoda	2.0	0.2	0.8	<0.1
Penaeidae	7.1	1.5	3.5	0.4
Parapenaeopsis hardwickii	1.0	0.1	0.6	
Parapenaeopsis tenella	6.1	1.4	2.9	
Caridea	66.7	21.4	9.5	22.5
Alpheidae	12.1	1.4	5.1	
Crangonidae	34.3	11.7	1.4	
Heptacarpus rectirostris	13.1	1.6	1.1	
Latretus planirostris	22.2	3.7	0.8	
Latreutes anoplonyx	4.0	0.5	0.2	
Leptochela sydniensis	4.0	0.4	<0.1	
Palaemonidae	1.0	0.1	<0.1	
Pandalus gracilis	1.0	0.1	<0.1	
Plesionika izumiae	1.0	0.1	<0.1	
Unidentified	14.1	1.9	0.9	
Brachyura	19.2	2.5	6.4	1.9
Cancer japonicus	1.0	0.1	3.0	
Charybdis bimaculata	1.0	0.1	0.2	
Oregonia gracilis	1.0	0.1	0.2	
Paradorippe granulata	4.0	0.4	0.4	
Pinnotheridae	3.0	0.3	0.2	
Pisoides bidentatus	1.0	0.1	0.3	
Pugettia quadridens	2.0	0.3	1.0	
Unidentified	9.1	1.1	1.0	
Teleostei	43.4	8.0	58.0	31.3
Apogon lineatus	1.0	0.1	2.9	
Callionymidae	1.0	0.2	0.3	
Chaeturichthys stigmatias	2.0	0.2	5.3	
Engraulis japonicus	4.0	0.7	11.2	
Gobiidae	1.0	0.1	0.0	
Hexagrammidae	1.0	0.1	2.5	
Sebastes schlegelii	15.2	3.5	4.4	
Thryssa spp.	3.0	0.6	26.9	
Unidentified	18.2	2.4	4.5	

A total of 99 stomachs that were accessed contained 13

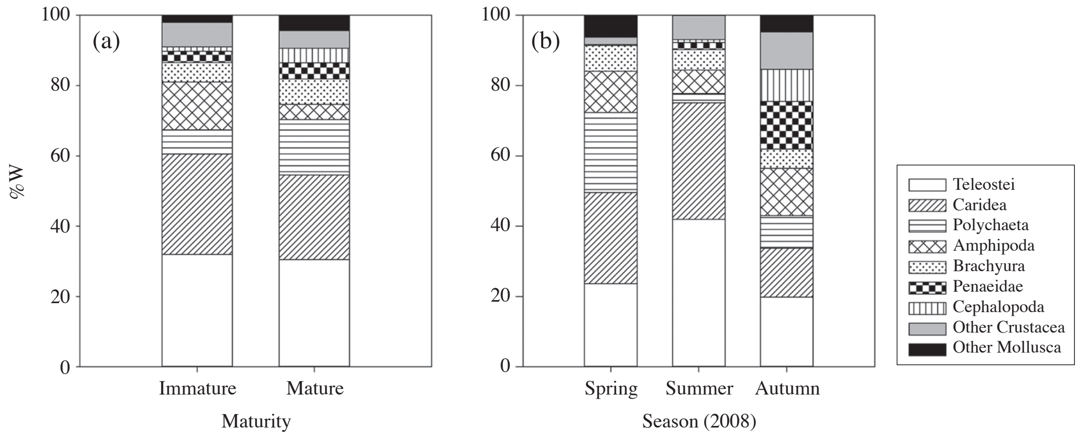

Fig. 2. Percentage mass contributions of the different dietary components of two maturity groups (Panel a) and three seasons (Panel b) for *Hexagrammos otakii* in the mid-western coast of Korea.

identifiable prey taxa (Table 1). In terms of %IRI, amphipods, carid shrimps and teleosts accounted for almost the entire diets (92.8%). Polychaetes and crabs were second in importance, comprising 3.4% and 1.9%, respectively, and all of remaining prey taxa constituted less than 1.0% in IRI.

2. Variations in diets

When the dietary data were examined by size class, teleosts and carid shrimps were the most common prey items in the diets of both immature and mature groups. Beside these prey taxa, the contribution of amphipods in dietary composition declined, while conversely, the contribution of polychaetes increased with increasing body size of *H. otakii* (Fig. 2a). Temporally, teleosts, carid shrimps and polychaetes dominated the diets during spring, with the former two prey taxa becoming more pronounced in summer (Fig. 2b). Autumn was season where various prey taxa contributed together entire diets of *H. otakii*.

3. Dietary variation analysis

The multivariate DISTLM test indicated that the diet of *H. otakii* was related with season, temperature and total length, with the most parsimonious conditional model for diet composition including these three predictors (Table 2). The model explained only 15.3% of the data variability (conditional test), indicating most of the variability in diet could not be explained by the predictors. Two-way crossed ANOSIM showed that the dietary composition of *H. otakii* differed with season ($P=0.001$), but not with maturity ($P=0.122$), and that the global R-statistic values were higher for season than maturity (global $R=0.256$ verse 0.062). Pairwise ANOSIM further demonstrated that all seasonal comparisons were significant, with being strongest between summer and autumn. SIMPER emphasized that carid shrimps and teleosts were present in relatively greater volumes in the diets of *H. otakii* in spring and summer, respectively, whereas the opposite trend was true for amphipods during autumn. The diet of *H. otakii* in summer was distinguished from those in spring and autumn by containing greater volumes of teleosts and lesser volumes of polychaetes, while carid shrimps contributed mostly to the dissimilarity between spring and autumn diets.

Table 2. Results of distance-based linear modeling (DISTLM) analysis relating *Hexagrammos otakii* 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 Korea

Predictor variables	R^2	P
Marginal test		
Season	0.035	0.009
Temperature	0.048	0.001
Total length	0.040	0.007
Maturity	0.018	0.114
Conditional test		
+Temperature	0.048	0.003
+Season	0.104	0.001
+Total length	0.153	0.001

DISCUSSION

In the present study, *H. otakii* was carnivorous fish that consumed various benthic invertebrates and teleosts (Table 1). Based on the %IRI values, the greatest contributors in the diets of *H. otakii* were epibenthic crustaceans (e.g. amphipods and carid shrimps) and teleosts, with the majority being benthic thus suggesting foraging close to or just above the surface of the substratum. The importance of these prey items in the diets was also reported elsewhere. For example, the diets of *H. otakii* from seagrass beds of southern Korea were mainly constituted by amphipods, decapods, polychaetes and teleosts (based on mass), but the dominance of polychaetes in the diets was higher than that of present study (Kwak *et al.*, 2005). Amphipods, carid decapods, mysids, polychaetes and teleosts were main food items for both smaller and larger *H. otakii* from the Jangbong tidal flat, western Korea (Seo and Hong, 2007). In the shelf off Iwate of north-eastern Japan, *H. otakii* consumed benthic crustaceans as well as pelagic and benthic fishes (Fuita *et al.*, 1995). Thus, although there were some region-specific differences in prey importance of each prey taxa, epibenthic crustaceans, polychaetes and bentho-pelagic teleosts are common food for *H. otakii* regardless of habitat differences.

Ontogenetic diet changes are common in fish species and are usually related to maximizing energy intake (Gerking, 1994), and can be related to onset of maturity and thus a transition from juveniles to adults (Lopez-Lopez *et al.*, 2011). The dietary composition of *H. otakii* changed significantly as total length of predator increase, but not maturity. The results indicate that the transition of diet shift did not occur at maturity size, but it was evident at any size either smaller or larger length of fish, or both. DISTLM also showed no significant variations in diets by maturity, because both maturity groups showed similar dietary compositions. Although carid shrimps and teleosts were major dietary component of both immature and mature groups, smaller *H. otakii* focused more on amphipods, whereas larger individuals targeted more polychaetes and cephalopods. This increase may be explained by the increase of ability to capture larger prey items as they grow, because larger fish may have increased ability to catch larger and/or mobile prey than smaller conspecifics (Park *et al.*, 2017).

"True" seasonal changes could not be assessed in this study due to the lack of seasonal replication and samples covering all seasons. Seasonal changes in fish feeding habits are associated with changes in food availability caused by environmental factors and physiological variation (Wootton, 1990). In the present study, *H. otakii* principally consumed carid shrimps and teleosts during summer, but there were decreased consumption of these prey taxa in spring and autumn. These temporal dietary differences were significantly related with seasonal variations in water temperature. Nonetheless, due to the lack of information on all season data and on seasonal abundance of prey items in the study area, our data did not show true temporal diet changes. Further studies are demanded to discover dietary habits of *H. otakii* in detail.

In conclusion, this study offers important insights into the diets of *H. otakii* that are abundant in the mid-western coast of Korea. Stomach contents indicated that they consume mainly epibenthic crustaceans and teleosts, with observing some ontogenetic and temporal changes in diets. Nonetheless, a lack of samples covering wider regions, all possible size ranges and/or all seasons imposed limitations on describing absolute diets of the species throughout their entire life histories. Studies on dietary habits of *H. otakii* are essential to understand prey-predator relationships in benthic ecosystems, and are important baseline studies for management and conservation efforts of fishes in western Korean sea.

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서해 중부 연안에 출현하는 쥐노래미(*Hexagrammos otakii*)의 성장과 계절에 따른 식성 변화

최희찬 · 허성회[1] *· 박주면*[2]

국립수산과학원 기후변화연구과, [1]부경대학교 해양학과, [2]맥쿼리대학교 생물학부

요 약 : 본 연구는 서해 중부 연안에 출현하는 쥐노래미 (*Hexagrammos otakii*)의 위내용물 분석을 통하여, 위내용물 조성과 성장 및 계절별 식성변화를 분석하였다. 쥐노래미의 전장 (total length, TL)은 5.6 cm에서 32.4 cm의 범위였다. 쥐노래미는 단각류 (amphipods), 새우류 (carid shrimps), 어류 (teleosts)를 주로 섭식하였고, 그 외 다양한 저서 갑각류와 무척추동물을 소량 섭식하였다. 새우류와 어류는 미성어 (immature)와 성어 (mature)의 위내용물에서 공통적으로 우점하였다. 단각류와 갯지렁이류 (polychaetes)는 그 다음으로 우점하였는데, 단각류는 작은 크기의 어류에서, 갯지렁이류는 큰 크기의 어류에서 그 비율이 더 높았다. DISTLM (distance-based linear modelling) 결과, 쥐노래미의 위내용물은 전장, 계절, 수온에 따라 유의하게 변하였지만, 성숙도와는 관련이 없었다.

찾아보기 낱말 : 위내용물, 식성 변화, 쥐노래미, 서해 중부 연안