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Vertical Distribution and Feeding Ecology of the Black Scraper, *Thamnaconus modestus*, in the Southern Sea of Korea

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Abstract

We investigated the vertical distribution and feeding ecology of the black scraper, *Tamnaconus modestus*, in the southern sea of Korea in the spring of 2009, 2010 and 2011 using otter trawl for 60 min per site. Fish abundance was significantly related to the basis of depth and temperature. *Thamnaconus modestus* occurred at depths ranging from 80 to 120 meter waters having temperature higher than 12°C. We found that 87% of the total catch were obtained at the depths between 80 and 120 meters (58% and 29% from 80–100 and 100–120 meters respectively). The total length of captured individuals ranged from 10.6 to 38.7 cm. Individuals captured at deeper were significantly larger than those from shallower sites. Diverse prey organisms, including algae, amphipods, gastropods, ophiuroids, and cephalopods, were found in *T. modestus* stomach. The main prey items in IRI value belonged to four groups: hyperiid amphipods, gastropods, ophiuroids, and algae. The fish showed ontogenetic diet shifts. In general, individuals in smaller size (< 20 cm) shifted their diets from vegetative food sources to animal ones when they reach the size bigger than 20 cm. Food diversity increased with fish size. Our findings suggested that *T. modestus* vertical distribution varied with depth and that fish showed ontogenetic diet shifts

Keywords: *Tamnaconus modestus*, Southern sea of Korea, distribution, stomach contents, PCR.

Introduction

Filefishes belonging to the family Monacanthidae (Order: Tetraodontiformes) include 95 species in 31 genera and are distributed in wide areas of tropical and subtropical oceans (Assadi and Dehghani, 1997). The black scraper, *Thamnaconus modestus*, of the family Monacanthidae is distributed in the coastal waters of Korea, Southern China, Japan, and Southern Africa (Kim *et al*., 2005). *T. modestus* is found in depths ranging from 50 to 110 m and temperature between 10 and 28°C (Baik and Park, 1989).

With advances in fishing gear and processing methods, *T. modestus* catches in Korea increased until the 1990s (Baik and Park, 1989) but then decreased dramatically until the 2000s because of poor management and overfishing (MIFAFF, 2008; Kim *et al*., 2011). A seedling release project has been conducted in specific areas to restore *T. modestus* populations, which requires understanding of the species' biological and ecological characteristics. To that end, the egg development, larval morphology (Lee et al., 2000), reproductive cycle (Lee et al., 2000), enzymatic hydrolysate properties (Suh, 1996), and fluctuating fishery conditions of *T. modestus* (Baik and Park, 1989) have been studied. However, there is no study about feeding ecology of *T. modestus* except study on the fishery biology in 28 years ago (Park, 1985). Therefore, we investigated feeding ecology of *T. modestus* with more various aspects and supplemented molecular analysis.

Analysis of stomach contents are the basis in understanding food chains and trophic levels in an ecosystem. In general, fish feeding ecology is studied by morphologically identifying stomach contents (Pilling et al., 2001; Santic *et al*., 2005). However, morphological identification of prey items in the stomach is not sufficient because of rapid prey digestion. To overcome this, molecular analysis has been combined with morphological analysis (Dunn et al., 2010; Jarman et al., 2002; Blankenship and Yayanos, 2005) and stable isotope analysis has been used in stomach contents analysis (Newsome *et al.*, 2009; Munoz *et al.*, 2011). In particular, polymerase chain reaction (PCR) has been used to analyze the DNA of digested prey items and feces. Mitochondrial DNA is well preserved after death and mitochondrial

primers are widely available (Lee, 2011). COI primers amplify a portion of the mtCOI gene, which appears to be the most conserved protein-coding gene in the mitochondrial genome of animals (Folmer *et al.*, 1994) and can be used to assess DNA from a wide range of phyla (invertebrates and vertebrates) (Blankenship *et al.*, 2005). The species of the family Monacanthidae are characterized by small mouths and specialized teeth (Kwak *et al.*, 2003), therefore, such molecular genetic analysis are needed to analyze the stomach contents of *T. modestus*

Therefore, we assessed *T. modestus* stomach contents by using morphological and molecular analyses. Our results will provide information for improving fisheries resource management.

Materials and Methods

Sampling

Survey was conducted at 42 stations in the southern sea of Korea in the spring of 2009, 2010, and 2011 (Figure 1). The sampling stations were located from 32°25′ to 35°25′ N and from 124°25′ to 129°25′ E. Samples were collected between the depths of 40 and 140 m by using an otter trawl with 20-mm mesh size and a mouth opening approximately 40 m in width and 4 m in height. The trawl was towed for 60 min at 3–4 knots by *Tamgu-20* of the National Fisheries Research & Development Institute (NFRDI). During sampling, the depth, salinity, and temperature were measured using a CTD profiler (Sea-Bird SBE 9). Specimen total length (TL, nearest 0.1 cm) and body weight were measured (nearest 0.1 g) and stomachs of 211 were removed and preserved in 94% ethanol.

Laboratory Analysis

Stomach contents were identified to the lowest taxonomic level under a microscope (JP/SZX 51 and JP/SZX 7; Olympus, Tokyo, Japan) by using previously published references (Hong *et al.*, 2006; Min *et al.*, 2004; Chihara and Murano, 1997). The number of prey items was counted, and their wet weight was measured to the nearest 0.01 g by using a scale (MS–300; Motex, Seoul, South Korea). Unidentifiable prey items in the stomachs were analyzed with molecular analysis methods after measuring the weight and number of each prey.

DNA was extracted using the DNeasy Blood & Tissue Kit (Qiagen, Venlo, The Netherlands). Universal primers amplifying portions of the mitochondrial cytochrome oxidase I (mtCOI) gene were used for PCR. Primer sequence and product lengths are shown in Table 1. PCR was conducted

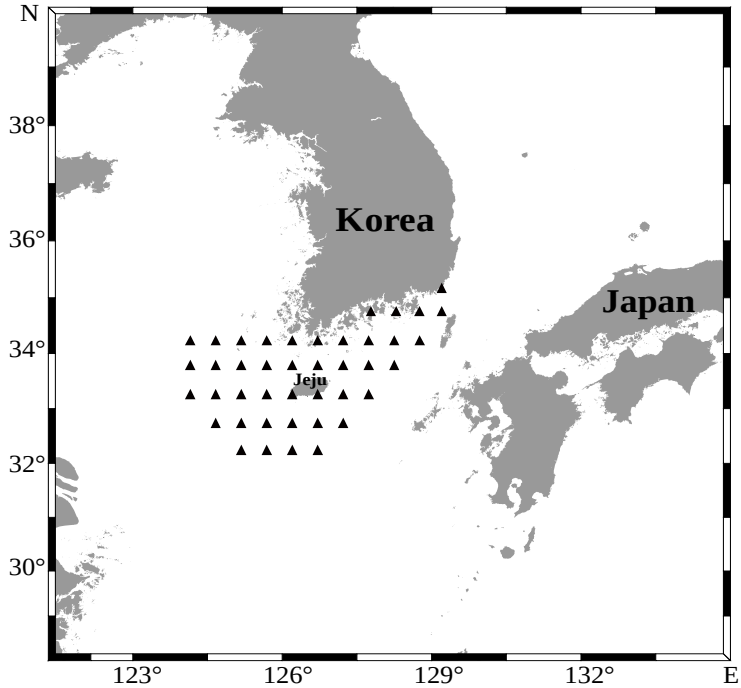

Figure 1. Study area. Triangles are the stations.

Table 1. Sequences and product lengths of mitochondrial DNA primers

Primer name	Primer sequence (5'–3')	Fragment length
LCO1490	5'-GGTCAACAAATCATAAAGATATTGG-3'	710 bp
HCO2198	5'-TAAACTTCAGGGTGACCAAAAAATCA-3'	710 bp

using a Bio-Rad Tetrad2 thermocycler (Hercules, CA, USA) under the following conditions: 11 min at 95°C, followed by 35 cycles at 95°C for 1 min, 50°C for 1 min, 72°C for 1 min, and 72°C for 5 min for the final extension. DNA sequence was determined using the ABI 3130xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA, USA). The DNA sequences were analyzed with the Basic Local Alignment Search Tool (BLAST) software provided at *http://www.ncbi.nih.gov* to identify the prevalence of each sequence.

Data Analysis

To evaluate the feeding ecology, we analyzed the vacuity index (VI), index of relative importance (IRI, %) and dietary breadth index (DBI). VI was calculated using the equation described by Molinero and Flos (1992):

$VI = N_1/N_2 \times 100$

where N_1 is the number of empty stomachs and N_2 is number of total stomachs.

IRI is used for describing major prey items (Pinkas *et al.*, 1970). IRI of each food item was calculated using the following equation:

$IRI = (\% N + \% W) \times \% O$

where % N is the number of each prey items as a percentage of the total number of prey items identified, % W is the percentage in wet weight of each prey item, and % O is the frequency of occurrence for each prey item in the total number of stomachs examined.

DBI was calculated using the following equation (Krebs, 1989):

$B_I = 1/n - 1\ (1/\sum_j P_{ij}^2 - 1)$

where B_I is Levin's standardized index for predator I, p_{ij} = proportion of diet of predator I that consumed prey j, and n = number of prey categories. This index ranges from 0 to 1; low values indicate diets dominated by a few prey items (specialist predators), and higher values indicate generalist diets (Gibson and Ezzi, 1987; Krebs, 1989). This value was regarded as low (0 – 0.39), intermediate (0.4 – 0.6), high (0.61 – 1) (modified of Grossman, 1986).

Statistical Analysis

Nonmetric multidimensional scaling (nMDS) and Cluster analysis were used to evaluate similarities among sampling sites with depth, bottom temperature, and salinity, using catches as a factor by statistical tests in PRIMER, version 5 (Ivybridge, UK). A similarity matrix was constructed using the Bray–Curtis similarity coefficient. This matrix was used in constructing two–dimensional ordinations of the multidimensional relationships among all samples. The stress value of below 0.2 was useful for interpreting relationships among samples (Clarke, 1993). Analysis of similarity (ANOSIM) was used to assess significant differences between groups separated by nMDS and Cluster analysis. Sampling depths were categorized randomly into 20-m intervals. TheVI and DBI of each size class was assessed using regression analysis. Kruskal–Wallis (hereafter K–W) tests in MINITAB, version 12 (State College, PA, USA) were used to compare the individual TLs and mean depths.

Results

Fish Abundance

T. modestus occurred at 19 of 42 sampling stations. Fish abundance significantly differed by depth (20-m intervals, K–W test, $P < 0.05$) (Figure 2); 87% of the total catch was obtained between 80 and 120 m, catch in depth of 80−100 m was more high (58% in 80−100 m and 29% in 100−120 m). Sampling stations were grouped by whether the fish

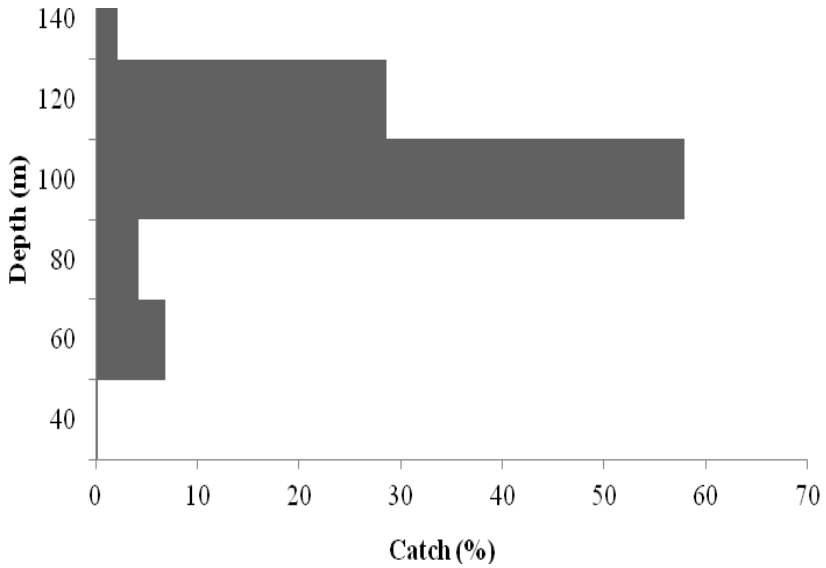

Figure 2. Total catch of *Thamnaconus modestus* by depth in 2009, 2010 and 2011.

was captured or not. Group A was stations where *T. modestus* was not captured, group B was stations where the fishes were captured. Main cause of the grouping was water temperature. The temperature was 12°C (nMDS analysis; Stress = 0.02, ANOSIM; Global R=0.155, P < 0.01) (Figure 3). *T. modestus* TL was significantly greater at deeper depths than at shallower depths (K–W test, P < 0.05) (Figure 4).

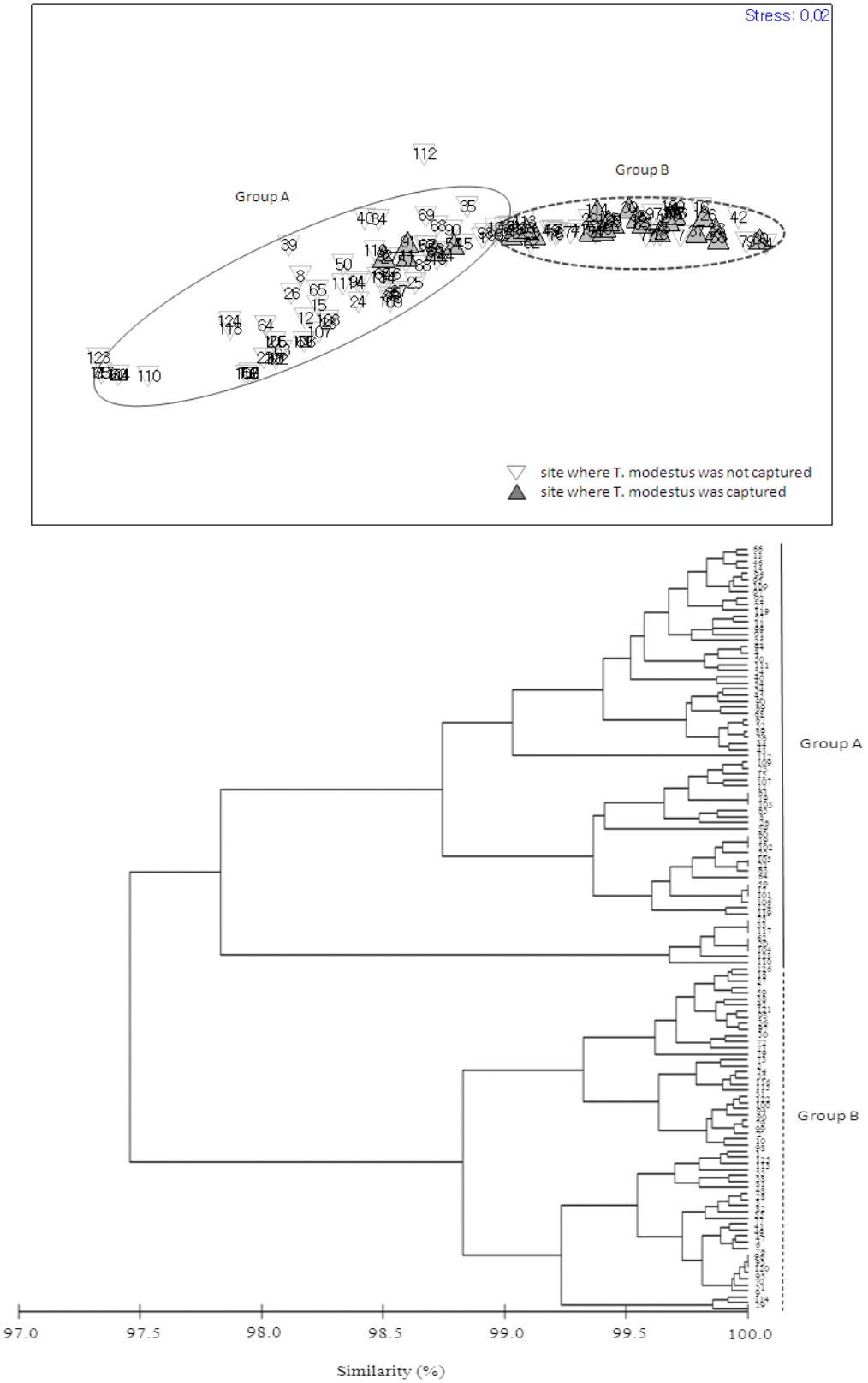

Figure 3. Dendrogram of Bray-Curtis similarity and nMDS ordination plots based on the community similarity of each station in 2009, 2010 and 2011. A circle show similarity between each station

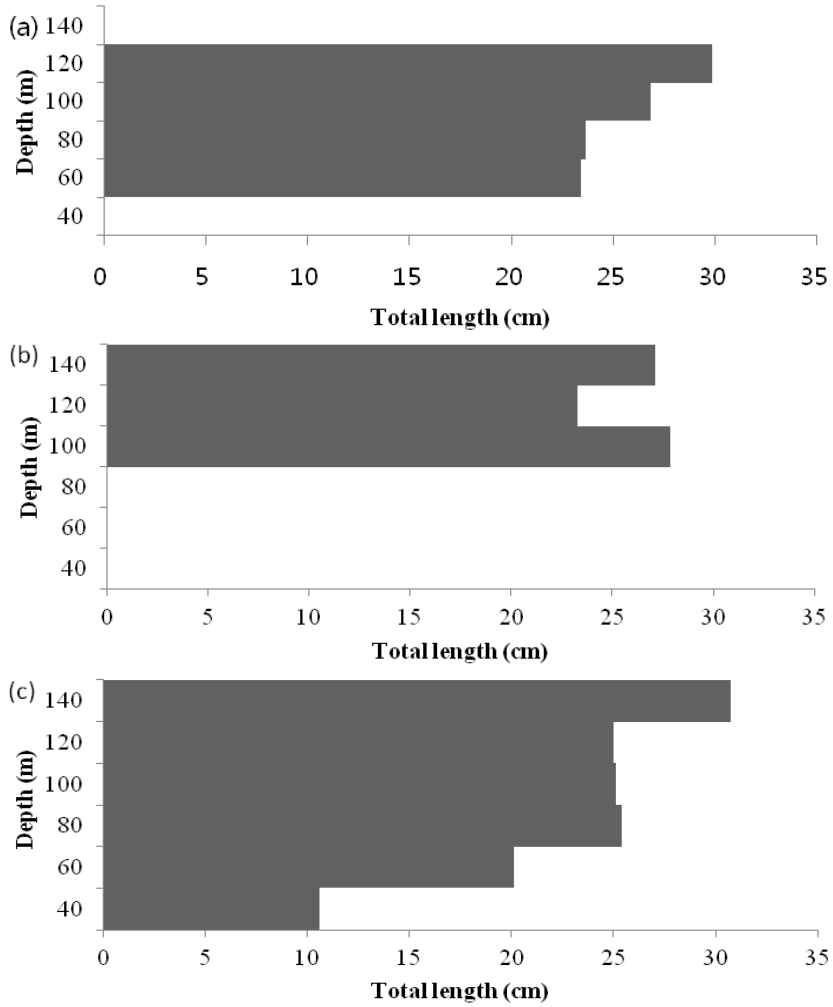

Figure 4. Length frequency distribution of *Thamnaconus modestus* by depth in 2009 (a), 2010 (b) and 2011 (c).

Prey Composition

A total of 48 prey items of which 46 and 2 morphologic and molecular analysis, respectively were identified, including 46 by morphological analysis and 2 by molecular analysis. Most of the prey items belonged to four groups: hyperiid amphipods, gastropods, ophiuroids, and algae. These 4 prey groups represented 93.6% of frequency of occurrence, 66.9% of IRI and 63.0% of total weight. Hyperiid amphipods accounted for 18.7% of IRI, 36.8% of frequency of occurrence and 2.0% of total weight. Ophiuroids made up 10.9% of IRI, 31.6% of occurrence frequency, and 22.3% of total weight. Algae accounted for 28.4% of IRI, 73.1% of occurrence frequency, and 23.6% of total weight. Gastropods made up 10.7% of IRI, 36.8% of occurrence frequency, and 15.0% of total weight. A small amount of fish was also found and accounted for 1.1% of total weight and 0.02% of IRI (Table 2).

Molecular analysis identified 60.1% of the unidentified prey items to the species level. Eight prey items unidentified by morphological analysis were successfully identified by molecular analysis. The eight prey items were classified under two taxon, these included *Todarodes pacificus* and *Nerita* spp., which accounted for 6.3% and 14.5% of the total weight, respectively. The sequence polymorphism of *Nerita* spp. ranged from 9 to 25 bp and that of *T. pacificus* was 4 bp.

Ontogenetic Diet Shifts

Amphipods and algae were found in the stomach of all size classes (Figure 5). Algae were the most important prey group in small size classes (≤20.0 cm in TL). In general, proportion of algae in stomach contents increased with decreasing body sizes. Algae accounted for 91.1% (≤15 cm), 62.6% (15–20 cm), 7.9% (20–25 cm), 19.8% (25–30 cm), 19.7% (30–35 cm), and 9.5% (35–40 cm) of the weight. Among prey items, hyperiid amphipods were also found in all body sizes with varying proportions: 8.9% (≤15 cm), 29.4% (15–20 cm), 36.5% (20–25 cm), 12.6% (25–30 cm), 25.4% (30–35 cm), and 9.1% (35–40 cm). The proportion of gastropods increased with increasing size. Nektonic cephalopods appeared in individuals > 20 cm. Stomach contents in the 25–30-cm size class were dominated by gastropods and ophiuroids, which accounted for 27.0% and 26.8% of the total weight,

Table 2. Prey composition by the supplement of molecular identification. (+ : less than 0.1%)

Prey organisms				%O	%W	%N	%IRI
Crustacea							
			Unidentified	5.3	3.9	2.6	0.5
	Amphipoda						
		Caprellidea		26.3	4.6	12.6	6.0
			Caprella aequilibra	4.1	0.1	0.9	
			Caprella kroyeri	8.2	0.5	4.6	
			Caprella scaura	0.6	+	0.3	
			Unidentified	14.0	4.0	6.9	
		Gammaridea		42.1	2.1	23.8	12.8
			Ampithoe valida	0.6	+	0.1	
			Atylus japonicus	0.6	+	0.1	
			Jassa sp.	4.1	0.1	0.9	
			Metopa sp.	15.2	0.5	10.5	
			Podocerus hoonsooi	3.5	+	0.4	+
			Pontogeneia rostrata	1.8	+	0.6	
			Stenothoe valida	0.6	+	0.1	
			Unidentified	17.5	0.4	9.2	
		Hyperiidea		36.8	2.0	29.0	18.7
			Brachyscelus crusculum	9.9	0.6	6.9	
			Oxycephalidea	0.6	+	0.1	
			Oxycephalus clausi	4.1	0.8	1.9	
			Phronima sedentaria	0.6	0.3	0.1	
			Platyscelus sp.	0.6	+	0.1	
			Proscina birsteini	0.6	+	0.5	
			Themisto sp.	40.9	1.3	21.3	
			Vibilia armata	0.6	+	0.1	
	Decapoda						
		Anomura					
			Galatheidae	2.3	0.1	0.5	+
		Brachyura		1.8	0.4	1.0	+
		Macrura		2.3	1.8	2.1	0.1
			Acetes sp.	0.6	+	0.1	
			Unidentified	2.3	1.7	2.1	
	Isopoda			0.6	+	0.1	+
Mollusca							
	Bivalvia			7.0	0.3	1.1	0.1
			Barbatia foliata	1.75	+	0.4	
			Hawaiarca uwaensis	2.3	+	0.3	
			Unidentified	2.9	0.3	1.0	
	Gastropoda			36.8	15.0	6.6	10.7
			Calliostoma multiliratum	0.6	+	0.1	
			Cavolinia inflexa	1.8	0.1	0.4	
			Clio pyramidata	1.2	0.1	0.6	
			Collonista amakusaensis	0.6	+	0.1	
			Columbellopsis bella	0.6	+	0.1	
			Cuvierina columnella columnella	0.6	0.1	0.2	
			Cypraeidae	0.6	+	0.1	
			Hybochelus cancellatus orientalis	1.2	+	0.2	
			Nerita sp.	29.2	14.5	3.5	
			Pyramidellidae	9.9	0.3	1.4	
			Teinostoma lucida	0.6	+	0.1	
	Cephalopoda			36.8	6.9	0.1	3.4
			Todarodes pacificus	37.4	6.3	4.5	
			Unidentified	0.6	0.6	0.1	
Pisces							
			Unidentified	0.6	1.1	2.3	+
Porifera				11.1	2.6	3.7	0.9
Polychaeta				1.2	0.1	0.4	+
Ophiuroidea				31.6	22.3	3.4	10.9
Algae				73.1	23.6	5.4	28.4
Miscellaneous items				29.2	13.3	5.4	7.3
			Egg	0.6	3.6	0.1	
			Unidentified items	29.2	9.7	5.2	
	Total				100.0	100.0	100.0

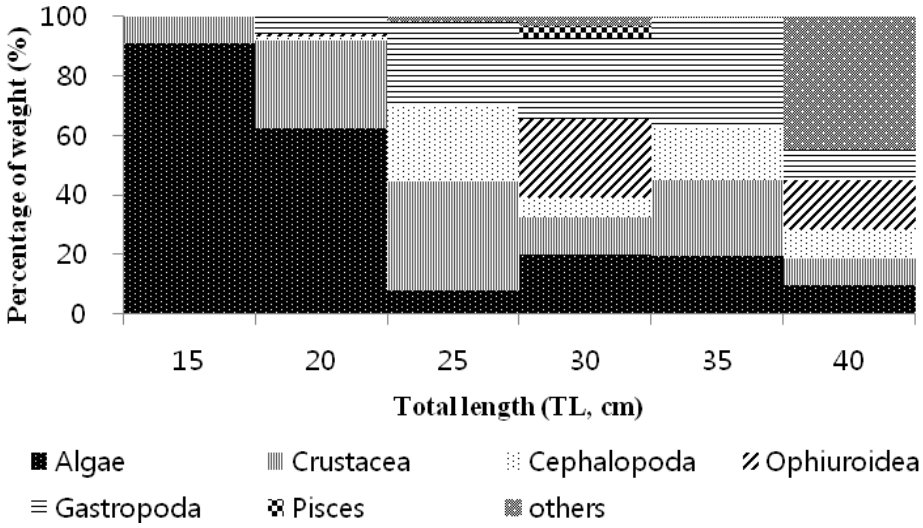

Figure 5. Ontogenetic diet variations of *Thamnaconus modestus.*

respectively. Ophiuroids were the most dominant prey items in the 25–30-cm and 35–40-cm size classes. Fish were found only in the 25–30-cm size class, accounting for 4.2% of the total weight.

Vacuity Index (VI)

The mean VI value was 19.0% and significantly decreased with increasing TL (Figure 6a). Half of the individuals < 15.0 cm had empty stomachs. VI was 25.8% in 15−20 cm, 17.9% in 20−25 cm, 22.4% in 25−30 cm, and 11.8% in 30−35 cm. Individuals in the 40−45-cm range did not have empty stomachs.

Dietary Breadth İndex (DBI)

DBI by fish size ranged from 0.08 to 0.19 (Figure 6b). DBI significantly increased with greater fish size ($R^2 = 0.952$, $P < 0.001$). DBI of individuals < 15 cm was 0.08, and it gradually increased with fish size (0.14 for 20–25 cm and 0.19 for 35–40 cm).

Discussion

The southern sea of Korea is influenced by the warm Tsushima current (Kondo, 1985), and the sea surface temperature is warmer than 10°C almost during throughout the year (Ahn *et al.*, 2005; Lee, 2003). The warm water provides ideal nurseries, permanent habitats, and migration routes for many fish and shellfish (NFRDI, 2005; Chung and Yang, 1991).

T. modestus is widely distributed in the low and warm latitudes (Assadi and Dehghani, 1997; Tatsuaki *et al.*, 1988), including the southern sea of Korea, where it plays an important role in marine ecosystem. In the present study, *T. modestus* was captured mainly in water warmer than 12°C. This is in accordance with findings published by Baik and Park (1989), who observed that *T. modestus* was mostly found in water ranging from 10 to 28°C. That we captured the most of the *T. modestus* in water >12°C confirms the findings of previous studies that *T. modestus* prefers warm water (Assadi and Dehghani, 1997; Tatsuaki *et al.*, 1988).

T. modestus is distributed in water depths ranging from 30 to 120 m (Baik and Park, 1989; Tatsuaki *et al.*, 1988; NFRDI, 2004). In the present study, 87% of the captured individuals were found in 80–120 m and 59% were found in 80–100 m. This result is similar to those of previous studies (Baik and Park, 1989; Tatsuaki *et al.*, 1988; NFRDI, 2004).

T. modestus spawns in shallow areas and juveniles migrate to deep areas where they grow into adults (NFRDI, 2009). In the present study, individuals in deeper areas were significantly larger than those in shallower areas. This finding implies that smaller individuals are distributed in shallower waters. In addition, after eggs are spawned in shallower waters, individuals moved to deeper waters as they grew.

Monacanthid species feeding ecology has been studied in various regions (Miyajima *et al.*, 2011; Kwak *et al.*, 2003; Bell *et al.*, 1978; Randall, 1967). Monacanthids consume various prey items such as seagrasses, algae, hydroids, mollusks, crustaceans, and polychaetes (Bell *et al.*, 1978; Peristiwady and Geistdoerfer, 1991; Randall, 1967). Like other monacanthids, *T. modestus* lives near the bottom of the seafloor (Randall, 1967). Accordingly, majority of the stomach contents in this study were benthic animals (e.g., gastropods, bivalves, crustaceans, sponges, and echinoderms), except cephalopods. A nektonic cephalopod consumed by *T. modestus* stays near the bottom during the daytime and ascends to the surface at night by diel vertical migration (Song *et al.*,

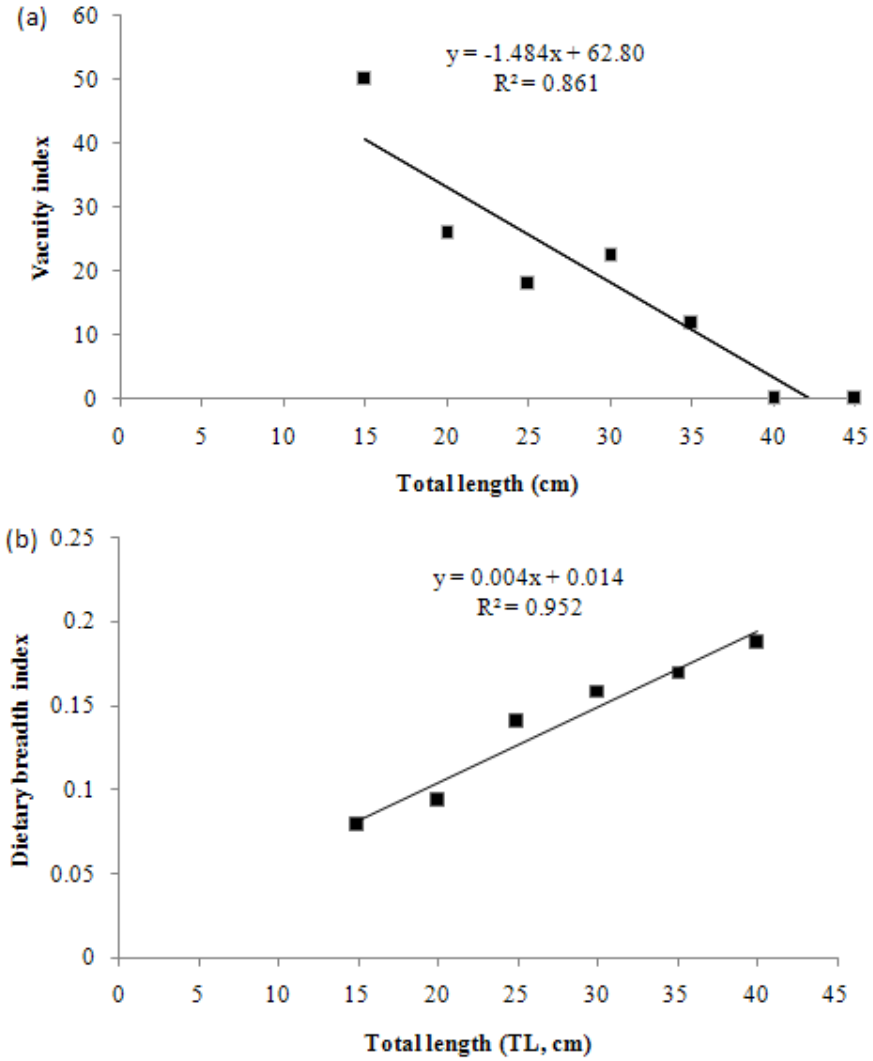

Figure 6. Vacuity index (a) and Dietary breadth index (b) of *Thamnaconus modestus* by total body length**.**

2006; Kawabata *et al.*, 2006). Therefore, *T. pacificus* fed on both benthic animals and epiplankton (Kim *et al.*, 2007; Song *et al.*, 2006). DBI value near the zero show *T. modestus* was specialist consuming algae and some benthic animals although there was ontogenetic change.

Among 20 species in the family of Monacanthidae, small individuals with TL between 4.6 and 6.5 cm (Randall, 1967) and between 8.0 and 10.9 cm (El-Ganainy, 2010) mostly consume algae. We observed that individuals < 20 cm fed on vegetable prey items and those >20 cm primarily consumed animal prey items. *T. modestus* with 50% maturity corresponded to 21.0 cm in TL (NFRDI, 2009; Baik and Park, 1989). We observed significant changes in prey composition at 20 cm in TL. Consumption of vegetative prey items was sharply decreased and animal prey items drastically increased in individuals > 20.0 cm. Our data did not elucidate the relationship between sex maturity and prey composition. However, the fact that small fish hatch in shallow areas rich in algae and migrate to deeper areas as they grow might be associated with the shift in prey composition. Settling in algae-rich shallow areas may be a survival tactic because juvenile fish are not strong swimmers and have small mouths (Randall, 1967). Small mouths may be better suited for algae feeding, but as the fish grow, they are able to consume small animals. Accordingly, they migrate to deeper areas inhabited by various marine animals.

20.8% of total prey weight has been identified by molecular analysis, but 9.7% of total prey weight still remains unidentified things. It was very difficult to know the taxon of the preys because preys different each other were mixed and digested well. Various methods and attempt for knowing food ecology of marine animals were needed. In general, species in the family Monacanthidae have lower VI values (Peristiwady and Geistdoerfer, 1991; Kwak *et al.*, 2003; Huh and Kwak, 1998). However, the VI value of *T. modestus* was 19.0%, which is higher than that of other monacanthids, although there is ontogenetic change. During the spawning season, fish typically reduce their feeding activity (Bond, 1979; Baeck *et*

al., 2007). The samples in the present study were collected in May and June, which is the *T. modestus* spawning season (Lee *et al.*, 2000; Park, 1985). The higher VI observed in the present study may be attributable to the fact that the samples were collected in the spawning period. In addition, feeding activity varies throughout the day (Sturdevant *et al.*, 2002; Kwak *et al.*, 2006; Zhang, 1988). If *T. modestus* fed heavily at night and was collected immediately, VI would be lower than that observed in the present study.

Beginning in the 1990s, large jellyfish blooms around the world damaged marine ecosystems and coastal industries (Uye, 2008; Daryanabard and Dawson, 2008; Purcell *et al.*, 2007; Chung *et al.*, 2012). For this reason, many policies have been implemented to control the jellyfish bloom (Chung *et al.*, 2012). In Korean coastal water, seedlings of *Stephanolepis cirrhifer* and *T. modestus* have been released to control jelly fish population since filefish are known the predator of jelly fish (Masuda *et al.*, 2008; Miyajima *et al.*, 2011). Jellyfish appearing frequently in Korean coastal seas include *Nemopilema nomurai*, *Aurelia aurita*, and *Spirocodon saltatrix* (Chung *et al.*, 2012). *N. nomurai* are distributed in areas shallower than 30 m. *S. saltatrix* and *A. aurita* are found in waters <10 m (Lee *et al.*, 2007; Hong *et al.*, 2006). As reported here and previously, adult *T. modestus* inhabits depths > 40 m. Therefore, filefish and jellyfish may not coexist in marine ecosystems, and seedling release may not be an effective method for controlling jellyfish blooms.

However, early juveniles inhabit shallower waters where jellyfish polyps are commonly found. The jellyfish body consist of mostly of water and is relatively well digested (Larson, 1986; Arai *et al.*, 2003). This makes it difficult to investigate stomachs for jellyfish, and other methods, such as stable isotope analysis and advanced laboratory experiments, are required. In addition, if *S. cirrhifer* and *T. modestus* juveniles consume jellyfish polyps at shallower depths, seedling release should be properly timed to coincide with the appearance of jellyfish polyps.

In conclusion, the results of this study provide new insights into the role of *T. modestus* in marine ecosystems and offer information that will be useful in guiding resource management efforts.

Acknowledgments

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