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Spawning Habits and Reproductive Isolating Mechanism of Two Closely Related River-Sculpins, *Cottus amblystomopsis* and *C. nozawae*

Akira Goto

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Abstract The spawning habits of two closely related river-sculpins, *Cottus amblystomopsis* and *C. nozawae* were compared. The spawning period partially overlapped between the two species though *C. amblystomopsis* began to spawn slightly before *C. nozawae*. There were no significant differences between the two species in micro-environmental conditions of the nest sites, such as the depth of water, current velocity, bottom substratum and the size of stones used as the nest. However, the spawning area in the course of the rivers was clearly geographically separated according to the species in most rivers studied; *C. amblystomopsis* spawned in the lower course of the river, while *C. nozawae* spawned in the upper course, distinctly apart from the spawning area of *C. amblystomopsis*. No noticeable differences were revealed between the two species in the pattern of spawning behavior, or the spawning time within the day. However, in mate preference tests, no mating occurred in heterospecific combinations between the two species, though conspecific combinations succeeded in mating in both species. From the present results, it appears that the two closely related species are reproductively isolated from each other, and it is suggested that geographical and ethological isolation may serve as important devices as isolating mechanisms.

The river sculpin, *Cottus nozawae* is one of the most common freshwater fish in Hokkaido, Japan, and is characterized as a nest spawner whose eggs are deposited on the under side of stones in the rivers during the spawning season (Okada, 1936; Goto, 1978). Recently, Goto (1975 a, b; 1977) found that there were two types of *C. nozawae* which were different from each other in ecological and morphological characteristics. They were named, for convenience, as the small-egg type and the large-egg type respectively. The small-egg type inhabits mainly the lower course of rivers and spawns a large number of small-sized eggs. In contrast, the large-egg type lives mainly in the middle and upper courses of the river and spawns a small number of large-sized eggs which produce well-developed benthic larvae. The ranges of geographic distribution of the two types broadly overlap in southern and eastern Hokkaido (Goto, 1980). Throughout the studies on morphology and life history of these two types of *C. nozawae*, Goto (1980) presumed that the two types are different species and systematically the small-egg type should be identified as *Cottus amblystomopsis*, first reported by Schmidt (1904) from Sakhalin, and the species named *C. nozawae* should be applied only to the large-egg type.

It is very important to clarify whether or not reproductive isolation exists between these two species, *C. amblystomopsis* and *C. nozawae*, in conditions of cohabitation, for this aspect is a key in deciding whether they are independent biological species or intraspecific variations. Up to date, however, there has been no study on reproductive isolating mechanisms not only between the two species of *C. amblystomopsis* and *C. nozawae*, but also among the other sympatrically occurring species of the genus *Cottus*, though some works have been done in some other freshwater fishes such as cichlids (Liley, 1966), threespine sticklebacks (Hagen, 1967), sunfish (Clark and Keenleyside, 1967; Steele and Keenleyside, 1971; Keenleyside, 1978) and dace (Bartnik, 1970).

In the present study, the spawning period, spawning area and the condition of nest sites of the two species, *C. amblystomopsis* and *C. nozawae*, were clarified by field observations. Observations on spawning behavior and mate preference were also carried out in the experimental aquarium, in order to show the reproductive isolating mechanism of the two species.

Study area and methods

Study area. The three study sites, the Hekiriji, the Ryukei and the Daitobetsu rivers, originate on Oshima Mountain in Hokkaido and drain into the Tsugaru Strait (Fig. 1). They are characterized by relatively high gradients and short length, about 24 km, 6 km and 17 km respectively. There are notched weirs approximately 3 km up from the river mouth in the Hekiriji River and about 2.5 km up from the river mouth in the Ryukei River. The water temperature of these rivers fluctuates from 1° to 25°C throughout the year.

These three rivers contain varied reach-types classified by Kani (1944). The Hekiriji and Ryukei rivers consist of Bb-Bc, Bb, Aa-Bb and Aa types. On the other hand, the Daitobetsu River is characterized by lacking the Bb-Bc type in the lower course when compared with the other two rivers. Bottom conditions in each river vary from unstable muddy and gravely sand to stable gravel or boulders.

Two other rivers studied for longitudinal distribution of the two species in the summer of 1979, the Tokachi River and the Saru River, are 156 km and 104 km long respectively and drain into the Pacific Ocean (Fig. 1).

Time and place of spawning. From December in 1976 to June in 1977, adult fish of the two species were collected from the Hekiriji River to acquire information on the development of gonads. Collections were made with dip nets or casting nets. Fish collected were preserved in 10% formalin, and measured for standard length, body weight and gonad weight in order to calculate the gonad index (G. W./S.L.^{3} × 1000).

During every spring from 1974 to 1978, extensive searching to locate newly deposited egg-clusters and gravid females having fully ripe eggs was carried out in the three rivers, to establish the spawning period more exactly, the range of spawning ground and the spawning site. When the deposited egg-clusters were found, the following measurements were made at the nest site: depth of water, current velocity and size of the stones with egg-clusters.

Spawning behavior and mate preference tests. In the spring of 1979 and 1980, mature fish of the two species captured in the Hekiriji River were kept in a stream at the Nanae Fish Culture Experimental Station of Hokkaido University.

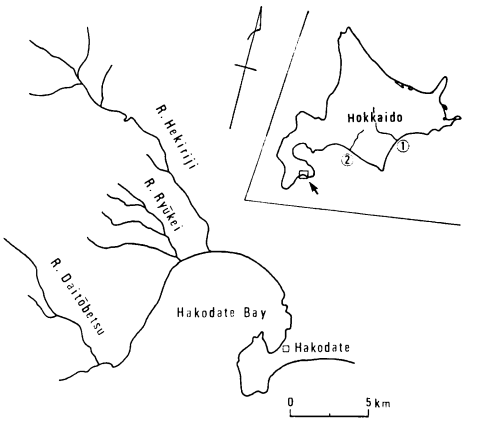

Fig. 1. Location and map of the rivers examined. ①, Tokachi River; ②, Saru River.

Then they were transferred into an aquarium placed on the bed of a streamlet flowing through the Station, to observe their spawning behavior and to test for heterospecific mating between the two species. The aquarium apparatus used for the observation was the same as that described in a previous paper (Goto, 1982).

In 1979, 2 males and 9 females of *C. amblystomopsis* were transferred from the holding corf to the experimental aquarium (Table 1). After 8 days, 9 females of *C. amblystomopsis* were replaced with 5 females of *C. nozawae*. In the aquarium, therefore, 2 males of *C. amblystomopsis* and 5 females of *C. nozawae* were raised. They were kept in the aquarium for 4 days, then the two males of *C. amblystomopsis* were taken out from the aquarium. Immediately after, 3 males of *C. nozawae* were transferred into the aquarium in order to make a conspecific combination.

In 1980, the mate preference tests were repeated, but the hetrospecific combination was reversed in sex to that in 1979 (Table 1).

Observations were made everyday in the morning, from 04:00 to 07:00 hrs, and occasionally at other times of the day, during the experimental periods.

Results

Spawning period. In 1977, the gonad indexes of two species collected from the Hekiriji River reached a peak during the period from late March to mid April in female *C. amblystomopsis* and in

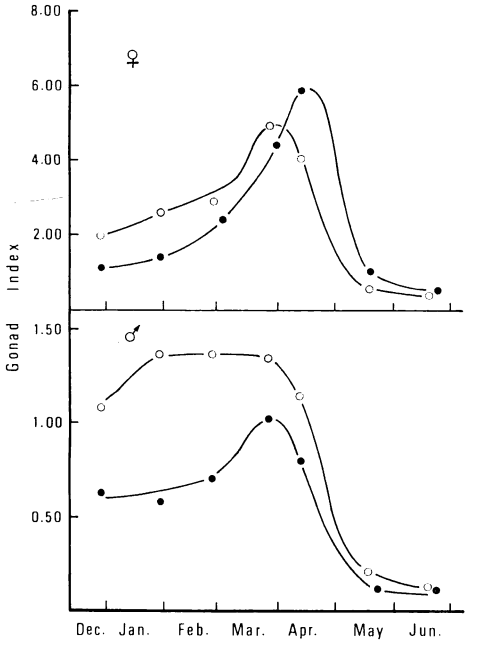

Fig. 2. Monthly changes of gonad index (G. W./S.L.^{3}×1000) in adults of the two species of *Cottus* in the Hekiriji River from 1976 to 1977. ○, *C. amblystomopsis*; ●, *C. nozawae*.

both sexes of *C. nozawae* from mid April to early May (Fig. 2). Although *C. amblystomopsis* reached a peak in the gonadal development about 2 weeks earlier than that of *C. nozawae*, a slight overlap in the peak between the two species still existed. This result may suggest that the spawning seasons of the two species partially overlap, because spawning commences either during, or just after, the time when the species reaches the maximum gonadal development.

Further evidence of such overlap for the spawning period was provided by the discovery of newly deposited egg-clusters found on the undersurfaces of stones and of females with fully ripe eggs. In both the Hekiriji and Ryukei rivers, nests with deposited egg-clusters were first found in mid-April in *C. amblystomopsis* and late April in *C. nozawae* in each year from 1976 to 1978 (Table 2). Females having fully ripe eggs were collected finally in early May in *C. amblystomopsis* and mid-May in *C. nozawae*.

From the results, the spawning period may range from mid-April to early May in *C. amblystomopsis* and from late April to mid-May in *C. nozawae*, though a little fluctuation may exist year by year. It is evident, therefore, that there is a considerable overlap in the spawning period of the two species.

Spawning area. The spawning areas of the two species are shown along the course of the three rivers (Fig. 3). In the Ryukei River and Daitobetsu River, the spawning areas of the two species were clearly separated from each other; *C. amblystomopsis* spawned in the lower course within approximately 3 km from the river mouth, while *C. nozawae* spawned in the upper course, completely separated from the spawning area of *C. amblystomopsis*. On the other hand, the spawning areas of the two species partly over-

Table 1. Number and body size of individuals of the two species of *Cottus* introduced into an aquarium. CA, *Cottus amblystomopsis*; CN, *C. nozawae*.

Date transferred	Species	Sex	Number of individuals	Standard length (mm)
Apr. 26, 1979	CA	Male	2	140.9, 159.6
	CA	Female	9	72.4～103.6
May 4. 1979	CA	Male	2	140.9, 159.6
	CN	Female	5	82.5～98.0
May 7, 1979	CN	Male	3	105.1～118.8
	CN	Female	5	82.5～98.0
Apr. 26, 1980	CA	Male	2	126.4, 149.9
	CA	Female	4	87.4～117.5
May 3, 1980	CN	Male	3	111.2～121.3
	CA	Female	4	87.4～117.5
May 9, 1980	CN	Male	3	111.2～121.3
	CN	Female	5	90.3～103.7

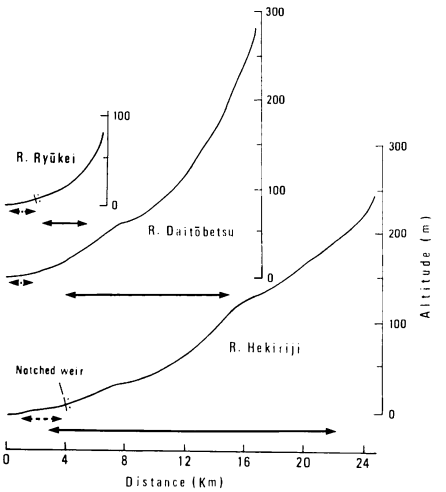

Fig. 3. Longitudinal distribution of spawning nests of the two species of *Cottus* in three rivers. Broken lines show distribution area in *C. amblystomopsis*; Solid lines show distribution area in *C. nozawae*.

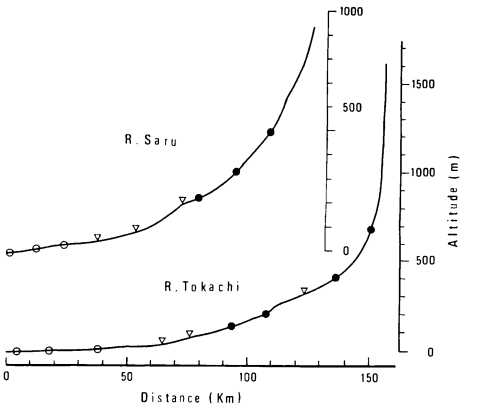

Fig. 4. Longitudinal distribution of the two species of *Cottus* in the Tokachi River and Saru River from August to September, 1979. ○, collecting sites of *C. amblystomopsis*; ●, those of *C. nozawae*; ▽, sampling sites where sculpins were not collected.

lapped in the Hekiriji River; the overlapping area was approximately 1 km long and located downstream from the notched weir in the lower course.

In addition, the distribution of the two species was also observed along the course of the other two rivers, though the spawning area was not checked (Fig. 4). In the Tokachi River, in the summer of 1979 *C. amblystomopsis* was distributed in the lower course within about 40 km from the river mouth, while *C. nozawae* inhabited the upper course, more than 90 km from the river mouth. The two species in the Saru River were also clearly separated from each other, as in the Tokachi River. These facts suggest that the spawning areas of the two species are separated from each other in these two rivers, because the adult fish carry on a sedentary life and never migrate far throughout the year.

Location of spawning nests. Deposited egg-clusters of both species were located mainly at

Table 2. Records on the date of the first observation of the egg-clusters and the last finding of mature females in the two species of *Cottus*.

	First egg-cluster	Last mature female
C. amblystomopsis		
R. Hekiriji	Apr. 9, 1976	May 10, 1976
	—	May 12, 1978
	Apr. 19, 1979	—
R. Ryukei	Apr. 22, 1975	May 10, 1975
	Apr. 12, 1976	May 6, 1976
	Apr. 20, 1978	—
C. nozawae		
R. Hekiriji	Apr. 30, 1975	May 21, 1975
	Apr. 28, 1976	May 14, 1976
	—	May 20, 1977
	May 4, 1979	—
R. Ryukei	Apr. 29, 1975	May 13, 1975
	Apr. 28, 1978	May 14, 1978
	Apr. 30, 1979	—

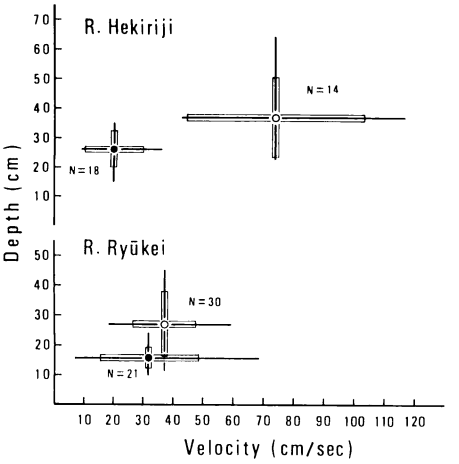

Fig. 5. Current velocity and depth of water at the spawning nests of the two species of *Cottus* in the Hekiriji River and Ryukei River in 1978. Numbers indicate the number of nests measured; thin lines indicate ranges and bold lines show standard deviations. ○, means in *C. amblystomopsis*; ●, those in *C. nozawae*.

the Hirase-rapid in the spawning area. In the Hekiriji River, the values (Mean±SD) of the water depth and current velocity in the spawning sites were 36.9±13.6 cm and 73.9±29.3 cm/sec in *C. amblystomopsis*, and in *C. nozawae* 26.0±13.6 cm and 19.7±9.9 cm/sec respectively (Fig. 5). In the Ryukei River, the water depth was 26.8±10.9 cm and the velocity was 37.1±10.5 cm/sec in *C. amblystomopsis*, and 15.6±3.5 cm, 31.8±16.6 cm/sec in *C. nozawae*. As shown in Fig. 5, the conditions of the water depth and current velocity at the spawning site were distinctly different between the two species in the Hekiriji River, whereas in the Ryukei River there was much overlap in these conditions. As for bottom substratum at the nest sites, no differences were revealed between the two species; both species spawned on the sandy gravel bottom or gravel bottom. The stones with egg-clusters were generally longer in axis than 20 cm in the both species, and considerable overlap in size existed between them (Fig. 6).

Thus, the micro-environmental conditions of the nest sites did not play an important role as the reproductive isolating mechanism between the two species.

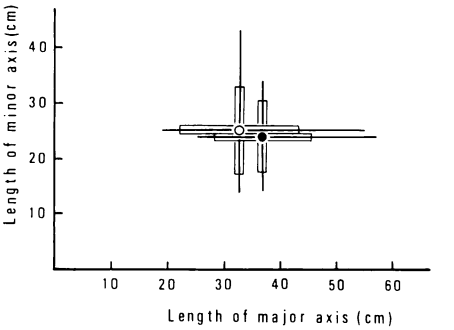

Fig. 6. Size of stones, on which egg-clusters were laid, in the Hekiriji River in 1978 Numbers, symbols and lines indicate the same as Fig. 5.

Mate preference tests. Spawning occurred three times in the aquarium where both sexes of *C. amblystomopsis* were introduced on April 26, 1979 (Fig. 7). One male, which was dominant over the other male and occupied the cavity beneath a stone, mated with three different females immediately after daybreak on April 30, May 1 and May 2 respectively. From May 4 to May 9, however, no spawning occurred in the aquarium containing two males of *C. amblystomopsis* and five females of *C. nozawae*, though they were fully matured. On the afternoon of May 9, two males of *C. nozawae* were introduced into the aquarium after removing two males of *C. amblystomopsis*. On the next morning, May 10, courtship behavior in a pair of *C. nozawae* was observed, though spawning did not follow. The first spawning occurred immediately after daybreak on May 11, and the second and the third spawnings were observed on May 12 at almost the same time of day as the first spawning.

In 1980, one spawning (April 30) occurred in the conspecific combination of *C. amblystomopsis*, and spawning was counted four times in the pair of *C. nozawae* (one time on May 11 and May 14, two times on May 12). On the other hand, no spawning occurred in the heterospecific combination of male *C. nozawae* and female *C. amblystomopsis* during a period from May 3 to May 9 (Fig. 7).

Spawning behavior. No noticeable differences in the pattern of spawning behavior and the spawning time of day were revealed in the conspecific mating of two species. In both species, fish began to move actively just after daybreak.

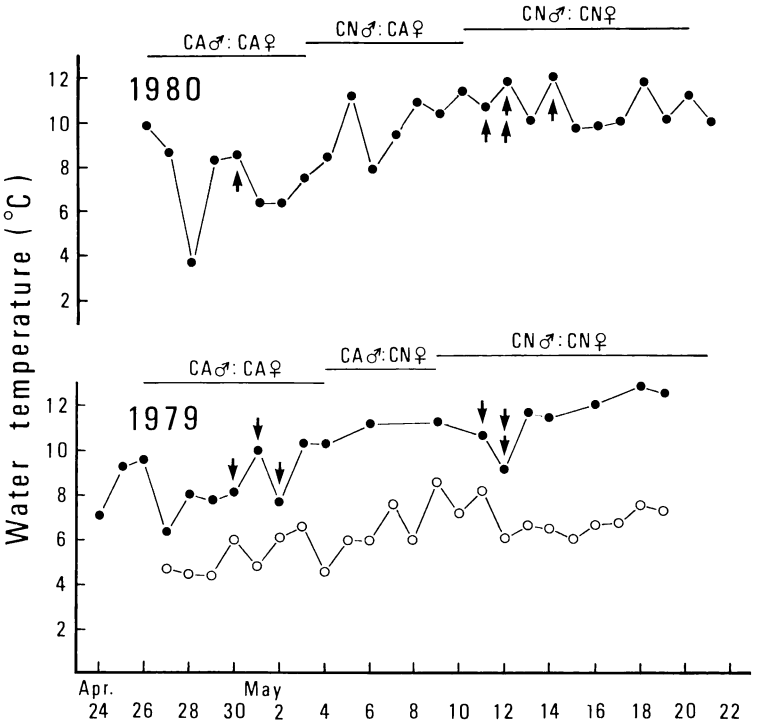

Fig. 7. Diagram showing the spawning success or failure in the artificial conspecific and heterospecific combinations of the two species of *Cottus*. Arrows indicate the spawning occurrence. CA, *C. amblystomopsis*; CN, *C. nozawae*.

A dominant male dashed from the shelter and approached a female when she came into sight. Then the male escorted her into the shelter and lay laterally with her. They maneuvered for about half an hour, then the female turned upside down to the under side of the stone. The male soon followed her, and in the inverted position he lay with his head underneath her body and with his body twisted so that his tail lay alongside hers. After a few minutes the male shivered his body very rapidly and simultaneously the female deposited her eggs on the ceiling of the nest. Then the spent female swam away from the nest, though the male remained in the nest in an upright position. The sequential spawning behavior finished when the spent female left the nest. The spawnings observed in the present study occurred between 4: 30 and 6: 30 a.m. in both species. Spawning behavior was almost the same as that described already in *C. nozawae* (Goto, 1982).

In both cases of heterospecific combination between male *C. amblystomopsis* and female *C. nozawae* in 1979, and male *C. nozawae* and female *C. amblystomopsis* in 1980, the male, hiding in a shelter, showed no response to the female and did not approach her when she arrived within his sight. Even if the male approached the female, she swam away from him. Consequently, no females entered the male's shelter and no heterospecific pairings were formed.

Discussion

Under natural conditions, one species is generally prevented from interbreeding with other species by some mechanism. Therefore, each species maintains its own biological characteristics, such as body form or mode of life (Mayr, 1963; 1969). It is very important, therefore, to study what isolating mechanism acts, or how it has developed, in order to consider the mode of existence of species and the process of speciation (Hubbs, 1961).

In the case of two species of *Cottus amblysto-*

mopsis and *C. nozawae*, the spawning periods of the two species considerably overlapped in the Ryukei and Daitobetsu rivers, though the spawning of the former species preceded that of the latter species by about two weeks. On the other hand, they were isolated from each other in the spawning area, for *C. amblystomopsis* spawned in the lower course of the river, and *C. nozawae* in the upper course, distinctly apart from the spawning area of *C. amblystomopsis*. In those two rivers, therefore, the two species have no chance of encountering each other for mating, and thus interspecific breeding between them will never occur. As pointed out by Goto (1980) and confirmed in the present study, the two species are distributed in different places in almost all rivers of Hokkaido where they are coexisting. Thus, it is reasonable to consider that two species distributed sympatrically in Hokkaido segregate each other along the course of a river, and this geographical isolation acts as an effective mechanism for reproductive isolation between them.

In the Hekiriji River, however, the two species partially overlapped both in the spawning period and in the spawning area. Thus, it may be possible for the two species to encounter each other for mating, though they never meet in the allotopic area. In fact, Goto (1977) reported that only one deposited egg-cluster, which might have been produced by interbreeding between the two species, had been observed in the overlapping area of the Hekiriji River. Because of such a rare finding, one interbred egg-cluster per several hundred, he supposed that in the cohabiting area of the Hekiriji River some isolating mechanisms had to prevent effective interspecific mating.

In the present study, the results on the spawning sites indicated that both species tend to spawn under similar conditions of water depth, current velocity, bottom substratum and size of nest stones. Therefore, it may be supposed that the micro-environmental conditions for the spawning sites are not involved in the reproductive isolation between the two species. On the other hand, the results of mate preference tests suggest that some incompatibility in the mating sequences exists between the two species and acts as an effective isolating mechanism. This is shown because mating never occurred in heterospecific combinations of the two species, in contrast with successes in the conspecific combinations. In the present mate preference tests, the males of each species responded differently to the conspecific and heterospecific females when they came into sight, though the patterns of reproductive behavior seemed to be basically similar between the two species. These facts suggest that each species has the ability to discriminate conspecific and heterospecific mates, and/or there is some slight difference in the reproductive behavior between the two species though it has not been revealed in the present observations. In most fish species, the patterns of courtship behavior are composed of a series of reciprocal or mutual signals involving both sexes. When individuals of two species meet, reproductive isolation may be maintained by the failure of one or both to give the appropriate signals and responses (Liley, 1966). Between the two species, *C. amblystomopsis* and *C. nozawae*, some differences in the signals and responses probably exist.

In any case, there must be powerful isolating mechanisms preventing the interbreeding between the two species of the genus *Cottus*, and geographical and ethological isolations may serve as important devices in these mechanisms. But they are not isolated over a fairly wide area in the Hekiriji River, so in this case, there is no geographical isolation. Habitat isolation should be also eliminated here, because the study has shown that depth, current velocity and substratum appear to be identical (i.e., habitat). 'Geographical isolation' is the general rule for the species pair and probably has contributed greatly to speciation; however, at the present time, the most important isolating mechanism seems to be behavioral.

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(Laboratory of Embryology and Genetics, Faculty of Fisheries, Hokkaido University, Hakodate 041, Japan)

淡水カジカの2種エゾハナカジカとハナカジカの産卵習性と生殖的隔離機構

後藤　晃

近縁な淡水カジカ2種，エゾハナカジカとハナカジカが共存する北海道南部の数河川で，産卵期・産卵場所・産卵巣の位置の微環境条件を調べ，さらに水槽内で両種の産卵行動の観察及び同種・異種間の雌雄の組み合せによる簡単な配偶者選好性実験を行なった．

産卵期は，生殖腺重量比の変化及び河川内での産出卵塊と孕卵雌魚の出現時期から，エゾハナカジカは4月中旬から5月中旬，ハナカジカは4月下旬から5月中旬までと推定された．産卵域は，1河川でのみ下流域上部に両種の混棲産卵域が存在したが，他の河川においては，エゾハナカジカは下流域，一方ハナカジカはそれより上部の中・上流域というように，両種間で分離して存在した．さらに，産卵巣の位置する微環境条件—水深・流速・底質・産卵巣として利用する石のサイズ—には，両種間で重複した特徴が認められた．両種の一連の産卵行動は酷似し，基本的な行動パターンに違いは認められなかったが，異種間の雌雄の組み合せでは，番い形成・産卵に至ることはなかった．

以上の結果から，エゾハナカジカとハナカジカは，かなり厳密に生殖的に隔離されており，その隔離機構としては，産卵域の分離と産卵行動上での何らかの不適合が重要な役割を果していると考えられた．

(041 函館市港町 3-1-1 北海道大学水産学部発生学・遺伝学講座)