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Habitat, diet, feeding and resting behaviour of the Korean endemic cobitid *Iksookimia hugowolfeldi* (Cobitidae, Pisces) in the wild

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Abstract. A study on the habitat use and diet of *Iksookimia hugowolfeldi*, which is endemic to Korea, was carried out focusing on feeding and resting behaviour in the wild. This study focused on a valley stream in a forest on Geogeum Island (Eojeon-ri, Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea) with a large population. The bottom substrate of this stream was mud (< 0.1 mm in diameter, 10 % relative abundance), sand (0.1-2 mm, 30 %), gravel (2-16 mm, 20 %), pebbles (16-64 mm, 20 %), cobbles (64-256 mm, 10 %), and boulders (> 256 mm, 10 %), with an Aa-Bb river type and a slow current (0.1 m/s). The food sources analyzed based on the index of relative importance (IRI) included Diptera (70.98 %), Phryganeidae (10.46 %), Branchiopoda (9.74 %), Harpacticidae (8.33 %), Ephemeroptera (0.47 %) and other (0.02 %). As a diurnal and benthic filter feeder, two main feeding behaviours were observed: feeding on surface (sand, cobble) and on sand-cobble digging. Resting behaviour predominantly occurred on the surface of sand and cobbles, and sometimes while buried in the sand. Daily feeding behaviour was more active at the highest water temperature of 22.1-23.7 °C from 14:00 to 15:00, and feeding behaviour occurred more frequently in July to August, at temperatures as high as 22-24 °C. Meanwhile, while the water temperature is below 8 °C, they are getting around in the water by being hid in or buried in the substrate. In that season, the empty stomach rate was higher than in other seasons.

Key words: Southern king spine loach, feeding ecology, diurnal fish, Korea

Introduction

The family Cobitidae includes approximately 177 species and 25 genera and is widely distributed throughout Europe and Asia (Nelson 2006). Among the 15 Korean cobitid species in five genera, *Iksookimia hugowolfeldi* occurs as an endemic freshwater species (Kim 2009). This southern king spined loach lives in very restricted area, including the River Yeongsangang and Tamjingang and smaller valley streams (Nalbant 1993, Kim 1997). The fish are directly or indirectly affected by physical, chemical, and biological characteristics of environment in their habitat preference. The physical characteristics such as water depth, water velocity and bottom substrate are main important factors in maintaining the community and its life cycle (Arthington et al. 2006, Hur et al. 2009). As feeding affects growth, reproduction, and health as well as responses to physiological and environmental stressors and pathogens, it is of ecological importance in fish species (Lall & Tibbetts 2009). Feeding behaviour is closely related to external morphology in fish, such as the position and structure of the mouth, and fin and body shape, and reflects environmental variables such as feeding substrate, water depth, and water flow (Tippets & Moyle 1978, Moyle & Cech 2004, Hildebrand et al. 2013). Resting behaviour after feeding also is related with a micro-refuge to protect them and conserve the energy (Emery 1973). Despite its ecological importance, little is known about the feeding and resting behaviour in the family Cobitidae in the wild. Meanwhile, *I. hugowolfeldi* is facing drastic population reduction due to artificial disturbances caused by stream reconstruction, water pollution, and dam construction (Ko et al. 2011). Thus, as part of *I. hugowolfeldi* conservation efforts, insight is needed into feeding and resting behaviour as well as substrates and food items.

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Study Area

The study was conducted from January to December 2015 in the independent stream of Geogeum Island (Eojeon-ri, Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea; 34°26′15″ N, 127°09′42″ E; Figs. 1-2), which has the largest known population of *I. hugowolfeldi* in Korea. The study site was approximately 2.5 km in length and 10-50 m in width, consisting of riffle, run and pool. Reeds and woody plants inhabited the shoreline, and there were farms and rice fields nearby. The habitat was classified as an Aa-Bb river type with 10-50 m river width and 0.5-3 m water width with repeating pools and riffles. The water depth was about 0.1-0.8 m, and the bottom substrate consisted of 10 % mud (< 0.1 mm), sand (0.1-2 mm, 30 %), gravel (2-16 mm, 20 %), pebbles (16-64 mm, 20 %), cobbles (64-256 mm, 10 %), and boulders (> 256 mm, 10 %). The water velocity was a maximum of 1.5 m/s in the riffle, and a minimum of 0 m/s in the pool (Kani 1944, Cummins 1962).

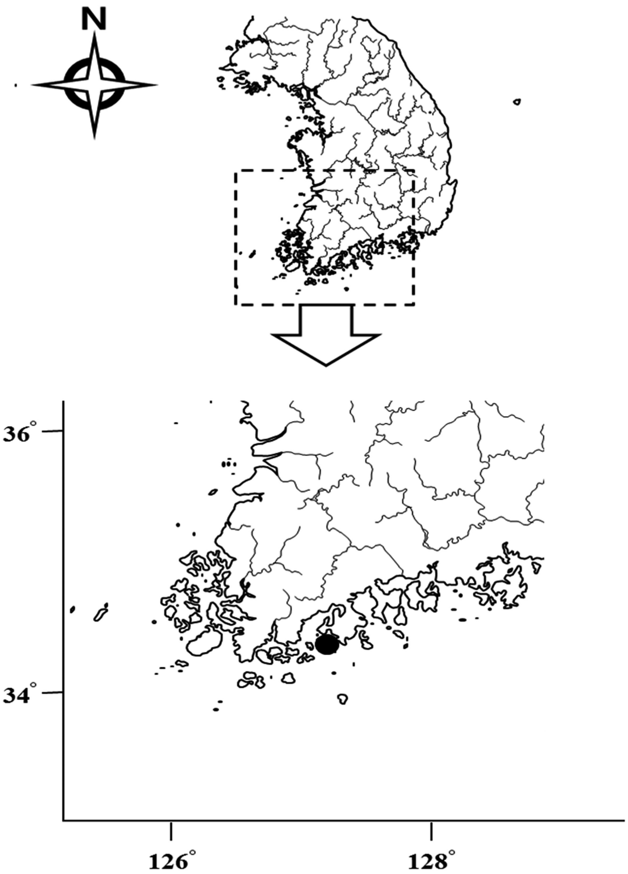

Fig. 1. A map showing the study site of *Iksookimia hugowolfeldi* in Eojeon-ri, Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea.

Fig. 2. Example of microhabitat and substrate in the study site, Eojeon-ri, Geumsan-myeon, Goheung-gun, Korea.

Material and Methods

Sampling was carried out with a cast net (mesh size, 7 × 7 mm, kick net, 4 × 4 mm and scoop net, 1 × 1 mm), and the identification key for sympatric fishes obtained from the study sites was taken from the classification of Kim & Park (2002). The analysis for phytoplankton was carried by surface water samples collected in the sites and the samples collected by scraping the surface of cobbles and pebbles with a brush, whereas invertebrate insects were sampled by using a kick net and a skimming net in the water, and by collecting those who were on cobbles and pebbles.

Analysis of stomach contents

At the end of each month, 20 fishes were selected and fixed in 10 % neutral-buffered formalin (240 specimens in total). In the laboratory, stomach contents were dissected and identified under a stereoscopic microscope to the lowest possible taxon according to Jeong (1993) and Won et al. (2005). To estimate the importance of food items, the index of relative importance (IRI) was calculated as follows:

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

%N is the numerical percentage, the number of items in each prey category; %W is the volumetric percentage, the wet weight of a prey category compared to the total weight of stomach contents; %F is the frequency of occurrence percentage, the ratio of stomachs containing a particular prey and expressed as a percentage (Pinkas et al. 1971), and IRI is expressed as a standarized IRI (%IRI; Cortes 1997).

Daily feeding and resting activity

Feeding and resting behaviour were observed for 72 hours using underwater photography equipment (Sony, 420TVL CCD 6LED Outdoor Camera Ip68, Japan) from June 24-26, 2015 during the spawning season, and the video footage obtained was analyzed. Considering the relationship between air and water temperatures, the best time to study feeding behaviour was set as late June. During this period, spawning occurs very actively because of higher air and water temperatures.

Evaluation of the frequency of feeding

From a video clip recorded in the wild, feeding frequency was counted as the number of operculum opening and

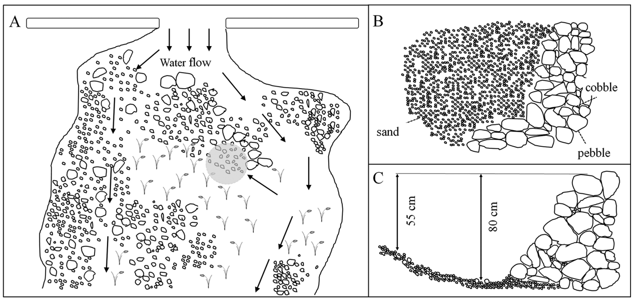

Fig. 3. The diagram showing the microhabitat of *Iksookimia hugowolfeldi* at the study site. A) general view of the area, B) detail of the ground (yellow circle) from above, C) cross section of the ground of the study site. Arrows, water flows, yellow circle, collective spot.

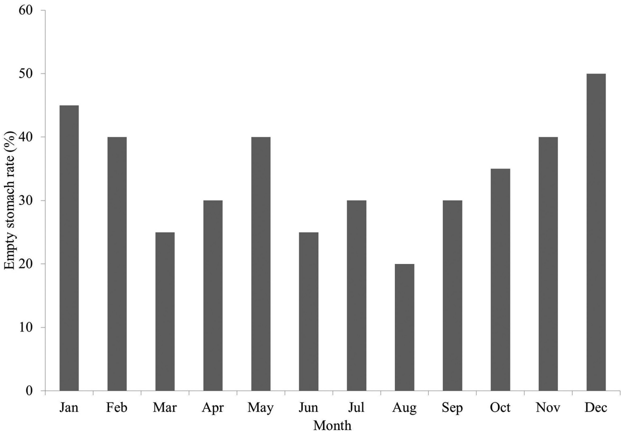

Fig. 4. Number of empty stomach (in percentage) for the each sampled month.

closing events where food items (substrate) and water were taken into the mouth and passed out via the gills. At two-hour intervals, only five individuals being confirmed exactly on the operculum movement were used for the feeding frequency for three complete days.

Results

Microhabitat

Most individuals are found at collective spots where are nearly located at pools being composed of sand and gravel with almost no water flows (Fig. 3). However, some individuals also occurred in riffles with a bottom substrate of sand and gravel as well as pebbles and cobbles with a relatively fast water flow. The collective spots of 0+ years were less than 0.3 m water depth and had almost no water velocity. At the collective spots, the population density was about 30-40 individuals per 50 cm^2.

Stomach contents

The monthly empty stomach rate was the highest in December, whereas it was lowest in August (Fig. 4). The stomach contents appeared to primarily comprise aquatic insects and crustaceans (Table 1). In terms of occurrence rates, they feed mainly on Chironomidae (52.63 %), Phryganeidae (16.13 %), Branchiopoda (15.68 %), Harpacticidae (13.38 %), and Ephemeroptera (1.86 %). Numerical rates were also highest for Chironomidae (42.86 %), followed by Harpacticidae (21.98 %), Branchiopoda (17.58 %), Phryganeidae (8.24 %) and Ephemeroptera (7.69 %). Each dry weight rates are Chironomidae (33.19 %), Phryganeidae (28.33 %), Branchiopoda (17.44 %), Harpacticidae (13.14 %) and Ephemeroptera (6.67 %). Chironomidae had the highest standardized IRI (%IRI) of 70.98 %, indicating that it is the preferred food for *I. hugowolfeldi*. An on-site survey of a frequency of occurrence of aquatic arthropods at the study site revealed Ephemeroptera 37.64 %, Phryganeidae 22.56 %, Chironomidae 19.28 %, Branchiopoda 8.51 %, Harpacticidae 6.24 %, and Coleoptera 5.77 % (Fig. 5). Compared the fauna of aquatic insects with the stomach contents, the frequency of Ephemeroptera in the habitat is relatively high.

Phytoplankton in the stomach contents consisted mainly of *Microcystis* sp., *Navicula* sp., *Cymbella* sp. and *Coconeis* sp. (Table 2), whereas the wild flora of the phytoplankton is similar, such as *Microcystis* sp., *Navicula* sp., *Cymbella* sp., *Coconeis* sp., and *Phormidium* sp. (Table 2).

Feeding and resting activities

Iksookimia hugowolfeldi is generally active during the day and twilight hours and less active or inactive

Table 1. The stomach contents in percentage by frequency of occurrence, number, dry weight and values of the standardized IRI in *Iksookimia hugowolfeldi*.

Prey organism	Occurrence (%)	Number (%)	Dry weight (%)	IRI (%)
Phryganeidae	16.13	8.24	28.33	10.46
Chironomidae	52.63	42.86	33.19	70.98
Branchiopoda	15.68	17.58	17.44	9.74
Harpacticidae	13.38	21.98	13.14	8.33
Ephemeroptera	1.86	7.69	6.67	0.47
Others	0.32	1.65	1.23	0.02

Table 2. Phytoplankton found in stomach contents and in the river.

Species	Frequency	
	Stomach contents	Habitat
Cyanophyceae		
Cosmarium sp.	+	
Chroococcus sp.		++
Microcystis sp.	+++	+++
Bacillariophyceae		
Navicula sp.	+++	+++
Cymbella sp.	+++	+++
Gomphonema sp.	+	
Pinullaria sp.	+	+
Synedra sp.	+	+
Fragilaria sp.	++	++
Coconeis sp.	+++	+++
Melosira sp.	++	++
Chlorophyceae		
Scenedesmus sp.	+	+
Oscillatoriaceae		
Phormidium sp.	++	+++
Oscillatoria sp.		+
Chaetophoraceae		
Cloniophora sp.		+
Euglenophyceae		
Euglena sp.	+	
Desmidiaceae		
Closterium sp.	+	

+: 1~50 individuals, ++: 50~100 individuals, +++: more than 150 individuals.

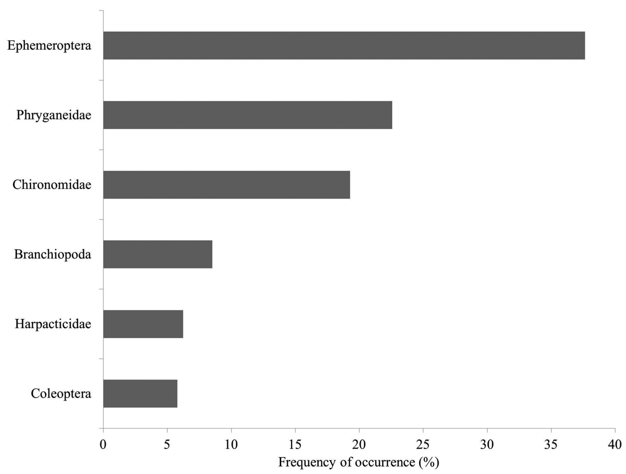

Fig. 5. Composition of benthos collected in the field, Eojeon-ri, Geumsan-myeon, Goheung-gun, Korea.

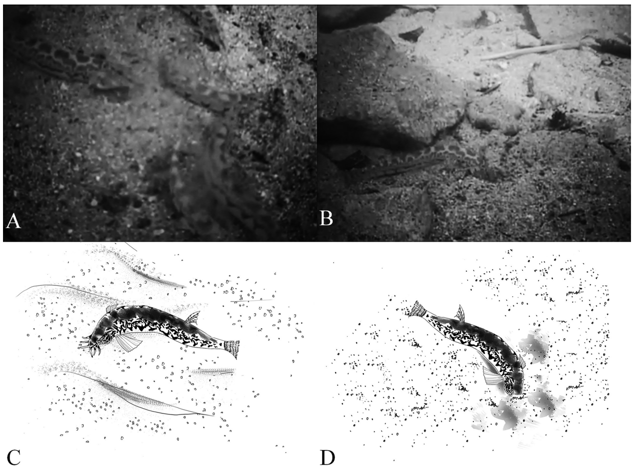

Fig. 6. Video clips (A, B) and illustrations (C, D) of natural feeding behaviours in sand. A and C, sand surface filter feeding; B and D, sand-digging filter feeding.

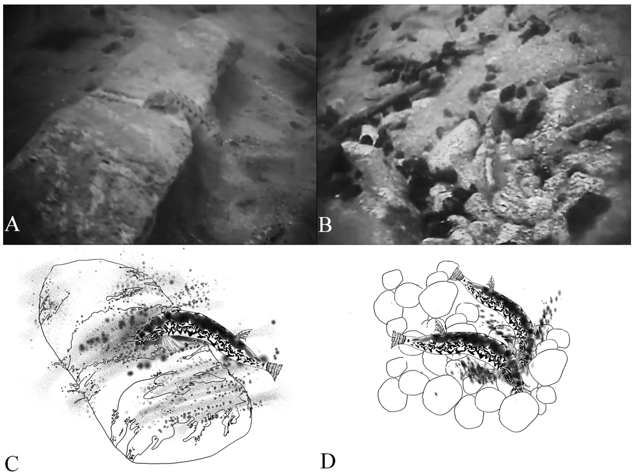

Fig. 7. Video clips (A, B) and illustrations (C, D) of natural feeding behaviours in cobbles. A and C, cobble surface filter feeding; B and D, filter feeding in between sand and cobbles.

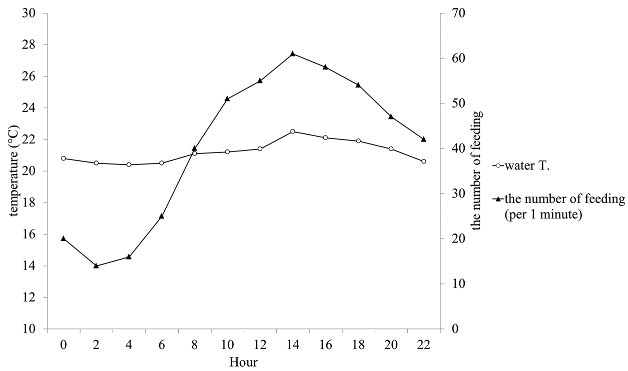

Fig. 8. Daily changes in the average water temperatures and the average number of feeding of *Iksookimia hugowolfeldi*, 24 to 26, June, 2015.

during the night, although there are individual differences. This fish is a benthic species and they little chase or select prey through active prey search.

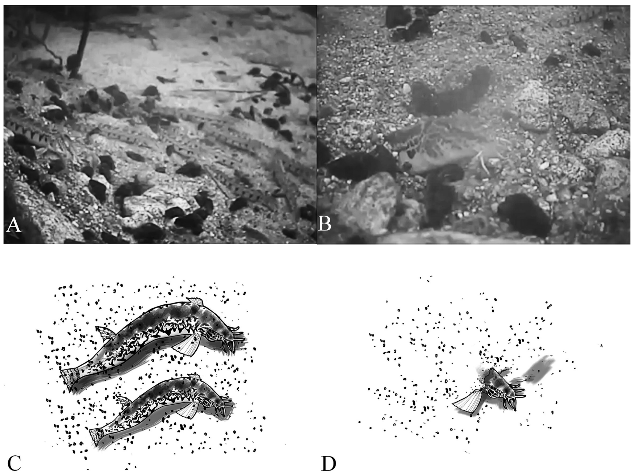

Fig. 9. Video clips (A, B) and illustration (C, D) of natural resting behaviours in sand. A and C, on sand in day; B and D, being half-buried in sand in day.

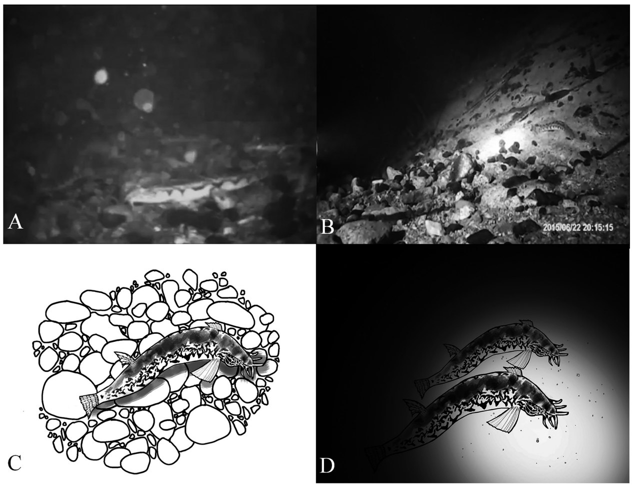

Fig. 10. Video clips (A, B) and illustration (C, D) of natural resting behaviours in cobbles and at night. A and C, on cobbles at night; B and D, on sand at night.

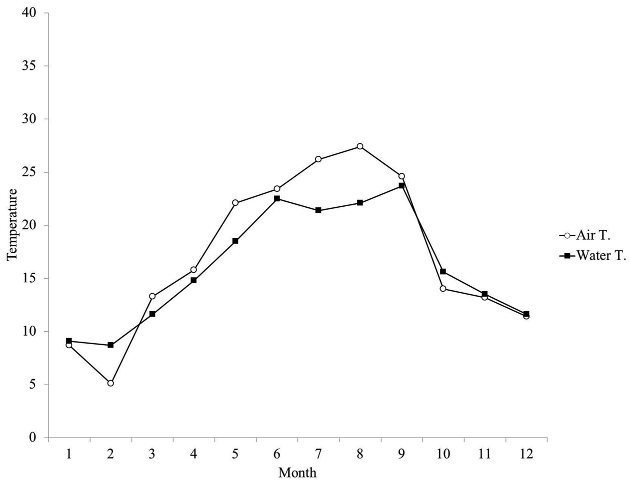

Fig. 11. Monthly changes of the average air and water temperatures of Eojeon-ri, Geumsan-myeon, Goheung-gun, Jeollanam-do, Korea from January to December, 2015.

Rather, feed on or near the bottom of a body of water, such as sand or gravel. Its behaviour consists of three patterns: resting, zigzag swimming and feeding. All the fish observed exhibited two behaviour types: 1) active (feeding activity, crawling along the bottom or swimming above the bottom), and 2) static (resting). Feeding activity starts with rapid inhalation of water together with small food particles above the bottom through the mouth and rapid exhalation from beneath the operculum. Such activity continues constantly until they swim in a zigzag pattern to another place to feed or rest. Resting involves little movement; fish burrow into or stay motionless on the substrate without any feeding activity.

Daily feeding behaviour

The feeding behavior of *I. hugowolfeldi* involved the following two main tactics: feeding on sediments in the surface of substrate (sand, cobble) and inside sand (digging, Figs. 6-7). First, most individuals exhibited sand surface filter feeding on the sand bottom, with sand taken into the mouth together with substrate particles and then expelled through the opercular opening. These repeated closing and opening operculum movements are very active. Through such filter feeding as a pump, the sand with particles is pulled into water, which it can be easily observed even outside water. Second, on cobbles or gravel, these fish feed by filtering sand or detritus. Third, it is just looking like diving. Some individuals dig down when feeding on the sand bottom, with the head buried in the sand by about a half of the head length, with the operculum exposed above the sand. The fourth pattern is filter feeding being carried out in between sand and cobbles, sometimes with their mouthparts embedded in the sand. On average, one individual feeds 14 to 61 times per minute. In particular, the most active feeding time is at the highest water temperature of the day at 14:00 to15:00, with up to 61 feeding events (*vs.* 8-18 times at 0:00 to 02:00 at below 21 °C water temperature, Fig. 8). As the water temperature decreases after sunset, the frequency of feeding also decreases.

Seasonal feeding behaviour

From late October, the average monthly air and water temperatures drop drastically below 14 °C from 24.6 °C and below 15.6 °C from 23.7 °C, and continue to decrease until February, which has a minimum 5.1 °C air temperature and a minimum 8.7 °C water temperature (Fig. 11). Under such environmental conditions, this fish is rarely seen swimming or moving about the bottom. In particular, under air temperatures less than 8 °C water temperatures under 10 °C, they hide or burrow into the substrate of the bottom such as sand and cobble. In Fig. 4, a higher empty stomach rate of 40-50 % (*vs.* 22-40 %) was seen from November to February, particularly in December.

Resting behaviour

Without showing any feeding activity such as swimming to seek out food or dynamic movement (opening and closing) of the operculum, resting involves remaining motionless except for breathing. Resting comes after feeding activity is finished. In the day time (sunrise to sunset), they rest predominantly on the surface of the sand and the cobbles, staying inactive or quiescent. Interestingly, some prefer to be buried in the sand with the anterior parts including the head and the pectoral fin or about the anterior half of its body exposed, with the rest being hidden.

At night, the resting pattern is the same as in the day. The duration of resting differed by individual, ranging from 10 seconds to more than an hour. After sunset, the resting time was longer and then *I. hugowolfeldi* is getting into the substrate (Figs. 9-10).

Discussion

Iksookimia hugowolfeldi has been thought to prefer gravel and pebble bottoms with rapids (Choi 2003) in the upper-middle portion of streams. Rather than such a substrate, however, it was confirmed that they inhabit pools with more sand than other substrates with a water velocity that is low or more or less stagnant. These preferences are similar to *I. yongdokensis*, *Cobitis choii*, and *C. tetralineata*, and differing from other cobitis fish such as *Koreocobits naktongensis*, *K. rotundicaudata*, *I. koreensis*, *I. pumila*, *I. longicorpa*, *C. lutehri* and *C. pacifica*, which inhabit pebble and cobble bottoms (Kim 1978, Kim & Lee 1984, Kim & Park 2002, Kim & Ko 2005, Kim et al. 2006, Byeon 2007, Choi & Byeon 2009, Ko et al. 2009, Hong et al. 2011, Ko 2015). This is a benthic species and they rarely chase or select prey through active prey search. But, it feeds by filtering sand or pebble bottoms (Kim & Park 2002). Based on stomach content analysis, insects swimming in the surface layer were not fed on (Won et al. 2005), whereas benthic macroinvertebrates were common in this fish's diet, indicating that they are a typical detritus feeder. Among other Korean cobitids, *I. koreensis* feeds on Rotifera (22.5 %), Chironomidae (22.1 %) and algae (Ko et al. 2009), and *I. pacifica* on aquatic insects, especially Chironomidae (76.7 %, Kim & Lee 1984, Ko 2015). Meanwhile, Amoebozoa and Chironomidae are the most common prey items for *I. longicorpa* (25.1 % and 24.4 %, respectively) and *C. tetralineata* (30 % and 21.3 %, respectively) (Kim & Ko 2005, Kim et al. 2006). *K. naktongensis* feeds mainly on Chironomidae (72.02 %) and Ephydridae (26.87 %, Hong et al. 2011). Among Korean cobitids, *I. hugowolfeldi* showed higher food selectivity toward Chironomidae than *I. koreensis*, *I. longicorpa* and *C. tetralineata*, but this was similar to *I. pacifica* and *K. naktongensis*. These results may be due to changes in the composition and appearance of food organisms (Baek et al. 2002, Choi 2002), and in the growth, the feeding behaviour of fish changes (Wootton 1976, Kim 1996). We confirmed that there is a slight difference between the occurrence of prey items in the habitat and the preference for prey in stomach contents. Such difference between invertebrate fauna appearing in stomach contents and natural habitat may be due to it's a small bottom-facing mouth, which is fit for eating more or less small preys on the bottom.

With regard to feeding activity, *I. longicorpa*, *I. pacifica* and *Cobitis tetralineata* are diurnal fishes (Kim & Ko 2005, Kim et al. 2006, Ko 2015). These findings, however, involved only simple observation carried during field work on fish fauna, without any elaboration or detailed information. Our study focused on feeding activity and revealed that *I. hugowolfeldi* swims actively to seek food between sunrise and sunset, and that at night they show little movement while buried into or remaining motionless on the substrate of the bottom without any feeding activity.

In *I. hugowolfeldi*, the timing of studying feeding behaviour was peak spawning in late June (June 24-26) for three complete days. This condition is typically closely related to feeding intensity, with increased feeding to obtain energy in preparation for spawning. In teleost fishes, water temperature and the light have an effect on growth, reproduction and feeding (Jonsson 1991). Hildebrand et al. (2013) described that mouth position and structure are closely related to feeding behaviour, especially for suction and filter feeding. Houlihan et al. (2001) explained that feeding behaviour is linked to feeding habits, food preferences and mechanisms of food detection. The feeding behaviour of *I. hugowolfeldi* is likely related to its mouth position at the bottom of the head and the barbels that carry tactile and chemosensory receptors (Kim et al. 2001). The feeding behaviour of Korean cobitis fishes has previously been described as filtering bottoms consisting of such substrates as sand and or cobbles, and by digging and gulping (Kim & Park 2002, Kim & Ko 2005, Kim et al. 2006, Ko 2015). The European species *C. taenia* takes food by filtering small organisms and detritus from the substrate while passing it through the mouth and out via the gills (Robotham 1982). The Japanese species *Misgurnus anguillicaudatus* has two feeding behaviours, digging in the bottom substrate and gulping sand by feeding crawl and twist (Watanabe & Hidaka 1983); however, that study was not performed in a natural feeding environment, but an experimental one.

This species rests predominantly on the surface of the sand and the cobbles, staying inactive or quiescent, sometimes buried in the substrate. The resting duration is from 10 seconds to more than an hour and varies between individuals. Such feeding and resting behaviours have not been described previously in the wild or in captive experiments. Fish rest to conserve energy (Emery 1973). Moyle & Cech (2004) also suggested that fishes move to safer shelters to rest, and resting patterns also appear to vary between species.

Meanwhile, during the winter season, late November to February, the average air and water temperatures are below 10 °C and 8 °C, respectively, and fish hide or burrow into the substrate. Due to these actions, it is likely that few individuals are moving around in the water, which leads to less participation in feeding behaviour. Fish thus have great difficulty seeking detritus under such conditions. Moreover, the empty stomach rate was higher than in other seasons. In the winter, other cobitid fishes rarely feed on prey to conserve energy (Kim et al. 2006). On exposure to low ambient temperature, the brown bullhead (*Ictalurus nebulosus*) and the largemouth bass (*Micropterus salmoides*) exhibit winter dormancy, a sleep-like state that leads to metabolic shutdown (Crawshaw 1984).

Based on field research, *I. hugowolfeldi*, a benthic filter feeder, has specific behaviours closely related to its habitat, including bottom substrate, and showed various types of feeding and resting behaviours. These approaches for this fish may play a fundamental role in protection from water pollution and reckless artificial development of the habitat.

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