

カマキリCottus kazikaの発音特性と発音筋の季節変化

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Sound Production in Fourspine Sculpin *Cottus kazika*, Cottidae: Sound Properties and Seasonal Variations of Sonic Muscle Size

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Abstract: Sound characteristics and the sonic muscle size in the catadromous fourspine sculpin *Cottus kazika* were examined in this study. The sound produced by this fish is composed of a series of 5 pulses lasting for 40–60 ms, with the peak frequency at approximately 100 Hz. The sonic muscle originates from the posteroventral surface of the cranium and inserts on the anterodorsal margin of the cleithrum. The sonic muscle somatic index (SMSI) value evidently indicated that the male sonic muscle was larger than that of the female throughout the year in both the wild and reared fish. In the wild fish, the mean SMSI value was 1.5 times larger in male's than in female's. Further, the mean SMSI value of the wild male fish significantly increased during the spawning season (December to March), suggesting reproductive phase related control of the sonic muscles size.

Key words: Fourspine sculpin; Sonic fish; Sonic muscle; Wild and reared fish

The fourspine sculpin *Cottus kazika* (Cottidae, Scorpaeniformes) is a catadromous fish with no swimbladder and endemic to Japan, being distributed from Akita prefecture to Kyushu. Although the sculpins are designated as a protected species, the numbers of these fish saliently tend to decrease in the rivers in Kyushu and Shikoku islands as well as in the Pacific Ocean (Japanese Environment Agency 1998). Therefore, stocking of the artificially produced fry and research on the ecology of this fish is being performed to recover the resource (Miyamoto 1997; Ueno et al. 1998).

In Fukui prefecture, the fish is an economically important indigenous product. Recently, the fish resource has also been in a downward trend as in other prefectures. Consequently, artificial seeding production and culture of this fish started in 1988 for the recovery of this important resource.

Knowledge on the ontogeny and ecology of a fish is fundamentally important to establish appropriate strategies for artificial seeding and subsequent aquaculture of the species. Several reports on such knowledge in the fourspine sculpin are available, developmental changes in body shape from juvenile to fry transition, and the days after hatching or size when the life style switches from planktonic to benthic (Sugita et al. 1995). Growth processes of juvenile and fry during the sea life and the body size in the fish of anadromous stage are also examined (Harada et al. 1999; Kinoshita et al. 1999). Regarding reproductive ecology, the female fourspine sculpin is known to spawn on tidal zone of a rock (Takeshita et al. 1998). The male fish, with the mouth turned to red color, forms a territory on the spawning season, and guards the spawned eggs (Kuroda 1947; Watanabe 1958). However, little is known on the precise

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processes that occur during the natural spawning and protection of spawned eggs with the male of this species. We observed the fish emits sounds upon artificial fertilization. It is well known that some foreign sculpins are soniferous. They have a pair of sonic muscles and produce sounds by the rapid contraction of these muscles (Barber and Mowbray 1956; Fish et al. 1952). It has been suggested that the sounds are involved in not only for agonistic behavior but also reproductive behavior (Connaughton et al. 1997; Ladich 1989, 1990; Whang and Janssen 1994). Although it is considered that the sounds of the fourspine sculpin are concerned with the spawn ecology, no investigations have been performed on sonic system in the fish. In the present study, as a first step to understand the reproductive ecology of fourspine sculpin from the light of vocalization, we analyzed the physical properties of the sound, seasonal (monthly) size variations of the sonic muscle, and the morphology of the sonic system.

Materials and Methods

Materials

The wild fourspine sculpins were caught with the net (called Ado ami) from November 2001 to January 2002 in Kuzuryu river (Fukui pref.). Some were also fished between July and August in Mimi river (Fukui pref.) in 2001 and 2002. The caught fish were maintained in aquaria filled with filtered freshwater at 12–17°C and fed with an artificial food (Mash for eel, Nihon-Haigoshiryo Co.).

The reared fish were 1–3 years old. They were raised from the larvae stage, hatched by artificial fertilization, wild and reared fish were used as parents, fed with rotifer *Brachionus plicatilis* during 0–50 days after hatching, and

with artificial mash (MF-2, 3, Riken vitamin Co.) from 20 days after hatching. They were maintained in aquaria filled with filtered seawater at 10–12°C until 100 days after hatching, and then were transferred to aquaria filled with filtered freshwater at 12–17°C. The under 1-year-old fish were fed with artificially crumble formula food (Ayu No.1 and No.3, Nihon-Nosan Co.), and the over 1-year-old fish were fed with blended pellet food (Flounder No. 1 and No. 3, Higashimaru Co.). Fish were reared in circular tanks (FRP, 1.5 m²).

Sound recording and analysis

The sounds were recorded in a soundproof room in January. Fish were carried from the rearing tank to this room with a bucket (15 l) and were taken up to a plastic case by hands, and we waited for the sounds of this fish at about 10°C. The wild and reared fish were recorded for sounds in air. The sounds emitted by the wild fish were obtained through a plain-talk microphone (unidirectional electret microphone, sensitivity: -9.5 dBV ± 5 dBV at 1.0 kHz relative 1.0 V/Pa) and recorded with the hard disk of a personal computer (Macintosh, sampling rate = 44.1 kHz). The microphone was placed within 5 cm from the fish. The recorded sounds were downsampled to 11 kHz and analyzed for frequencies, waveforms and sound intervals with Sound Edit Pro (Macromedia, Inc.). Sonograms and Frequency distributions were calculated using fast Fourier transform.

Sonic muscle somatic index (SMSI)

The fish ($n=583$) were euthanized with an overdose of tricaine methanesulfonate (MS222), then fixed and preserved in 10% formalin for about a month. Total length, body weight and gonad weight were then measured. The

Table 1. Fourspine sculpin fish samples used in this study

Month		Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Wild fish	♂	12	14	2	2	2	2	8	14	4	11	9	11
	♀	7	8	2	4	4	6	5	3	5	5	5	23
Reared fish	♂	5	29	20	3	11	4	18	35	42	28	19	16
	♀	10	24	27	12	16	13	6	10	13	30	11	13

bilateral sonic muscles were also removed and weighed. To control for the variation in body size, sonic muscle-somatic index (SMSI) was calculated: $(\text{total sonic muscle mass} / \text{total body mass}) \times 100$ following (Connaughton et al. 1997). SMSI was compared among four groups: wild males ($n=91$), wild females ($n=77$), reared males ($n=230$) and reared females ($n=185$). The values of SMSI in each group were sorted per sampling month, and mean values were calculated for each month. In some specimens ($n=325$), Gonad-somatic index (GSI) was also calculated as $(\text{gonad mass} / \text{total body mass}) \times 100$. The samples size is shown in Table 1.

Morphology of the sonic system

From the fish used in studying the SMSI, some specimens were also used to investigate the morphology of the sonic system. The fish were dissected under a stereomicroscope before the sonic muscles were removed. The origin and insert of the sonic muscle were determined by carefully removing the connective tissue. The nerves innervating the sonic muscle were stained by 1% osmic acid and traced back to the stem root to identify the peripheral nerve that innervates the sonic muscle. Definition of the peripheral nerves is after Parenti and Song (1996) as modified by Onuki and Somiya (2007).

Statistical analysis

Differences in SMSI among the four groups calculated for each month were analyzed with student *t*-test and Tukey-test (considered statistically significant with $P<0.05$). Groups showing unequal variance were re-checked with Welch-test. Regression lines were as determined with least squares method, and were analyzed with *F*-test for as statistically significant differences ($P<0.05$). Correlation index was also calculated using *F*-test for statistically significant differences ($P<0.05$).

Results

Sound analysis and sonic muscle characteristics

The fourspine sculpin produced sounds in

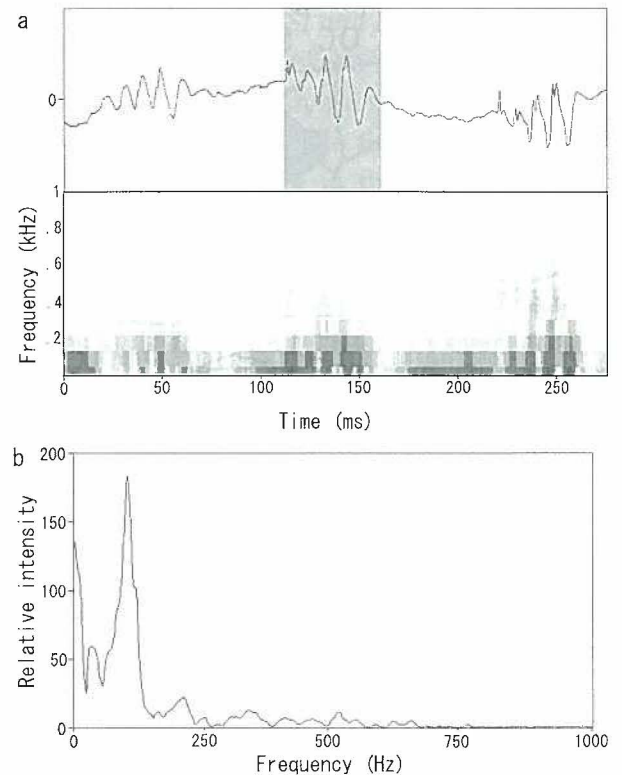


Fig. 1. Sound characteristics of the fourspine sculpin. Representative oscillogram and sonogram (a), and frequency distribution (b). Gray area in a indicates rough duration of a knock.

air, accompanied with the abduction of the gill cover. Two types of sounds were observed, single knock and trains of knocks. The trains of knocks were composed of repeated “single knocks” with about 50 ms intervals (Fig. 1a). The sounds of wild fish were easily recorded in air when they were handled in the laboratory. However, the sounds of reared fish were scarcely recorded, and even if recorded they were too feeble to be analyzed further. Sounds were recorded from 3 males and 4 females of the 35 wild fish caught in July 2001. A representative sonogram, oscillogram and frequency distribution of the three knocks emitted by a female fish (TL, 111 mm) are shown in Fig. 1. The knock consists of a series of 5 pulses, with the duration ranging from 40 to 60 ms (Fig. 1a). The duration of single pulses were approximately 10ms. The main frequencies of the sound ranged between 50 and 150 Hz, with the peak at about 100 Hz (Fig. 1b). No sexual differences were detected in the physical properties of the sounds, sonogram, oscillogram and frequency distribution, produced by these fish.

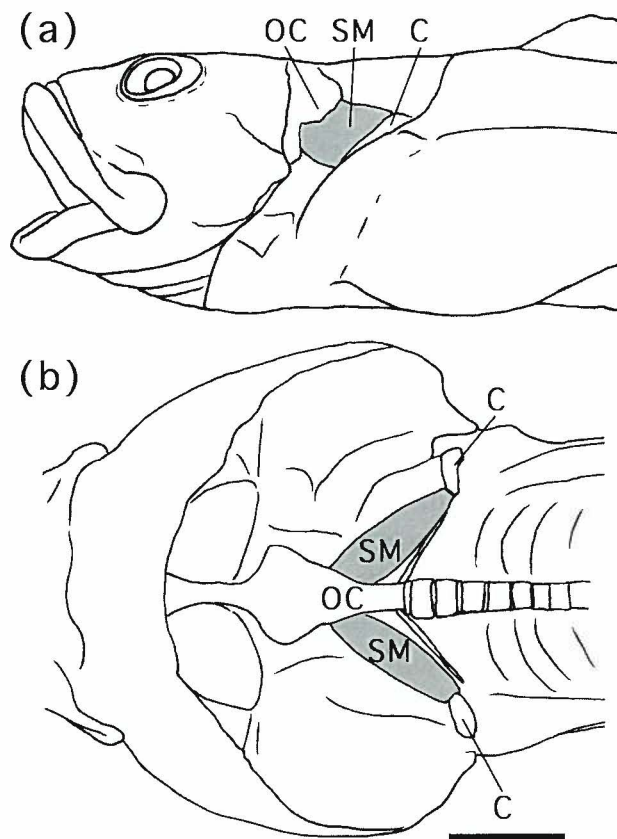


Fig. 2. Lateral (a) and ventral view (b) of the sonic muscles of the fourspine sculpin (TL = 17.6cm, male). SM, sonic muscle; OC, occipital region of cranium; C, cleithrum. Scale bar = 1 cm.

Morphology and innervation of the sonic muscles

The sculpin possesses a pair of sonic muscles originating from the ventral side of the basi-occipital and inserted on the anterior edge of cleithrum (Fig. 2). The nerve innervating the sonic muscle emerged from a foramen in the occipital bone and was identified as the occipital nerve (Fig. 3). Branches of the occipital nerve innervated the sonic muscles, while there was no sign of spinal innervation.

Sonic muscle size and SMSI

The scatter diagrams for the total length (TL, mm) and the sonic muscle weight (g) of the wild and reared fish in both sexes are shown in Fig. 4. This figure indicates that the male sonic muscle was heavier than that of the female. Relationship between SMSI and the total length, that was female and male fish each reared and wild fish, was constant. The scatter diagrams between the TL (mm) and SMSI

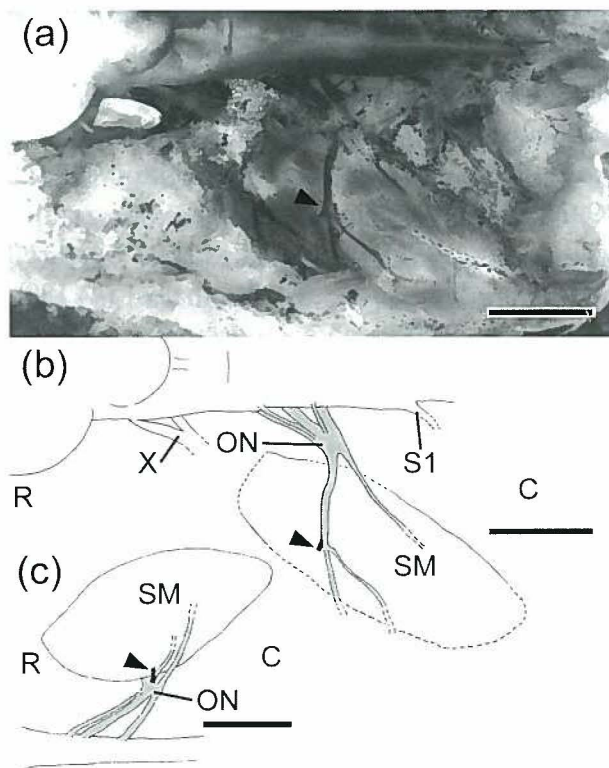


Fig. 3. Occipital nerve innervation of the sonic muscle of the fourspine sculpin. Photograph (a) and diagrammatic drawing (b) showing dorsal view of innervation. A sonic nerve branch (arrowhead in a&b) of occipital nerve enters into the sonic muscle from its dorsal surface. Diagrammatic drawing (c) showing ventral view of innervation. A sonic nerve branch (arrowhead in c) of occipital nerve enters into the sonic muscle from its ventral surface. Terminals of occipital nerve branches indicated by dashed lines innervate lateral trunk muscles. C, caudal; ON, occipital nerve; R, rostral; S1, first spinal nerve; SM, sonic muscle; X, vagal nerve. Scale bars = 5 mm.

in the wild and reared fish (in both sexes) are shown in Fig. 5. In the wild fish (Fig. 5a), sexual dimorphism is evident in the SMSI. SMSI of males is approximately 1.5 times greater than that of females at any length examined (Fig. 5a). In the reared fish (Fig. 5b), sexual dimorphism, evidenced in the sonic muscle size, was recognized only for larger fish (TL, 125–200 mm).

Seasonal (monthly) changes in SMSI

Seasonal changes of the SMSI are shown in Fig. 6. The SMSI values of the males were predominantly higher than those of the female throughout the year in both wild and reared fish. In the wild male fish, the SMSI values start to increase in December, reach a peak in

March, and then decrease significantly in April. While in the reared male fish, no prominent increase in SMSI was observed, in the female, there were slight seasonal changes in both the wild and the reared fish.

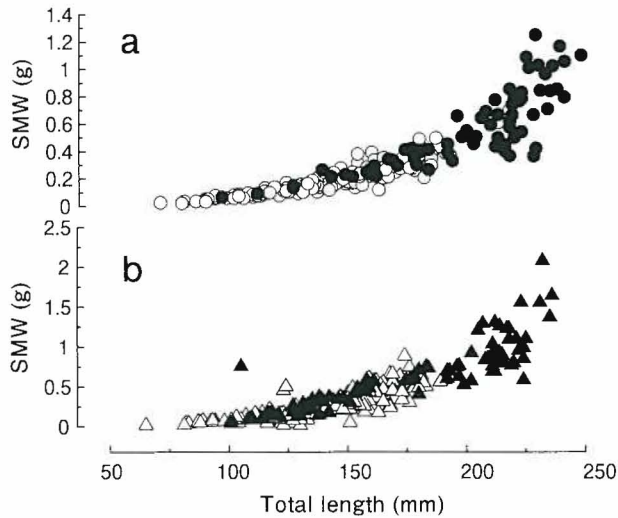


Fig. 4. Relationship between total length and sonic muscle weight (SMW) in the fourspine sculpin. Each correlation is sufficiently significant ($P < 0.05$). a, female (●, wild fish; $r = 0.924$; $SMW = 1.92E - 0.07 \times TL^{2.792}$; ○, reared fish; $r = 0.956$; $SMW = 1.03E - 0.07 \times TL^{2.897}$); b, male (▲, wild fish; $r = 0.919$; $SMW = 7.15E - 0.08 \times TL^{3.061}$; △, reared fish; $r = 0.865$; $SMW = 8.75E - 0.09 \times TL^{3.452}$).

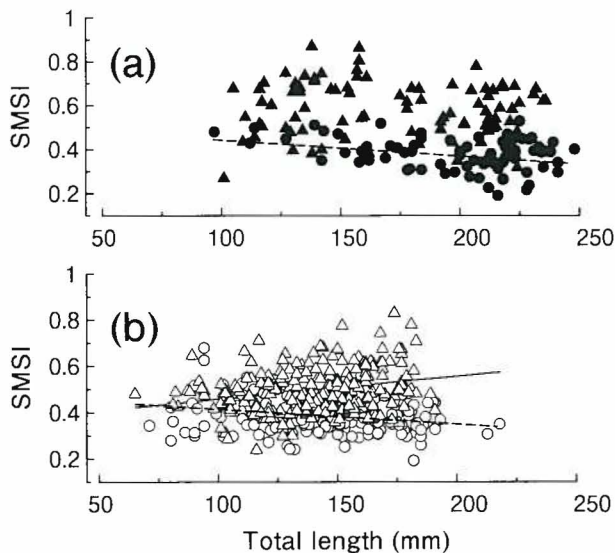


Fig. 5. Relationship between sonic muscle somatic index (SMSI) and total length in the fourspine sculpin. (a), wild fish (●, female; $r = -0.336$; ▲, male; $r = -0.106$), (b), reared fish (○, female; $r = -0.224$; △, male; $r = 0.256$). Sufficiently significant correlation ($P < 0.05$) was recognized in the female of wild fish and reared fish (broken line) and male of reared fish (solid line).

Relationship between GSI and SMSI

Scatter diagrams between GSI and SMSI in the male and female fish are shown in Fig. 7. In both wild and reared male fish significant correlations were detected in the indices (F -test, wild fish: $n = 36$, $r = 0.374$, $P < 0.05$; reared fish: $n = 107$, $r = 0.378$, $P < 0.05$). In the males, there was a tendency of increasing SMSI values with increased GSI. In the female, however, no significant correlation was observed (F -test, wild fish: $n = 57$, $r = 0.041$, $P > 0.05$; reared fish: $n = 125$, $r = -0.024$, $P > 0.05$).

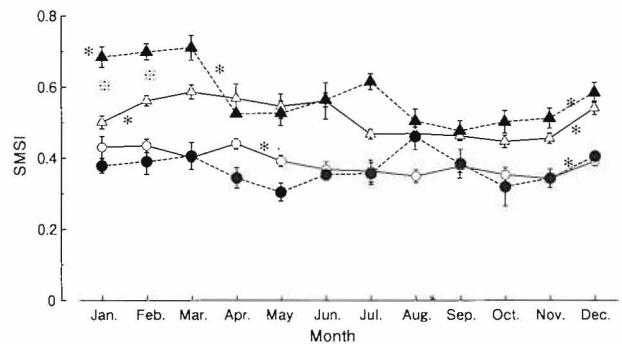


Fig. 6. Seasonal changes in sonic muscle somatic index (SMSI) in the fourspine sculpin. Values are mean $\pm$ SEM. ●, wild female; ○, reared female; ▲, wild male; △, reared male. * means significant differences among the last month and the following month ($P < 0.05$). ** means significant differences between the reared male and the wild male ($P < 0.05$).

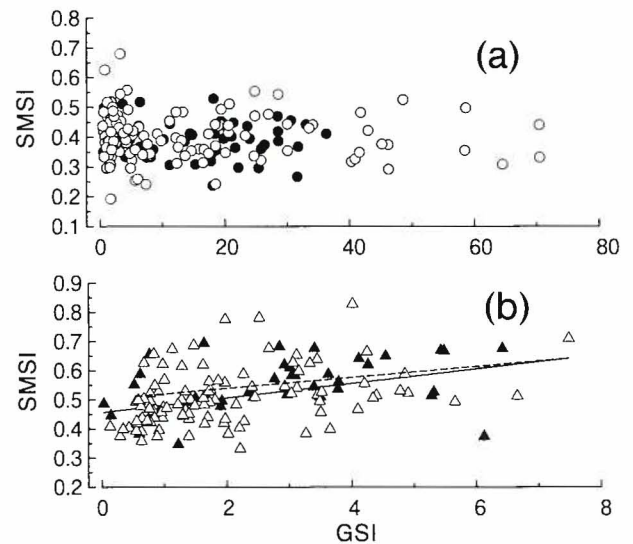


Fig. 7. Relationship between gonad somatic index (GSI) and sonic muscle somatic index (SMSI) in the fourspine sculpin. (a), female (●, wild fish; $r = 0.041$; ○, reared fish; $r = -0.024$); (b), male (▲, wild fish; $r = 0.374$; △, reared fish; $r = 0.378$). Sufficiently significant correlation ($P < 0.05$) was recognized in the male of wild fish (broken line) and reared fish (solid line).

Discussion

Sonic system and sound analysis

In the present study we found that the four-spine sculpin possesses a pair of sonic muscle as in other sculpins examined for far (see Introduction). Unlike the sonic muscle in many sonic species that inserts to the swim bladder, the sonic muscle of the four-spine sculpin inserts to the bone (cleithrum). This appears to be a conserved trait in sonic sculpins. The sonic muscle is innervated by the occipital nerve as in other fishes of Scorpaeniformes, *Prionotus* (Evans 1973), *Leptococcus* (Bass and Baker 1991). Involvement of the occipital nerve in the sound production may be a common character in Scorpaeniformes.

In this study, we found that the sounds of four-spine sculpin are single knock and trains of knocks, and the main frequency of the single knocks was about 100 Hz with a duration of about 50 ms. In the case of the river bullhead *Cottus gobio* the frequency of the single knock was between 50 and 500 Hz with an average duration of about 50 ms (Ladich 1989). Similarly, we suppose that four-spine sculpin would produce sounds in agonistic behavior. Three kinds of sounds in mottled sculpin *C. bairdi* were observed and these kinds of sounds were distinguished from the behavior (Whang and Janssen 1994). Because we observed the sounds of single knock and trains of knocks in four-spine sculpin in this study, these kinds of sounds were no distinguished from the behavior. So we would like to develop the relationship between these kinds of sounds and the behavior.

Sexual dimorphism in sonic muscle size

The sonic muscle of males was about 1.5 times greater than that of females throughout the year as assessed by SMSI. Sexual dimorphism in sonic muscle size has been described in a number of families, including the Batrachoididae (Fine et al. 1977; Fine 1997; Park et al. 1994), Gadidae (Templeman and Hodder 1958; Park et al. 1994), Ophidiidae

(Courtenay 1971), Macrouridae (Jones and Marshall 1953), Pempheridae (Takayama et al. 2003) and Sciaenidae (Connaughton et al. 1997). This is the first report that Cottidae also exhibits sexual dimorphism in sonic muscle size. The sculpin is now added to the growing list of sexual dimorphism in fish sonic muscles.

Seasonal changes in sonic muscle size

The sonic muscle of the wild male significantly increased in December, reached a peak in March, and declined in April. The breeding season of the sculpin is from December to March (Takeshita et al. 1998). It is known that the GSI of males shows seasonal changes similar to those of SMSI (Iwatani 2006) and SMSI shows significant correlation with the GSI (present study). Parallel increases in the sonic muscle size, therefore, suggest that the muscle plays important roles in reproduction. In the breeding season, the sculpin builds nest, attracts a female for mating and guards nest with eggs against predators (Takeshita et al. 1998). Sounds may be utilized for such territorial, courtship, and aggressive behavior. Janssen and his colleagues (Janssen 1990; Whang and Janssen 1994) described the sound produced by the mottled sculpin as "the vibrational communication system". This description is very reasonable because the sculpin has no swimbladder and hear the sounds or vibrations with the otolith or the lateral line. The four-spine sculpin may also have the same vibrational communication system as pertains the mottled sculpin. Whang and Janssen (1994) further discussed that sound or vibration may provide information on size of the fish. Indeed, in the case of the mottled sculpin, the female chooses their mates based on size (Downhower and Brown 1980; Downhower et al. 1983). In the four-spine sculpin, the hypertrophy of the male sonic muscle only in the breeding season also suggests the sexual selection. Possibly, the louder acoustic display by male during the breeding season may have fitness benefit, by reinforcing threat displays to other males as well as attracting and communicating spawning readiness to the female (Rowe 2004).

As mentioned above, the sound production of the fourspine sculpin may have critical roles in their reproductive success. The resources of sculpins, despite their being designated as a protected species in Japan, are still decreasing. We are currently trying the artificial seedling production and culturing, but the less developed sonic muscle in the cultured male compared to the wild male during the breeding season is one big challenge. Reproductive success of the cultured fish in relation to their bioacoustics will be the target of our future research.

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カマキリ *Cottus kazika* の発音特性と発音筋の季節変化

岩谷芳自・大貫敦嗣・宗宮弘明

降河回遊魚であるカマキリ (*Cottus kazika*) の鳴音および発音筋について検討した。鳴音は雌雄差がなく連続する5パルスからなり、継続時間は40~60ミリ秒で、周波数は50~150 Hz でピークは100 Hz であった。発音筋は、頭骨の後腹側の表面から上鎖骨の耳石背部の縁に付着して存在する。発音筋体指数は、天然魚および養殖魚において雄魚が雌魚より周年高く、天然の雄魚は産卵期である12月から3月に高くなることが分かった。