Thesis for the Degree of Master of Science

# Distribution and feeding ecology of the file fish, *Thamnaconus modestus* in the southern sea of Korea


by

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# (한국 남해안에 서식하는 말쥐치의 분포와 섭식생태 연구)

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by

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## Abstract

*Tamnaconus modestus* was collected in the southern sea of Korea. *T. modestus* distribute in depth ranging 80~120 m and warmer water temperature than 12℃. The size of specimens ranged from 10.6 to 38.7 cm in total length (TL). Body length of the fish found in deeper depth was significantly larger than that of more shallow depth. To understanding feeding habits of *T. modestus*, stomach contents was analized. The main method of analysis in present study was analysis of stomach contents based on morphology. Molecular analysis by polymerase chain reaction (PCR) of DNA was surrogated because morphological identification accompany often difficult due to digestion and ingestion. *T. modestus* was

omnivorous, consuming diverse prey such as algae, amphipods, gastropods, ophiuroids and cephalopods. Diet composition showed slightly ontogenetic fluctuations. Food diversity increased with increasing fish size.

# 1. Introduction

The filefishes (Order Tetraodontiformes, Family Monacanthidae) include approximately 95 species in 31 genera and distribute in the wide area of tropical and subtropical oceans (Assadi and Dehghani, 1997). Filefish, *Thamnaconus modestus* of family Monacanthidae exists in the coastal waters of Korea, southern China, Hokkaido, Japan and southern Africa (Kim et al., 2005). *T. modestus* distributes at the depth range of 50 to 110 m with a temperature range from 10 to 28℃ (Baik and Park, 1989; Bang, 2005).

*T. modestus* harvest was avoided before the development of processing industry. After the development of processing technology, catches of *T. modestus* had increased until 1990 (Baik and Park, 1989; Lee, 1999). Dried and processed *T. modestus* was consumed by people as a snack. The catches sharply decreased until 2000s because of poor management and overfishing (MIFAFF, 2008; Kim et al., 2011). After the great decrease of catch, seed release project has been carried out in several areas to recover the population. To recover the population, it's very important to understand their biological and ecological characteristics. Despite its

importance, little has been studied for the ecology of the fish. There had been a few studies of egg development and larval morphology (Lee et al., 2000), reproductive cycle (Lee et al., 2000), properties of enzymatic hydrolysates (Suh, 1996) and fluctuation of fishing conditions of *T. modestus* (Baik and Park, 1989).

However, no studies about feeding habits of *T. modestus* were conducted. Studies of the feeding habits based on the analysis of stomach contents are the basis in understanding partial food chain and trophic level in an ecosystem. The main method of feeding habit study is to indentify stomach contents based on morphology (Pilling et al., 2001; Santic et al., 2005). However, morphological identification accompany often difficult due to digestion and ingestion. For these reasons, new techniques such as the use of stable isotopes (Newsome et al., 2009, Munoz et al., 2011) and molecular genetic analysis (Dunn et al., 2010; Jarman et al., 2002; Blankenship and Yayanos, 2005) are required in addition to existing morphology analysis. In particular, polymerase chain reaction (PCR) of DNA for the analysis of feces and digested prey items was often used to surrogate the shortness of morphological analysis. Molecular identification method is especially needed to analyze stomach contents of *T. modestus* because monacanthids have a small mouth, but well developed teeth (Kwak et al., 2003).

The present study analysed stomach contents of *T. modestus*, using morphological and genetic analyses. This study will provide basic information for ecosystem based resource management.

# 2. Materials and Methods

## 2.1. Sampling

### 2.1.1. Fish sampling

*Thamnaconus modestus* was collected at 42 stations in the southern sea of Korea (Fig.1). The sampling stations were located from 35°25′ to 32°25′ N and from 124°25′ to 129°25′ E. Sampling was performed with depths ranging from 40 to 140 m during 3 years in March and April 2009, March and April 2010, March, April and May 2011. Samples were collected using an otter trawl having 20 mm mesh size and a mouth opening of approximately 40 m wide and 4 m height. Hauling time was 60 minutes and towing speed ranged from 3 to 4 knots by *Tamgu-20* of the National Fisheries Research & Development Institute (NFRDI). The total length (to the caudal fin) and body weight of the specimens were measured (nearest 0.1 cm and 0.1 g, respectively), and then fish stomach was taken off and preserved in 94% ethanol.

### 2.1.2. Oceanographic sampling

Zooplankton was double obliquely collected using a bongo net with 60 cm in diameter of 333 μm mesh. Flow meters were placed inside the bongo nets to determine the filtered water volume. Zooplankton samples were preserved in RCL2 after removing any large organisms. The depth, salinity and temperature were measured with a CTD profiler (Sea-Bird SBE 9).

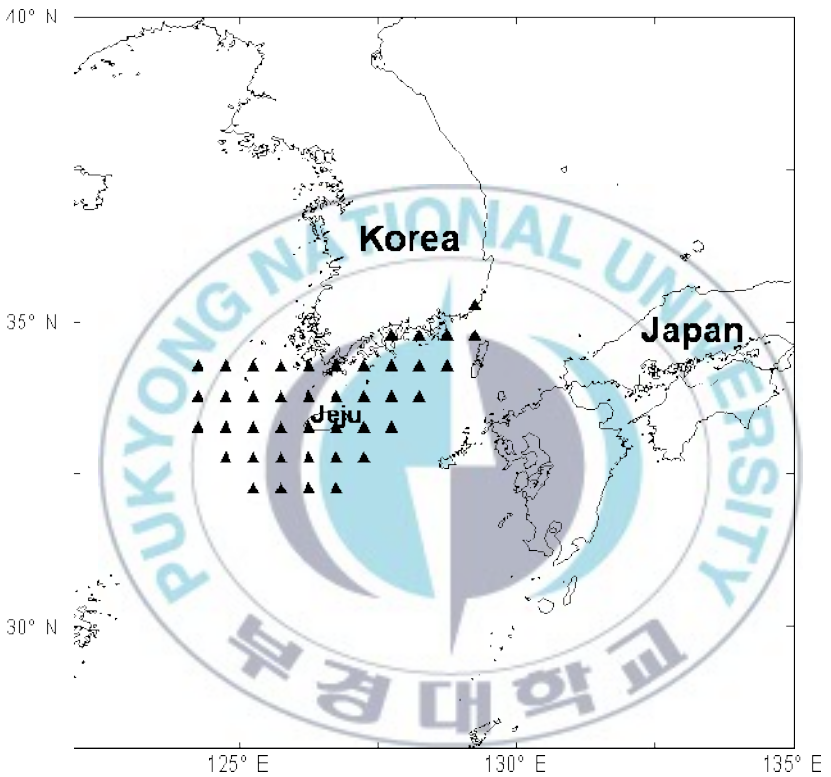


Fig.1. Map showing sampling area. Triangles indicate sampling stations.

## 2.2. Laboratory analysis

### 2.2.1. Plankton biomass

Displacement volume (DV., ml) of total plankton was estimated: sample was brought to a jar (500 ml) by adding water, and then filtered through 200 μm mesh until liquid no longer dropped into the below container. DV was estimated by subtracting the volume of graduated liquid from the sample-liquid starting volume. DV was converted to standing stock divided by the volume of filtered water based on the flowmeter revolutions per haul.

### 2.2.2. Morphological identification of stomach contents

211 stomachs captured only in 2011 were analyzed. Stomach contents were identified to lowest taxonomic level under the microscope (JP / SZX 51, Olympus and JP / SZX 7, Olympus) as much as possible using references (Hong et al., 2006; Min et al., 2004; Mitsuo and Masaaki, 1997) (Fig 3). For each prey item, the number of prey item was estimated and wet weight of prey item was measured to the nearest 0.01 g using a scale (MS–300, Motex). Prey item which was not

possible to determine their exact number due to poor morphology were counted as a single prey item. Pieces of body were counted as an individual if a pair of eyes, a piece of tail and beak was found. Stomach contents which were not able to be identified, even phylum level (Fig. 4) were removed for molecular analysis. Weight and number of each prey which was not able to be identified were measured to aggregate with the result of molecular analysis.

### 2.2.3. Molecular identification of stomach contents

#### 2.2.3.1. DNA extraction

Tissue which was not able to be identified because of damage by digestion and ingestion were identified using molecular analysis. DNA was extracted from the tissue preserved in ethanol using a DNeasy Blood & Tissue Kit (Qiagen). Extracted DNA solution was preserved -20℃ for PCR amplification.

#### 2.2.3.2. Primer selection and PCR amplification

Universal primers amplifying portions of the mtCOI gene were employed in PCRs for *T. modestus* diet analysis. The primer lengths ranged from 25 and 26 bp. Sequence and product length of primer was shown in the table 1.

The components of these 20 μL PCRs were 2 μL template DNA, 2 μL 10 x buffer, 0.4 μL 10 mM dNTPs, 0.8 μL 10 pmol forward primer, 0.8 μL 10 pmol reverse primer and 0.2 μL HS tag (TNT research, Korea). PCR was conducted using a Bio-Rad Tetrad2 thermocycler under the following conditions: 11 min at 95℃, followed by 35 cycles at 95℃ for 1 min, 50℃ for 1 min, 72℃ for 1 min and 72℃ for 5 min as final extention. Amplified PCR products were identified by 1.5% agarose gel electrophoresis (Fig 5). PCR products visualized by electrophoresis were purified using PCR purification kit (Qiagen): 5 x PB buffer was added and centrifuged at 17900 g for 1 min. 750 μL PE buffer was added and centrifuged at 17900 g for 1 min, and then 20 μL EB buffer was added and incubated for 30 min at room temperature. The column was centrifuged for 1 min.

**2.2.3.3. DNA sequencing**

The components of these 10 μL sequencing PCRs were 1 μL template DNA, 1.5 μL 5 x buffer, 0.6 μL big dye (Applied Biosystems) and 1.6 μL 1 pmol forward primer. The cycle profile was 25 cycles at 96℃ for 10 sec, 50℃ for 5 sec and 60℃ for 4 min in Bio–Rad Tetrad2 thermocycler. In cycle sequencing products, 1 μL 125 Mm EDTA, 1 μL 3 M sodium acetate and 25 μL 100% EtOH were added, and then incubated for 15 min at room temperature. The mixture was centrifuged at 2250 g for 45 min and the supernatant was removed. 35 μL 70% EtOH was added to the mixture and the mixture was centrifuged at 1650 g for 15 min. The supernatant was removed. It was air–dried at room temperature and added 15 μL Hidi (Applied Biosystems). DNA sequence was determined by ABI 3130 x 1 Genetic Analyzer.

The DNA sequences were analyzed with the Basic Local Alignment Search Tool (BLAST) software provided at *http://www.ncbi.nih.gov* to identify the provenance of each sequence. The p-distance matrix among each sample was constructed using MEGA4 software.

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 |

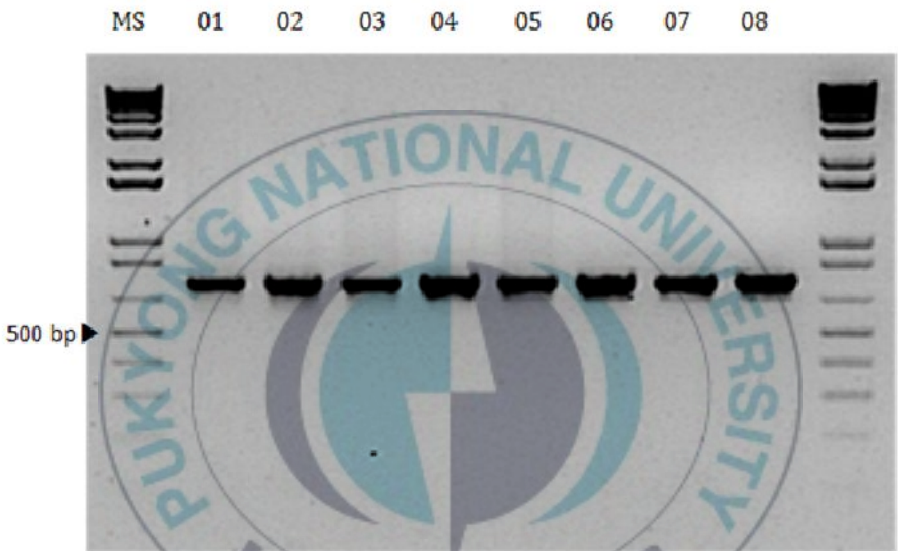


Fig. 2. PCR optimization by agarose gel electrophoresis. The lane numbers indicate different prey items . MS was marker.

### 2.3. Data analysis.

To evaluate the feeding ecology, vacuity index, stomach contents weight, index of relative important (%) and dietary breadth were analyzed. For these data analysis, 211 individuals sampled in 2011 were used.

#### 2.3.1. Vacuity index

The vacuity index was calculated as the number of empty stomach divided by the total number of stomach examined * 100 (Molinero and Flos, 1992).

$$VI = N_1 / N_2 * 100$$

Where $N_1$ is number of empty stomach and $N_2$ is number of total stomach.

#### 2.3.2. Stomach content weight

Stomach contents index (SCI) of each specimen was calculated using following equation :

$$SCI = SCW\ (g) / BW\ (g) * 100$$

Where SCW is stomach content weight and BW is the body weight of fish.

### 2.3.3. Index of relative importance (IRI) and occurrence (%)

Index of relative importance (IRI) was used for describing major prey item of fish (Pinkas et al., 1970). IRI of each food item was calculated using following equation:

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

Where % N is the number of each prey item 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.

### 2.3.4. Dietary breadth index

Dietary breadth index was calculated using following equation:

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

where $B_I$ is Levin's standardized index for predator I, $p_{ij}$ = proportion of diet of predator I that is consumed prey j, and n = number of prey categories. This index ranges from 0 to 1; low values indicate diets dominated by few prey items (specialist predators) and higher values indicate generalist diets (Gibson and Ezzi,

1987; Krebs, 1989).

### 2.4. Statistical analysis

Non–metric multi dimensional scaling (nMDS) was used to evaluate similarities among each site including depth, bottom temperature, salinity. The catch was used as a factor. 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). The relationships of body weight by total body length and total body length specific dietary breadth index were analyzed with regression analysis. Kruskal–Wallis test was used to compare a stomach contents weight of each size class and total length of individuals by mean depth classes. Prior to Kruskal–Wallis test, Levene's test for homogeneity of variance was evaluated. Results from this examination were used to determine the analyses on data. The assumption for non–parametric test was supported, and Kruskal-Wallis test was conducted. To evaluate vacuity index

of each size classes and mean dietary breadth index of each size class, Statistical analyses were accomplished in PRIMER version 5 and MINITAB Version 12.

# 3. Results

### 3.1. Fish abundance

Depth specific abundance was investigated from three year surveys. The survey was conducted at 42 stations. Specimens were captured at only 19 sampling stations. Fish abundance was significantly different by depth class (interval 20 cm) (Kruskal–Wallis test, $P < 0.05$). Individuals captured in 80~120 m accounted for 87% of total catch: 58% in 80~100 m and 29% in 100~120 m (Fig 3). *T. modestus* was found in specific sites in every year (Fig. 4, 5, 6). nMDS analysis showed difference of stations that the fish was found or not (Stress = 0.01) (Fig. 7). Though depth of water affected individuals size but it did not affects whether or not individuals appear and salinity was either. All stations were grouped by temperature and standard of the temperature was 12℃. *T. modestus* was captured mainly in warmer water temperature than 12℃. Fish size was different by depth (Kruskal–Wallis test, $P < 0.05$) (Fig. 8a, c). Body length of the fish found in deeper depth was significantly larger than that of more shallow depth.

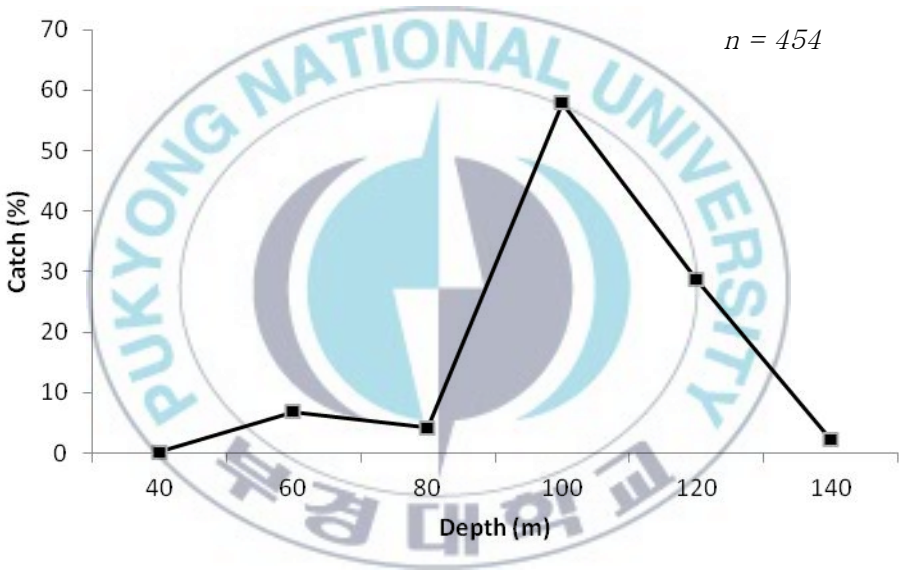


Fig. 3. Total catch of *Thamnaconus modestus* by depth in 2009, 2010 and 2011.

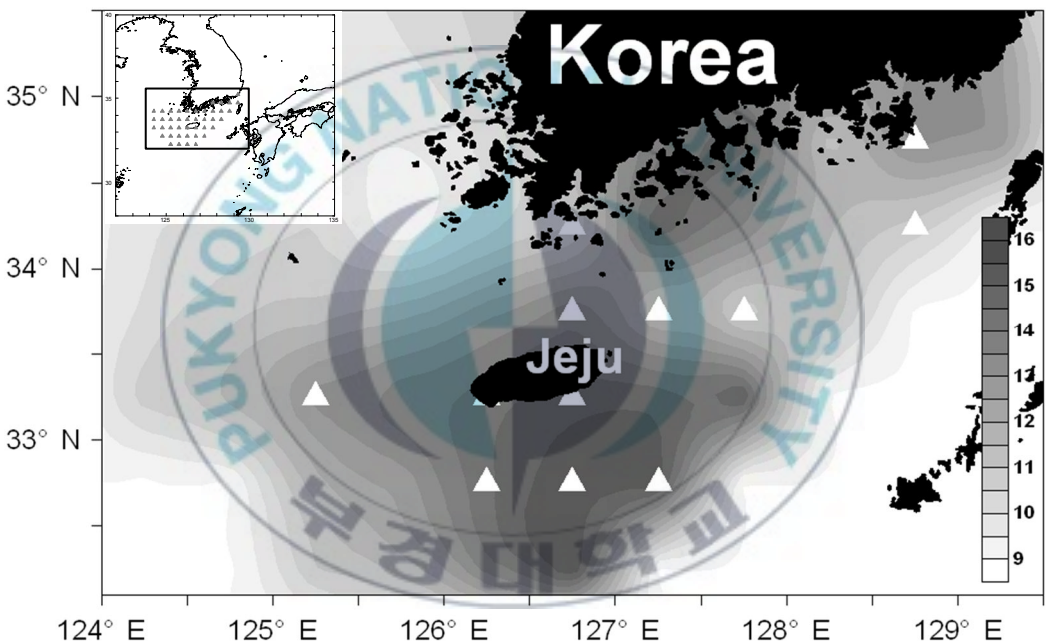


Fig. 4. Sampling stations where *Tamnaconus modestus* was captured in 2009. The color contour indicates bottom temperature.

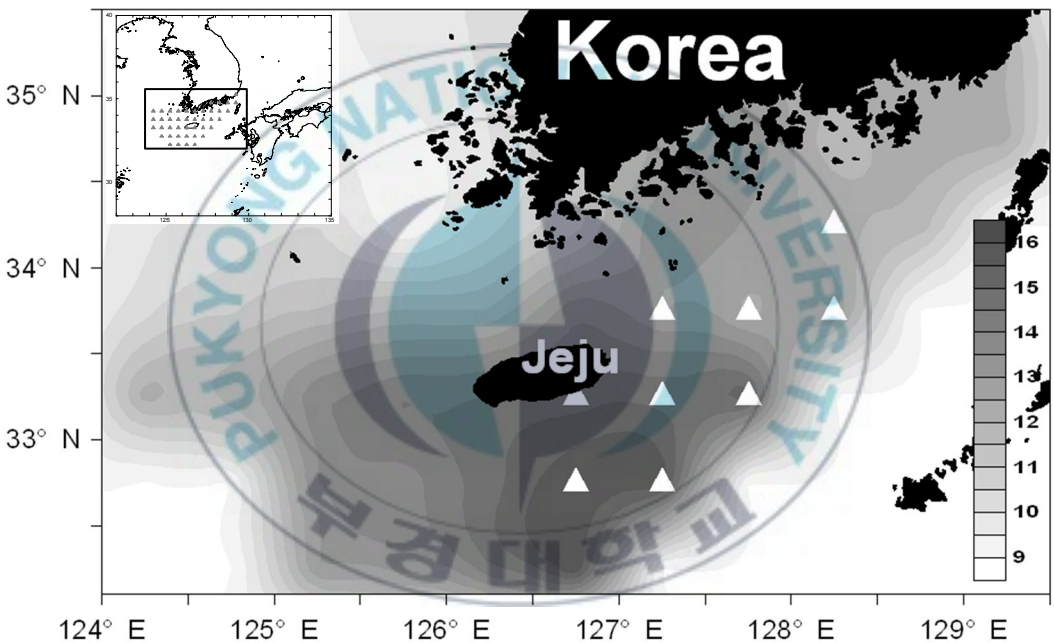


Fig. 5. Sampling stations where *Tamnaconus modestus* was captured in 2010. The color contour indicates bottom temperature.

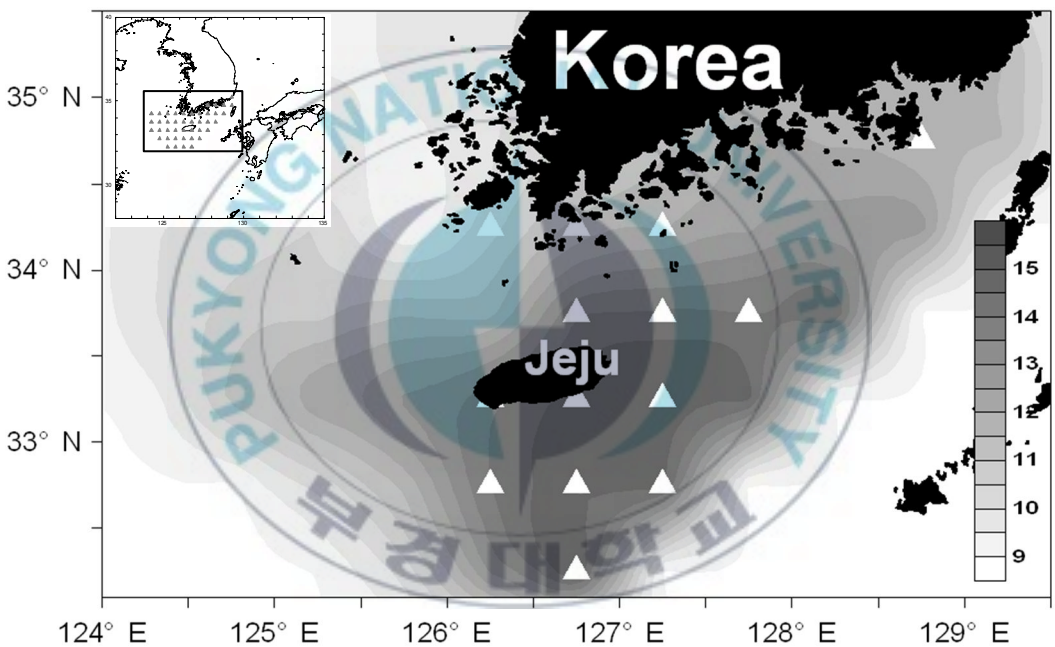


Fig. 6. Sampling stations where *Tamnaconus modestus* was captured in 2011. The color contour indicates bottom temperature.


Fig. 7. nMDS ordination plots of *Thamnaconus modestus* catch. A: stations where *T. modestus* was captured, B: stations where *T. modestus* was not captured.

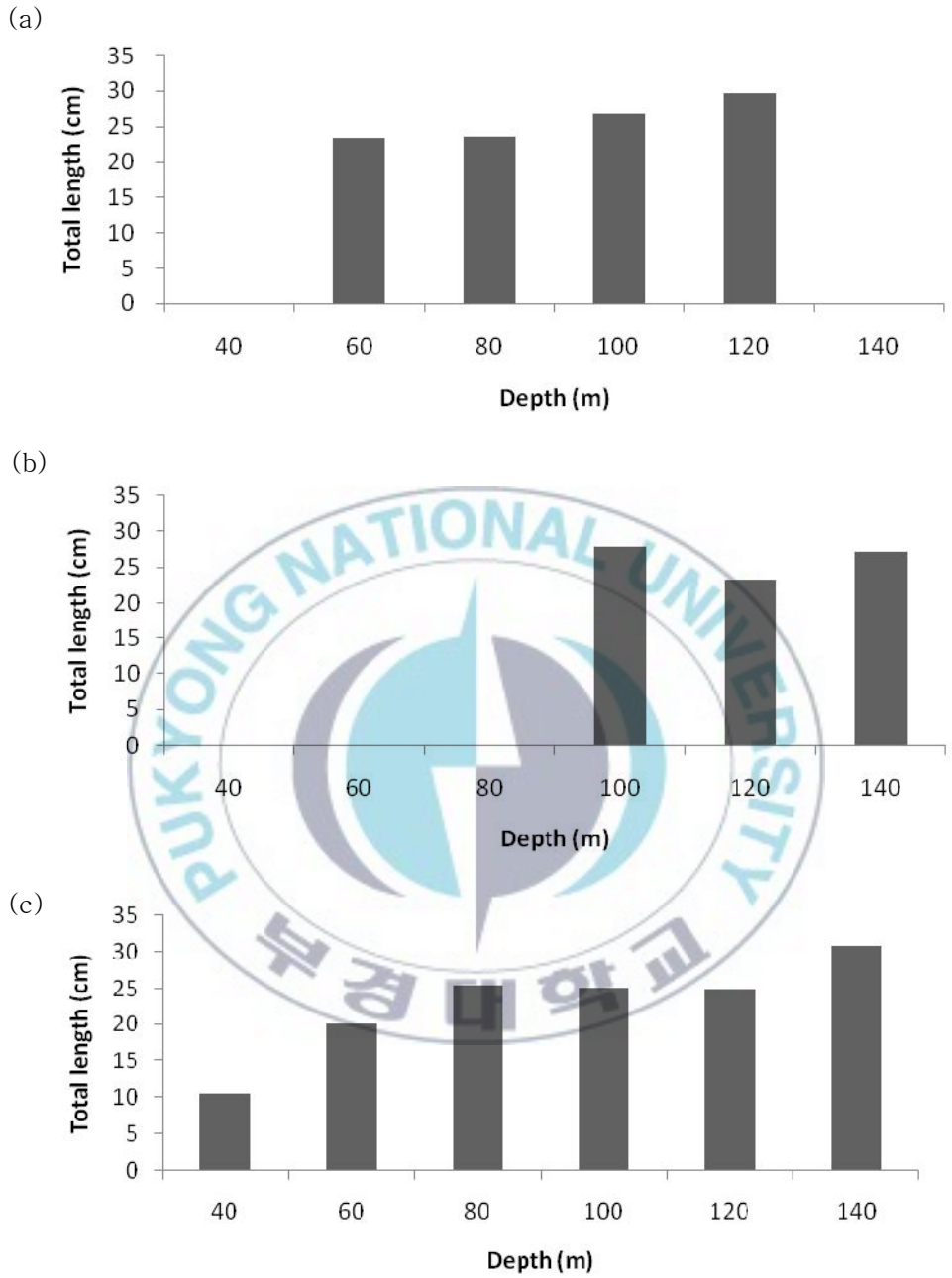


Fig. 8. Length frequency distribution of *Thamnaconus modestus* by depth in 2009 (a), 2010 (b) and 2011 (c).

### 3.2. Relationship between total length and body weight

Total length and body weight were significantly correlated ($R^2 = 0.9004$, $P < 0.001$) (Fig. 9).

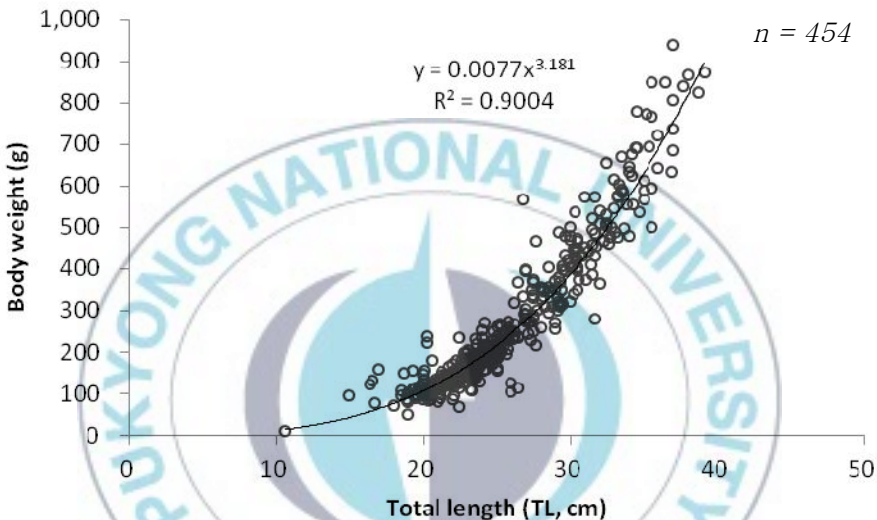


Fig 9. Length–weight relationship in *Thamnaconus modestus* in total catch in 2009, 2010 and 2011

### 3.3. Oceanographic characteristics

#### 3.3.1. Depth, temperature and salinity

The mean depth of sampling stations during the sampling period ranged from 27 to 135 m (Fig. 10). The mean bottom temperature during the sampling period ranged from 9.0 to 15.3℃ (Fig. 11). Lowest bottom salinity was 33.6 psu and highest bottom salinity was 34.6 psu (Fig. 12).

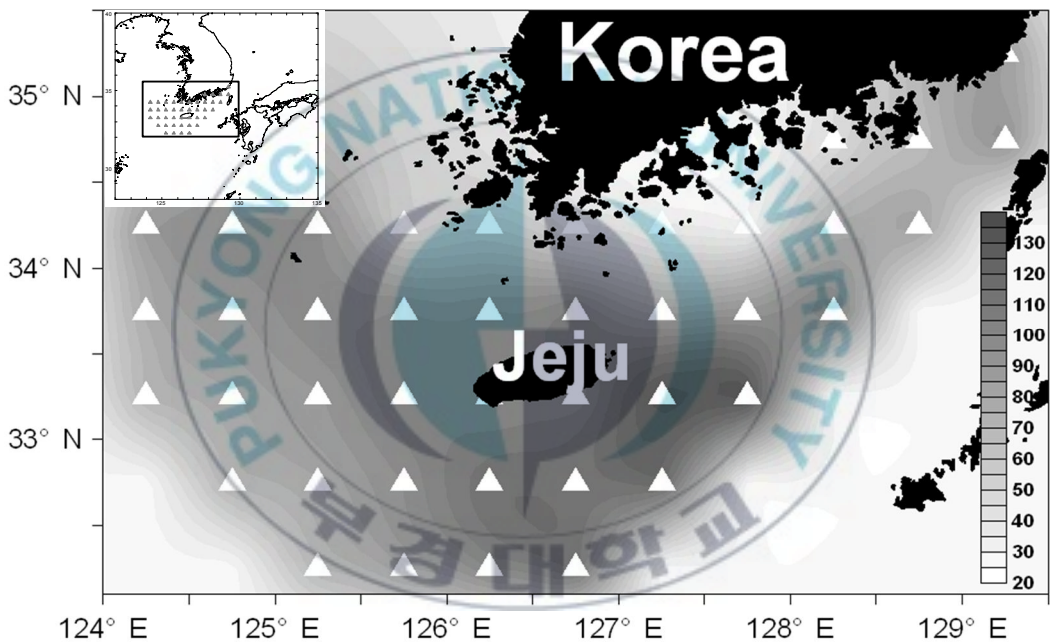


Fig. 10. Depth distribution of sampling area.

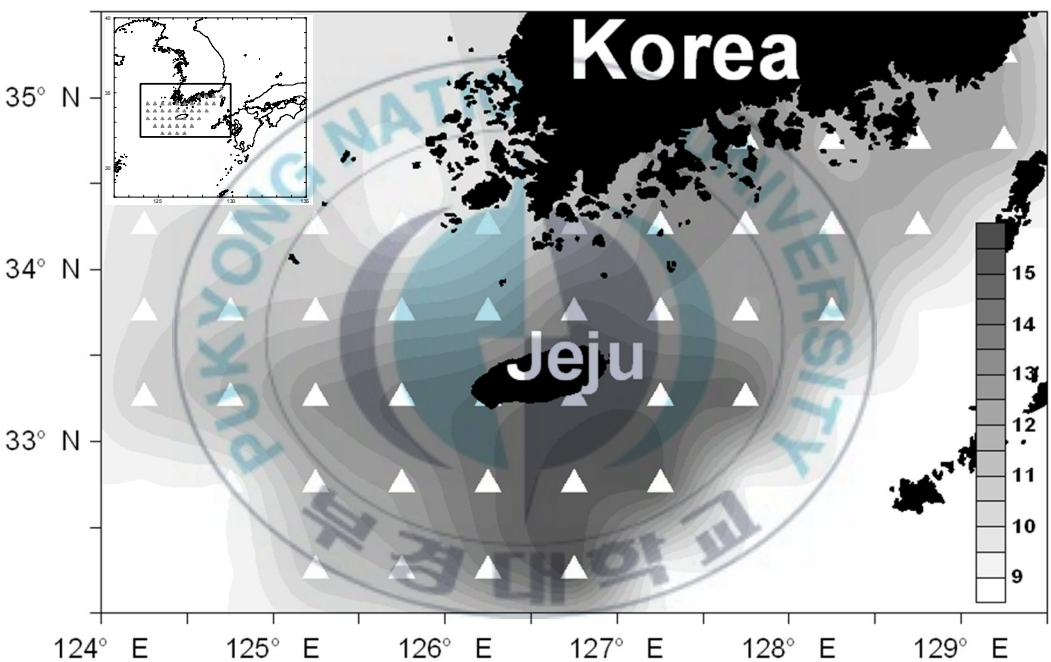


Fig. 11. Bottom temperature distribution of study area. Color contour indicates the isotherm of bottom temperature.

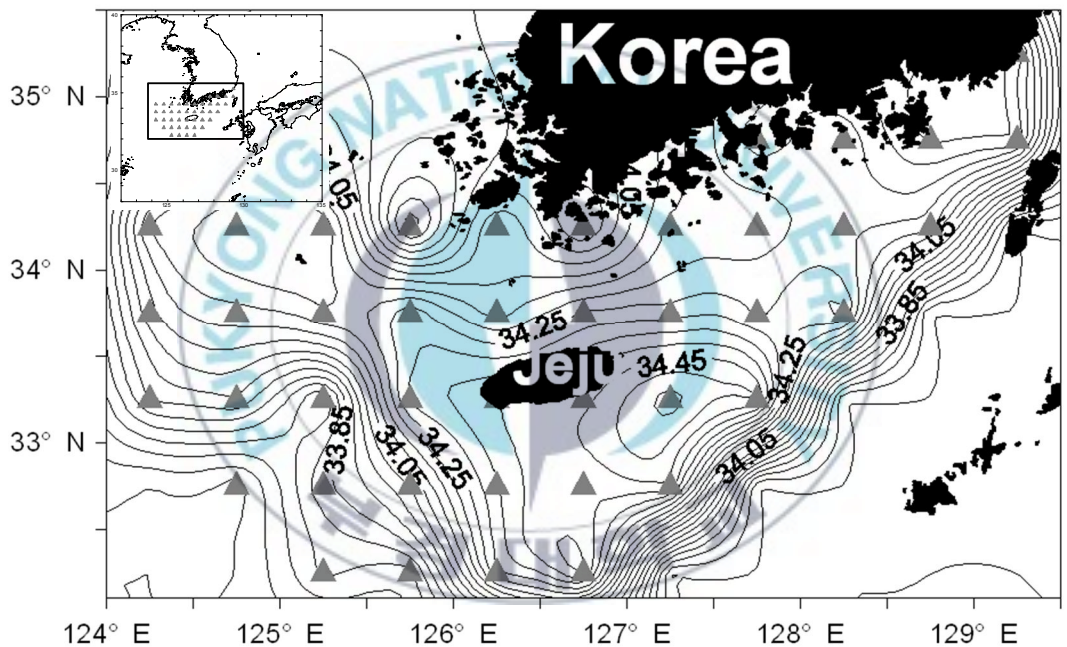


Fig. 12. Bottom salinity distribution of study area.

### 3.3.2. Biomass of zooplankton

The displacement volume (DV) varied with sampling stations ranging from 0.08 to 10.44 ml/$m^3$ (Fig. 13). In general, zooplankton biomass was relatively higher at warmer stations than colder stations.

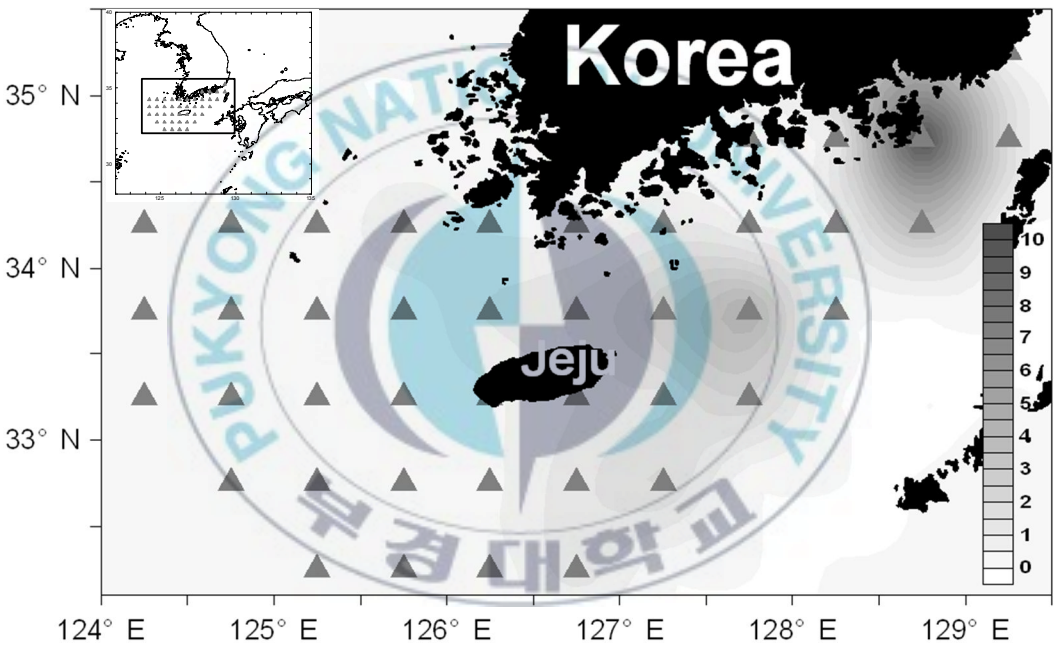


Fig. 13. Distribution of zooplankton biomass in the sampling area.

### 3.4. Analysis of stomach contents

#### 3.4.1. Size composition of *Thamnaconus modestus*

To evaluate the ontogenetic variation of stomach contents, total length of *T. modestus* was measured (Fig. 14). Individuals in the size category in 20~30 cm were 64.5%: 37.0% in 20~25 and 27.5% in 25~30 cm. Individuals in the size category in 30~35 cm were 16.1%, followed by 14.7% in 15~20 cm and 3.8% in 35~40 cm. Individuals smaller than 15.0 cm took up only 0.9%.

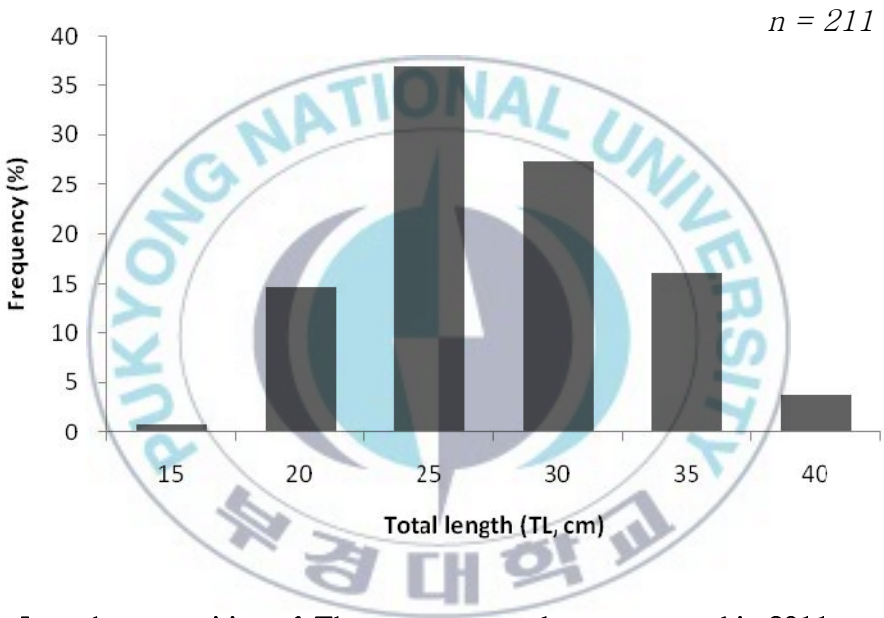


Fig. 14. Length composition of *Thamnaconus modestus* captured in 2011

### 3.4.2. Vacuity index (VI)

The mean vacuity index was 19.0%. There was significant difference of VI among six size groups (Fig 15). VI diminished with increasing body length. Half of individuals smaller than 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 40~45 cm did not have empty stomachs.

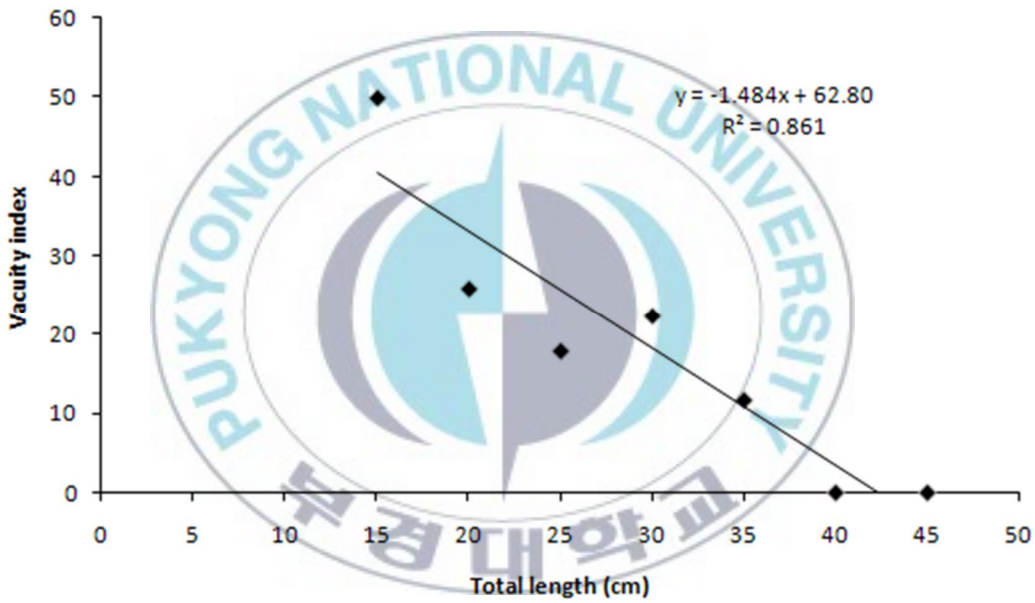


Fig. 15. Vacuity index of each size category.

### 3.4.3. Stomach contents index (SCI)

Stomach contents index (SCI) varied with size category (Fig. 16)**.** SCI was lowest in the size category in 15~20 cm (SCI = 0.22) and highest in 35~40 cm (SCI = 0.42). SCI was 0.3% in the size category smaller than 15.0 cm, 0.3% in 20~25 cm and 0.3% in 25~30 cm. There was no significant difference in SCI among different size categories (Kruskal–Wallis test, P = 0.11).

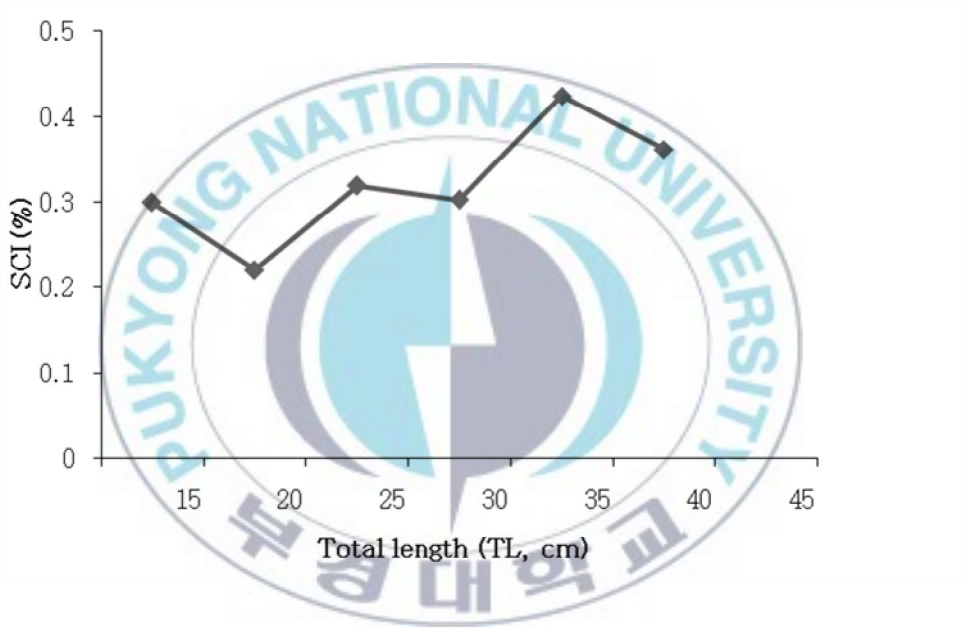


Fig. 16. Stomach contents weight of each size category.

### 3.4.4. Prey composition

#### 3.4.4.1. Prey composition by morphological identification

Stomach contents of *T. modestus* consisted of 46 different prey items (Table 2, Fig. 17). Main prey items belonged to three groups: hyperiid amphipods, ophiuroids and algae. These three prey groups represented 51.9% of total % IRI and 52.2% of total weight. Algae in the stomach contents occupied 26.8% of total % IRI and 73.1% of frequency of occurrence and 25.7% of total weight. Hyperiids occupied 16.5% of % IRI and 40.9% of frequency of occurrence and 3.4% of total weight. Amphipods consisted of gammarids, hypheriids and caprellids. Of these, hyperiids most frequently occurred. Despite the high rate of frequency of occurrence and % number of hyperiids, % weight was not high owing to their small sizes. Ophiuroids occupied 10.3% of % IRI, 31.6% of frequency of occurrence and 24.3% of total weight. Gastropods occupied only 0.5% of % IRI because of their small size although they occurred in large number. Fish were consumed with a small quantity, occupying 1.1% of total weight and 0.02% of % IRI. All of them were not adult fish, but juvenile fish. Fish could not be identified to species level because they were much digested.

A large proportion of preys remained unidentified (Fig 18). Due to the advanced stage of digestion and minimal amount of tissue, the majority of remaining prey could only be classified to more higharchy taxonomic groups.

Table 2. Prey composition by morphological identification

| Prey organisms | %O | %W | %N | %IRI |
|---|---|---|---|---|
| Crustacea | | | | |
| Unidentified | 5.3 | 4.3 | 2.5 | 0.4 |
| Amphipoda | | | | |
| Caprellidea | 26.3 | 5.0 | 12.5 | 5.4 |
| *Caprella aequilibra* | 4.1 | 0.1 | 0.8 | |
| *Caprella kroyeri* | 8.2 | 0.6 | 4.6 | |
| *Caprella scaura* | 0.6 | + | 0.3 | |
| Unidentified | 14.0 | 4.3 | 6.8 | |
| Gammaridea | 42.7 | 1.1 | 21.7 | 11.4 |
| *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 | 10.4 | |
| *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 | 40.9 | 3.4 | 30.8 | 16.5 |
| *Brachyscelus crusculum* | 9.9 | 0.7 | 6.8 | |
| Oxycephalidea | 0.6 | + | 0.1 | |
| *Oxycephalus clausi* | 4.1 | 0.9 | 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 | 1.4 | 21.3 | |
| *Vibilia armata* | 0.6 | + | 0.1 | |

Table 2.( Cont'd)

| Prey organisms | %O | %W | %N | %IRI |
|---|---|---|---|---|
| Decapoda | | | | |
| Anomura | | | | |
| Galatheidae | 2.3 | 0.2 | 0.5 | + |
| Brachyura | 1.8 | 0.4 | 1.0 | + |
| Macrura | 2.3 | 1.9 | 2.1 | 0.1 |
| *Acetes* sp. | 0.6 | + | 0.1 | |
| Unidentified | 2.3 | 1.9 | 2.0 | |
| Isopoda | 0.6 | + | 0.1 | + |
| Mollusca | | | | |
| Bivalvia | 7.0 | 0.4 | 1.1 | 0.1 |
| *Barbatia foliata* | 1.8 | + | 0.4 | |
| *Hawaiarca uwaensis* | 2.3 | + | 0.3 | |
| Unidentified | 2.9 | 0.3 | 0.5 | |
| Gastropoda | 12.3 | 0.5 | 3.1 | 0.5 |
| *Calliostoma multiliratum* | 0.5 | + | 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 | 0.21 | |
| Cypraeidae | 0.6 | + | 0.1 | |
| *Hybochelus cancellatus orientalis* | 1.2 | + | 0.2 | |
| Pyramidellidae | 9.9 | 0.3 | 1.3 | |
| *Teinostoma lucida* | 0.6 | + | 0.1 | |
| Cephalopoda | 0.6 | 0.6 | 0.1 | + |
| Pisces | | | | |
| Unidentified | 0.6 | 1.1 | 2.3 | + |
| Porifera | 11.1 | 2.8 | 3.7 | 0.9 |

Table 2.( Cont'd)

| Prey organisms | %O | %W | %N | %IRI |
|---|---|---|---|---|
| Polychaeta | 1.2 | 0.1 | 0.4 | + |
| Ophiuroidea | 31.6 | 24.3 | 3.4 | 10.3 |
| Algae | 73.1 | 25.7 | 5.4 | 26.8 |
| Miscellaneous items | 62.0 | 28.3 | 9.6 | 27.6 |
| Egg | 0.6 | 3.9 | 0.1 | |
| Unidentified items | 62.0 | 24.4 | 9.5 | |
| Total | | 100.0 | 100.0 | 100.0 |

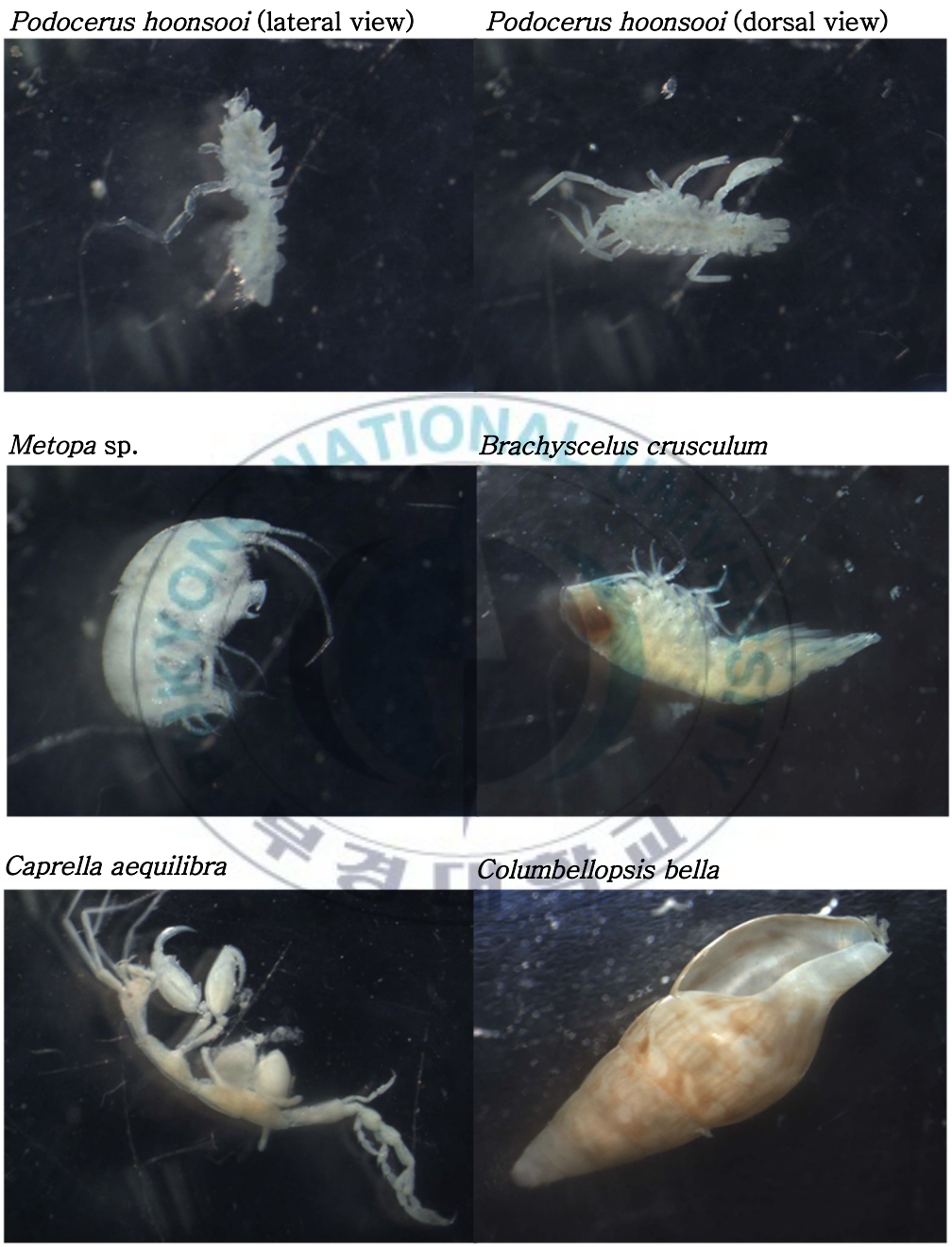


Fig. 17. Prey items that were identified in morphological analysis.

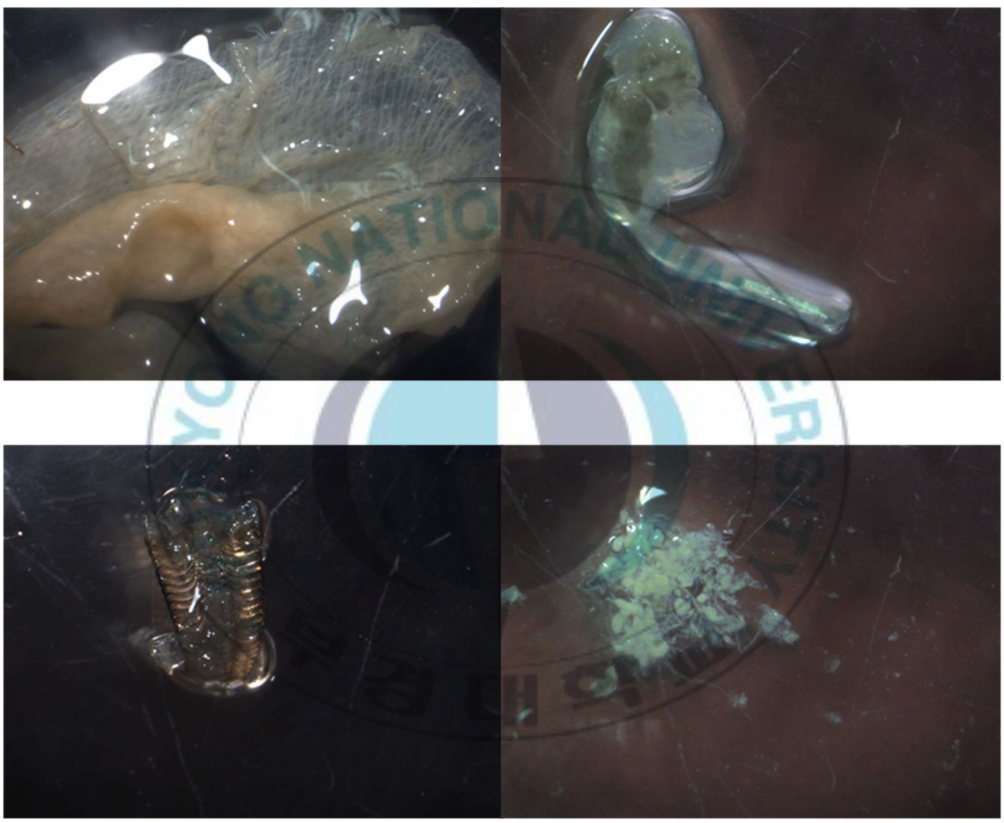

Fig.18. Prey items that were not identified in morphological analysis

### 3.4.4.2 Prey composition by the supplement of molecular identification

eight prey items which were not able to be identified in mophological analysis were identified by molecular analysis. One was *Todarodes pacificus* (Fig. 19) and others were *Nerita* sp. (Fig. 20). The sequence polymorphism of *Nerita* sp. ranged from 9 to 25 bp and that of *T. pacificus* was 4 bp. The p-distance among each *Nerita* sp. was 0.004 ~ 0.055 (Fig. 21). The phylogenetic tree of cytochrome c oxidase subunit I gene from *Nerita* sp. in stomach of *T. modestus* was generated using neighbor-joining method (Fig. 22). *Nerita balteata* was considered *Nerita* sp. because sequences did not show 100% identity with the NCBI database of *Nerita balteata.*

After two additional preys were identified by molecular analysis, total prey consist of 48 prey items. Two prey items identified by molecular analysis were added to 46 prey items identifed by morphological analysis. % weight of prey items identified by molecular analysis had been calculated in morphological analysis, and species name were identified by molecular identification. Gastropods were added to the higher taxonomic prey categories: hyperiid amphipods, gastropods, ophiuroids and algae. These four prey groups represented 66.9% of % IRI and 63.0% of the total weight (Table 3). Hyperiids occupied 18.7%

of % IRI and 36.8% of frequency of occurrence and 2.0% of total weight. Ophiuroids occupied 10.9% of % IRI and 31.6 % of frequency of occurrence and 22.3% of total weight. Algae occupied 28.4% of IRI, 73.1% of frequency of occurrence and 23.6% of total weight. Gastropods increased the % IRI from 0.5 to 10.7% after DNA analysis. The frequency of occurrence of gastropods increased from 12.3% to 36.8%. The % weight increased from 0.5% to 15.0% after molecular analysis. Molecular analysis identified 60.1% of unidentified prey items to species level. Among the gastropods, *Nerita* sp. was totally unidentified in morphological analysis but it occupied 14.5 % of total weight after molecular analysis. In addition, except for a few cephalopod beaks, anything of cephalopod was not identified in morphological analysis. However, cephalopods occupied 3.4% of %IRI, 36.8% of frequency of occurrence and 6.9% of total weight after molecular analysis. In cephalopods, *T. pacificus* was only species identified. It ranked 13.8% of % IRI, 37.4% of frequency of occurrence and 6.3% of total weight.

Table 3. Prey composition by the supplement of molecular identification.

| 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 | |

Table 3.( Cont'd)

| Prey organisms | %O | %W | %N | %IRI |
|---|---|---|---|---|
| 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 | |

Table 3. (Cont'd)

| Prey organisms | %O | %W | %N | %IRI |
|---|---|---|---|---|
| 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 |


Fig. 19. Sequences alignment of 493-bp sequences of the cytochrome c oxidase subunit I gene in tissue of unidentified prey. The number 8 indicates a sample number of unidentified prey. Dots indicate identical bases.

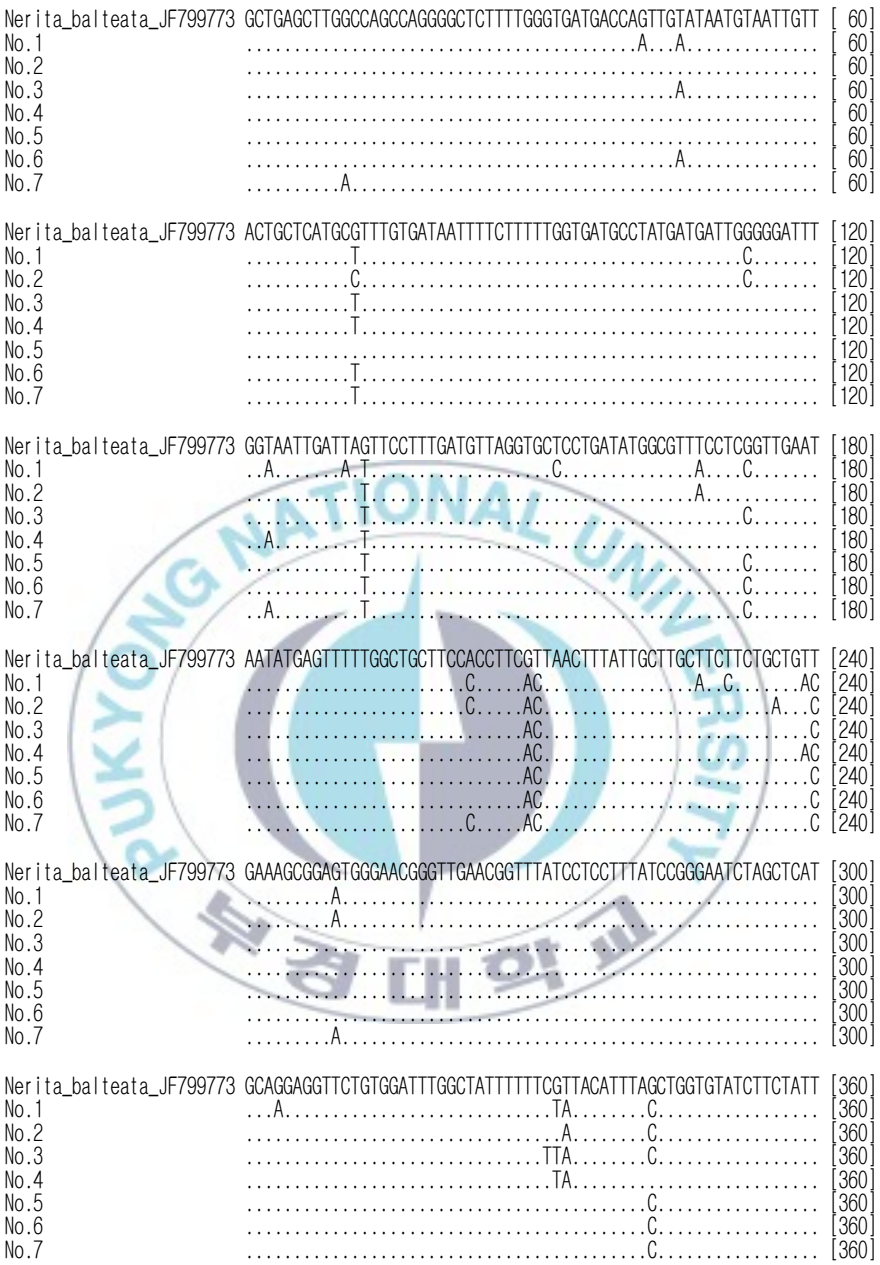
Nerita_balteata_JF799773 GCTGAGCTTGGCCAGCCAGGGGCTCTTTTGGGTGATGACCAGTTGTATAATGTAATTGTT [ 60]
No.1 .....A...A..... [ 60]
No.2 ..... [ 60]
No.3 .....A..... [ 60]
No.4 ..... [ 60]
No.5 ..... [ 60]
No.6 .....A..... [ 60]
No.7 .....A..... [ 60]
Nerita_balteata_JF799773 ACTGCTCATGCGTTTGTGATAATTTTCTTTTTGGTGATGCCTATGATGATTGGGGGATTT [120]
No.1 .....T.....C..... [120]
No.2 .....C.....C..... [120]
No.3 .....T..... [120]
No.4 .....T..... [120]
No.5 ..... [120]
No.6 .....T..... [120]
No.7 .....T..... [120]
Nerita_balteata_JF799773 GGTAATTGATTAGTTCCTTTGATGTTAGGTGCTCCTGATATGGCGTTTCCTCGGTTGAAT [180]
No.1 ..A.....A.T.....C.....A....C..... [180]
No.2 .....T.....A..... [180]
No.3 .....T.....C..... [180]
No.4 ..A.....T..... [180]
No.5 .....T.....C..... [180]
No.6 .....T.....C..... [180]
No.7 ..A.....T.....C..... [180]
Nerita_balteata_JF799773 AATATGAGTTTTTGGCTGCTTCCACCTTCGTTAACTTTATTGCTTGCTTCTTCTGCTGTT [240]
No.1 .....C.....AC.....A..C.....AC [240]
No.2 .....C.....AC.....A...C [240]
No.3 .....AC.....C [240]
No.4 .....AC.....AC [240]
No.5 .....AC.....C [240]
No.6 .....AC.....C [240]
No.7 .....C.....AC.....C [240]
Nerita_balteata_JF799773 GAAAGCGGAGTGGGAACGGGTTGAACGGTTTATCCTCCTTTATCCGGGAATCTAGCTCAT [300]
No.1 .....A..... [300]
No.2 .....A..... [300]
No.3 ..... [300]
No.4 ..... [300]
No.5 ..... [300]
No.6 ..... [300]
No.7 .....A..... [300]
Nerita_balteata_JF799773 GCAGGAGGTTCTGTGGATTTGGCTATTTTTTCGTTACATTTAGCTGGTGTATCTTCTATT [360]
No.1 ...A.....TA.....C..... [360]
No.2 .....A.....C..... [360]
No.3 .....TTA.....C..... [360]
No.4 .....TA..... [360]
No.5 .....C..... [360]
No.6 .....C..... [360]
No.7 .....C..... [360]

Fig. 20. (Cont'd)

```
Nerita_balteata_JF799773 TTAGGTGCTGTAAATTTTATTACTACAATTATTAATATGCGGTGGCAAGGGATGCAATTT [420]
No.1                     ..............................................G.T..... [420]
No.2                     ..............................................C.T..... [420]
No.3                     ..............................................G..... [420]
No.4                     ..............................................G..... [420]
No.5                     ..............................................G..... [420]
No.6                     ..............................................G..... [420]
No.7                     ..............................................G..... [420]

Nerita_balteata_JF799773 GAGCGATTGCCTCTTTTTGTTTGATCAGTGAAGA [454]
No.1                     ....................A..... [454]
No.2                     ....................A.....A..... [454]
No.3                     ....................A.....A..... [454]
No.4                     ....C...............A.....A..... [454]
No.5                     ....................A.....A..... [454]
No.6                     ....................A.....A..... [454]
No.7                     ....................A.....A..... [454]
```

Fig. 20. Sequences alignment of 454-bp sequences of the cytochrome c oxidase subunit I gene in tissue of unidentified prey. Numbers 1 through 7 indicate the sample numbers of unidentified prey. The dots indicate identical bases.

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|
| [No.1] | 0.055 | | | | | | |
| [No.2] | 0.035 | 0.033 | | | | | |
| [No.3] | 0.031 | 0.033 | 0.026 | | | | |
| [No.4] | 0.029 | 0.035 | 0.029 | 0.015 | | | |
| [No.5] | 0.020 | 0.040 | 0.022 | 0.011 | 0.018 | | |
| [No.6] | 0.024 | 0.035 | 0.024 | 0.007 | 0.018 | 0.004 | |
| [No.7] | 0.031 | 0.033 | 0.022 | 0.018 | 0.020 | 0.011 | 0.011 |

Fig. 21. p-distance between *Nerita balteata* individuals found in stomach contents.

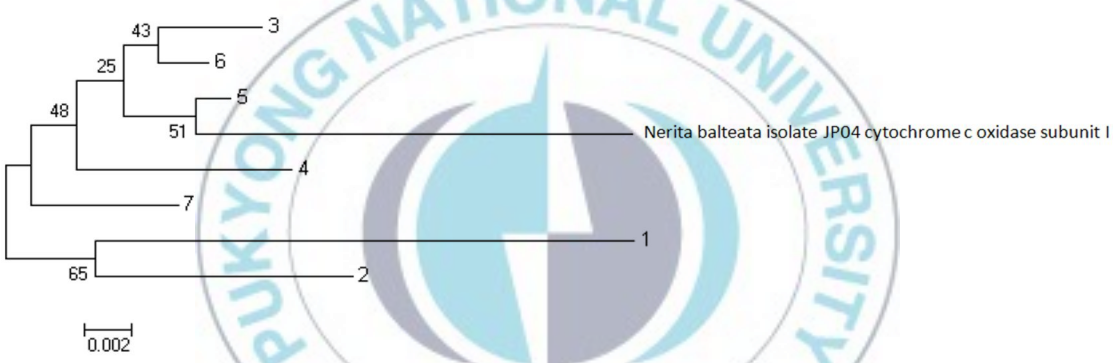


Fig. 22. Phylogenetic tree of cytochrome c oxidase subunit I gene from *Nerita balteata* in the stomach of *Thamnaconus modestus.*

### 3.4.4.3. Prey composition in relation to fish size

Amphipods and algae were preys groups present in the diet of all size classes (Fig. 22). Algae were the most important prey group in small size classes (≤20.0 cm, TL), while proportion of the other prey items was comparatively low. Dominant prey items of the small size classes (≤20.0 cm, TL) were species having relatively low mobility or smaller crustaceans such as amphipods. Algae were present in all size classes, but percentage of wet weight significantly decreased with increasing body length. It occupied 91.1% (≤ 15 cm), 62.6% (15 ~ 20 cm), 7.9% (20~25 cm), 19.8% (25~30 cm), 19.7% (30~35 cm), 9.5% (35~40 cm). Crustaceans, particularly amphipods were also found in all body length with varying proportion: 8.9% (≤ 15 cm), 29.4% (15~20 cm), 36.5% (20~25 cm), 12.6% (25~30 cm), 25.4% (30~35 cm), 9.1% (35~40 cm). The frequency of gastropods was increased with increasing size. Cephalopods having high mobility appeared from individuals over 20 cm size classes. Stomach contents of individuals in 25 ~30 cm size class were dominated by gastropods and ophiuroids: 27.0% of total weight in gastropods and 26.8% of total weight in ophiuroids. Ophiuroids mostly appeared in 25~30 cm and 35~40 cm size classes except for 0.9% in 15~20 cm. Fish were found only in 25~30 cm, occupying 4.2% of total weight.

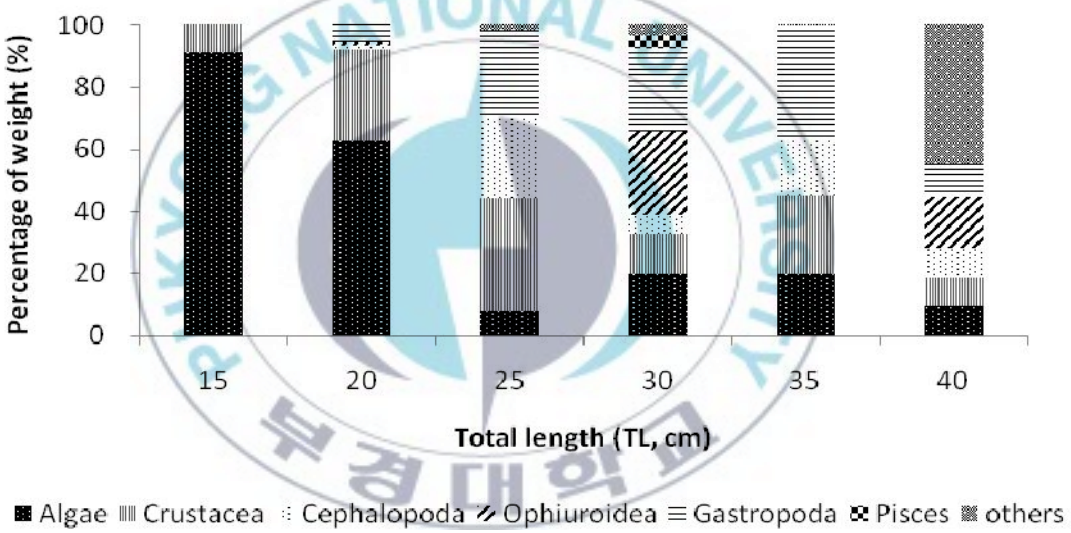


Fig. 23. Ontogenetic variations in the prey composition of *Thamnaconus modestus*

### 3.4.5. Dietary breadth index (DBI)

DBI by fish size ranged from 0.08 to 0.19 and the value was relatively low in comparison with other fish species (Fig. 23). There was a significant increase of the dietary breadth index by increasing fish size ($R^2 = 0.952$, $P < 0.001$). DBI in smaller than 15 cm size class was 0.08 and gradually increased with increasing fish size. It took up 0.14 in 20~25 cm size group and 0.19 in 35~40 size group.

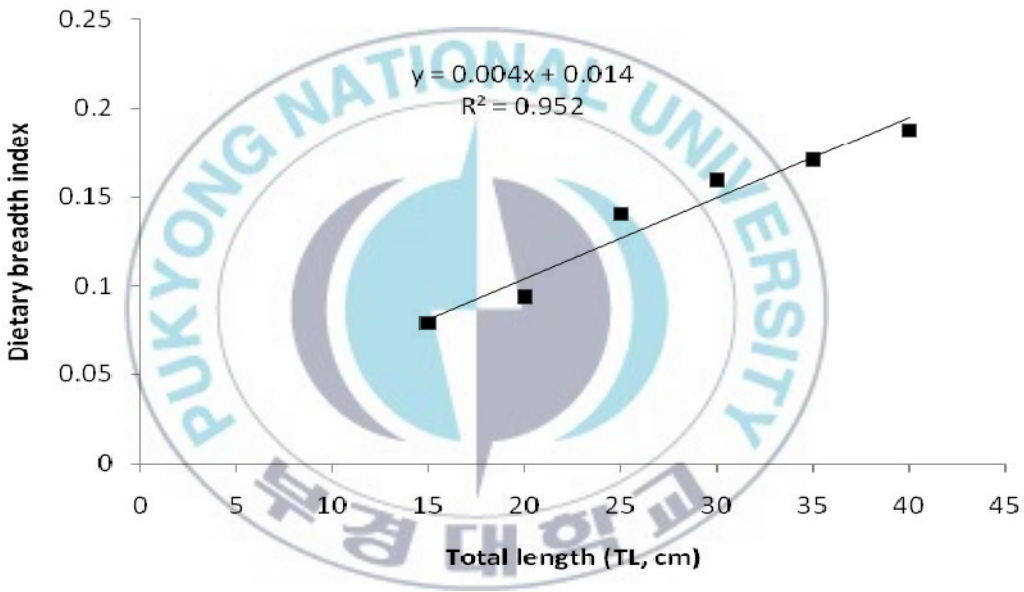


Fig. 24. Dietary breadth index of *Thamnaconus modestus* by total body length.

# 4. Discussion

Filefish is important fish resource as a commercial fish. After the development of processing technology in 1970s, demand of the fish had increased and the catches had increased until 1990. By poor management and overfishing, abundance of population sharply decreased until 2000s. To recover the population, it's very important to understand their biological and ecological characteristics. However, little has been studied for the ecology of the fish. The main goal of present study was to determine their ecological and biological characteristic of the fish by studying distribution characteristic and feeding habit.

It was reported that *T. modestus* distributes ranging from 10 to 28℃ water temperature (Baik and Park, 1989). The fish were dominant in layer that was covered by warm water mass (Tatsuaki et al., 1989). The fish was captured mainly in warmer water temperature than 12℃ in present study. It is considered that *T. modestus* inhabit ranging from 10~28℃ water temperature and prefer warmer temperature than 12℃. It was reported that distribution depth of *T. modestus* was 50~100 m (Tatsuaki et al.1989) and 30~120 m (Baik and Park, 1989). Present

study showed 87% of captured individuals was found in 80~120 m and 59% was found in 80~100 m. *T. modestus* spawn shallow area and individuals migrate from shallow area to deep area with increasing body length (NFRDI, 2009). In present study, body length of individual found in deep area was significantly larger than that of shallow area. It was assumed that *T. modestus* distributes roughly 30~120 m depths and mature individuals distributes more deep area, 80~120 m depths.

Feeding habit studies of monacanthidae species were carried out in diverse areas (Miyajima et al., 2011; Kwak et al., 2003; Bell et al.,1978; Randall., 1967). Bell et al. (1978), Peristiwady and Geistdoerfer (1991), Randall (1967) stated monacanthidae species were omnivorous and consumed diverse prey such as seagrass, algae, hydroids, mollusks, crustaceans and polychaetes. Species of family monacanthidae inhabiting *zostera marina* bed in Jindong bay, Korea also consumed diverse animal prey items as well as vegetable prey items *zostera marina* (Kwak et al., 2003). *T. modestus* was also omnivorous, although there was variation between fish size classes by present study. Major prey items of individual which was longer than 20 cm were animal prey items, but vegetable prey items were also consumed. Vegetable prey items occupied large percentage in individual which was smaller than 20 cm, but animal prey items also was

consumed. The present study clearly showed that *T. modestus* was omnivorous.

Randall (1967) stated that species of family monacanthidae were “not such strong swimmer and thus are more closely associated with the bottom”. Many of animal prey items except for cephalopods were benthic animals (such as gastropods, bivalves, crustacean, sponge and echinoderms). However, *T. pacificus* occupying 6.3% of total weight are not a benthic animal, and it has swimming ability in relation to other benthic animals. By Song et al., 2006, the *T. pacificus* stay in bottom during daytime by diel vertical migration. Kim et al. (2007) reported that major prey items of *T. pacificus* were benthic animals. It is assumed that *T. modestus* fed on *T. pacificus* when *T. pacificus* stayed in bottom by diel vertical migration.

Randall (1967) carried out stomach contents analysis of 20 *Cantherhines pullus* (family monacanthidae). The stomach of 2 individuals consisted entirely of sea algae. Total length of the 2 individuals was 4.6 and 6.5 cm, which were smallest size among 20 *Cantherhines pullus*. By research of El-Ganainy (2010), sea algae also were consumed largest amount in smallest size class (8.0~10.9 cm). In present study, small size individuals (≤20 cm) consumed large amount of sea algae and the sea algae were main prey item. It was assumed that the favorite prey

of small size fish was vegetable food.

Total length at 50% maturity of *T. modestus* was 21.0 cm (NFRDI, 2009, Baik and Park, 1989). Prey composition of *T. modestus* sharply changed from total length at 50% maturity in present study. Vegetable food sharply decreased and diverse prey items were consumed in ≥20.0 cm size classes.

Unidentified materials had been classified into eight items by its morphology and the eight items were identified by molecular analysis. In polymerase chain reaction (PCR) of DNA, universal primer amplifying portions of the mitochondrial cytochrome c oxidase gene (mtCOI) gene was employed. Mitochondrial DNA is preserved well after death by character not decomposed well (Lee, 2011). Furthermore, mitochondrial primers is well known in comparison with that of nuclear DNA. COI primer amplified portion of the mtCOI which appears to be the most conservative protein-coding genes in the mitochondrial genome of animals (Folmer et al. 1994) and amplifying DNA from widest range of phyla (invertebrates and vertebrates) (Blankenship et al. 2005). *Nerita* sp. and *Todarodes pacificus* were identified by using COI primer in molecular analysis. It was difficult to identify the *T. pacificus* by morphological analysis because it remained just a piece. But for molecular analysis, *T. pacificus*

wouldn't have been identified. The species of family monacanthidae were characterized by small mouth and specialized teeth, which make it difficult to study about feeding habits of monacanthidae species. Unidentified prey items in stomach of *Monacanthus tuckeri*, family monacanthidae (Randall, 1967), ranked 41.3% of total weight. In present study, 24.4% of total weight was unidentified in morphological analysis. Thus, molecular genetic analysis is very useful and essential method in feeding study of family monacanthidae.

The vacuity index of *T. modestus* was (V.I) 19.0%. It was low in relatively to that of other piscivore (Park et al., 2007, Huh et al., 2006, Choi et al., 2011). However, it was high in relatively to that of other omnivorous species (Peristiwady and Geistdoerfer, 1991; Kwak et al., 2003; Huh and Kwak, 1998). If *T. modestus* fed heavily at night, V.I of individuals captured at nighte would be low. Sampling in present study was conducted at daytime. To observe accurately, sampling conducted in all of day and night was needed. Food diversity increased with increasing fish size. It was assumed that food diversity increased by improving of predation ability. Large individuals were more good hunter that possesses good swimming ability and high prey selectivity.

# 5. Acknowledgement

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