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# Spawning and Subsequent Copulating Behavior of the Elkhorn Sculpin *Alcichthys alcicornis* in an Aquarium

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**Abstract** The spawning and subsequent copulating behavior of the elkhorn sculpin (*Alcichthys alcicornis*) were observed in an aquarium. Soon after the female was introduced into the tank, the male approached and initiated courtship behavior by unfolding all fins. Females which carried ovulated eggs eventually accepted the male and spawning progressed. Females which had not ovulated, repeatedly rejected the male's advances. The onset of spawning in the female was indicated by fluttering of the pectoral fins and successive horizontal undulations of the posterior trunk which served to sweep the spawning site. The laying of several thousand eggs was preceded by the female taking several large inhalations of water through the mouth and a slight raising of the posterior trunk. The male continued various behavior related to courtship while the female approached spawning. After spawning, the female smoothed the egg mass into a thin layer by using the posterior trunk and anal fin. After spawning, and while the female flattened the egg mass, the male copulated repeatedly. No copulation was observed prior to spawning. Semen was observed to be emitted from the tip of the penis and to leak from the female's genital pore. Eggs were fertilized externally by the emitted sperm in the case of females which had no prior copulating experience in the present breeding season. The entire reproductive behavior of the pair ceased, as the female left the spawning site after flattening the egg mass. The male remained near by, but did not aerate the eggs. During the breeding season, the area around the female's genital pore and along both sides near the base of the anal fin became slightly distended and soft by the accumulation of a serous fluid. It is presumed that this edematous modification serves to flatten the egg mass for softness without crushing the eggs.

Several studies have noted that some cottids copulate (Bolin, 1941; Morris, 1952, 1956; Krejsa, 1964; Hubbs, 1966; Lamp, 1966; Shiogaki and Dotsu, 1974; Shinomiya, 1985; Ragland and Fischer, 1987). The elkhorn sculpin *Alcichthys alcicornis* also have been proved to copulate because fertilized eggs have been obtained from a tank in which females had been kept without a male (Munehara and Mishima, 1986). It was concluded from preliminary observations of elkhorn sculpin's reproductive behavior that spawning and subsequent copulation are performed as a series of reproductive actions. The present study was undertaken to explain the pattern of reproductive behavior and the mode of fertilization in elkhorn sculpin. This information is indispensable for the study of cottid's reproductive modes, for understanding whether the sculpin is a copulating fish or not, and to clarify when and where fertilization takes place. The reproductive behavior and sexual dimorphism relating to the reproductive behavior of elkhorn sculpin is also described.

## Materials and methods

One male and ten female adult elkhorn sculpin used were captured by trammel net sets from the shore of Usujiri, southern Hokkaido, on 31 March and 5 April 1986. The mean standard length and body weight were 308 mm and 810 g in male and 219 mm (range 170–252 mm) and 296 g (range 120–470 g) in females. Fish of the two sexes were segregated in two round polycarbonate tanks of 1,000 *l*. No substrate was placed on the bottom of the tank. During the experimental period, 4–14 April 1986, water temperatures ranged from 2.9 to 4.4°C.

Observations of reproductive behavior were carried out by the following procedures: A female held in a transparent acrylate resin case (70×25×25 cm) was placed on the bottom of a tank in which a single male was kept. After 30 minutes' acclimation, the female was released from the case. Following the completion of all reproductive behavior, the deposited eggs were left in the tank

with the male and the female was removed. To assure accuracy of conclusions, the experiments were repeated with twelve pairs consisting of one male and each of ten different females. Behavior of each pair was recorded by direct observation and video tape.

The elkhorn sculpin is a multiple spawning and copulating fish. Furthermore, the season of copulation and spawning overlaps and the reproductive behavior may change according to the reproductive condition of the female. For these reasons females were grouped into four categories prior to the experiment according to reproductive conditions as follows:

1. Nonovulated-nonimpregnated female (NNF): having neither ovulated eggs nor copulating experience in the present breeding season.
2. Ovulated-nonimpregnated female (ONF): having ovulated eggs but no copulating experience in the present breeding season.
3. Nonovulated-impregnated female (NIF): having no ovulated eggs but having copulating experience in the present breeding season.
4. Ovulated-impregnated female (OIF): having both ovulated eggs and copulating experience in the present breeding season.

In the course of 12 experiments involving 10 females, these reproductive conditions were found 3, 5, 2 and 2 times, respectively. Ovulation was detectable by pressing slightly on the abdomen and observing whether eggs spilled from the genital pore. The presence of sperm in the ovarian fluid collected by syringe indicated copulating experience in the present breeding season. Additionally, about 100 eggs were collected with the ovarian fluid from ovulated females (ONF and OIF) and reared in sea water to confirm the lack of copulating experience. The eggs from OIF developed normally, while those of ONF did not proceed over the blastodisc stage.

## Results

Sexual dimorphism: Like many other cottids, elkhorn sculpin exhibit considerable sexual dimorphism. The ground color of females is reddish brown, and that of males is dark or grayish brown. Males are larger than females in body size. An adult male is 27–32 cm in standard length and 600–900 g in body weight. On the other hand, the range of an adult female's body size and weight is 15–25 cm and 100–500 g, respectively (Munehara, unpubl. data). There are three marked sexual differences in external morphology. First, the male has an anal papilla which functions as a penis (Fig. 1A). It is cone-shaped, and approximately 30 mm in length. Secondly, the first dorsal fin is longer with the tips modified from spines into tassels in males. Thirdly, the areas around the female's genital pore and along both sides of the body near the base of the anal fin are slightly distended and soft with a serious fluid between the dermis and subcutaneous layer (Fig. 1B). This edematous modification occurs only during the breeding season.

Reproductive behavior: The male did not take food during this study, and usually remained stationary when alone. When a female was introduced into the tank, the male erected all fins and initiated courtship. The first dorsal fin was unfolded so that the first spine reached near the interorbital space. The male subsequently approached the female with operculum and branchiostegals erected (Fig. 2A).

The male sometimes approached the female, displayed its lateral side, moved the head right and left, undulated the body, or spasmodically shook the operculum with the branchiostegals erected. Initial reaction on the part of the female was to swim freely along the side of the tank, remain still, or flee from the male's approach. The male chased and violently bit the side of the females (Fig. 2B). In successive experiments, the male crawled on or swam back and forth between the newly introduced female and eggs which had been deposited by previous females. This behavior lasted for 20–200 minutes, ending with ovulated females (ONF and OIF) finally approaching the male or the spawning site where eggs were already present. This response by ovulated females signified the successive acceptance of the male's courtship. Nonovulated females (NNF and NIF) which continued to reject the male were exhaustively chased and bitten. Two conclusions are possible from these observations: (1) the nonovulated female will not assent to copulate, and (2) the male cannot distinguish the female's reproductive conditions.

Having accepted the male's courtship, the female did not maintain composure and sometimes shifted position for several minutes. As the female was gradually motivated to spawn, the fish ceased

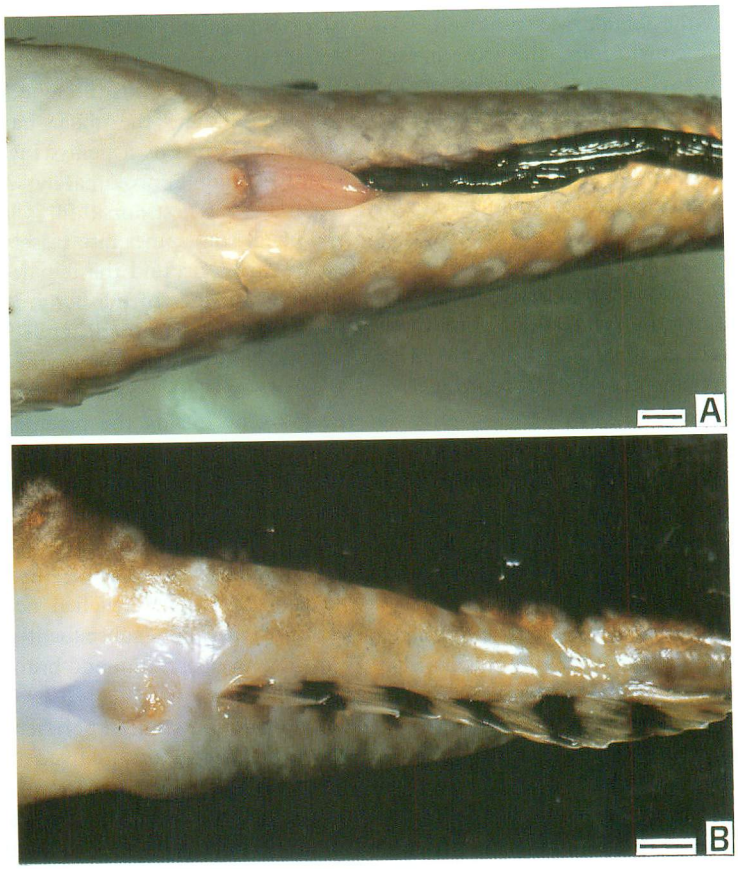


Fig. 1. Penis of the male (A) and fluid-filled modification of the female occurring during the breeding season (B) of the elkhorn sculpin *Alcichthys alcicornis*. Scale is 10 mm.

to shift and frequently fluttered the pectoral fins and successively undulated the posterior trunk right and left (Fig. 2C). The duration of the strokes of the posterior trunk was several seconds and functioned to clean the area of the tank bottom with the pectoral fins, the anal fin and the posterior trunk. The male swam around the female and repeated the display of its lateral side, occasionally approached the female's genital pore and spasmodically shook the operculum with the branchiostegals erected, rolled the body right and left, or bit the female.

The laying of several thousand eggs was preceded by the female taking several large inhalations of water through the mouth and a slight raising of the posterior trunk (Fig. 2D). Eggs were discharged with viscous ovarian fluid as a flexible and spherical mass.

Following spawning, the female undulated the posterior trunk right and left as had been seen in pre-spawning, but in post-spawning this action was slower (20–30 seconds) and functioned to gradually flatten the egg mass. This was repeated about 5–15 times for 3–15 minutes. When this behavior finished, the egg mass had been transformed into a thin layer of eggs which firmly adhered to the bottom of the tank.

Copulation was never observed prior to spawning. The first copulation was performed by the male approaching the female's genital pore several seconds after spawning. With the penis bent forward, copulations were repeatedly performed by the male mounting the female as the egg mass was flattened (Fig. 2E). Semen was emitted from the tip of the penis just before and after insertion, and leaked from the female's genital pore so that the vicinity of the spawned eggs was slightly obscured for a moment (Fig. 2F). Eggs spawned by nonimpregnated females (ONF) were fertilized externally by the emitted sperm.

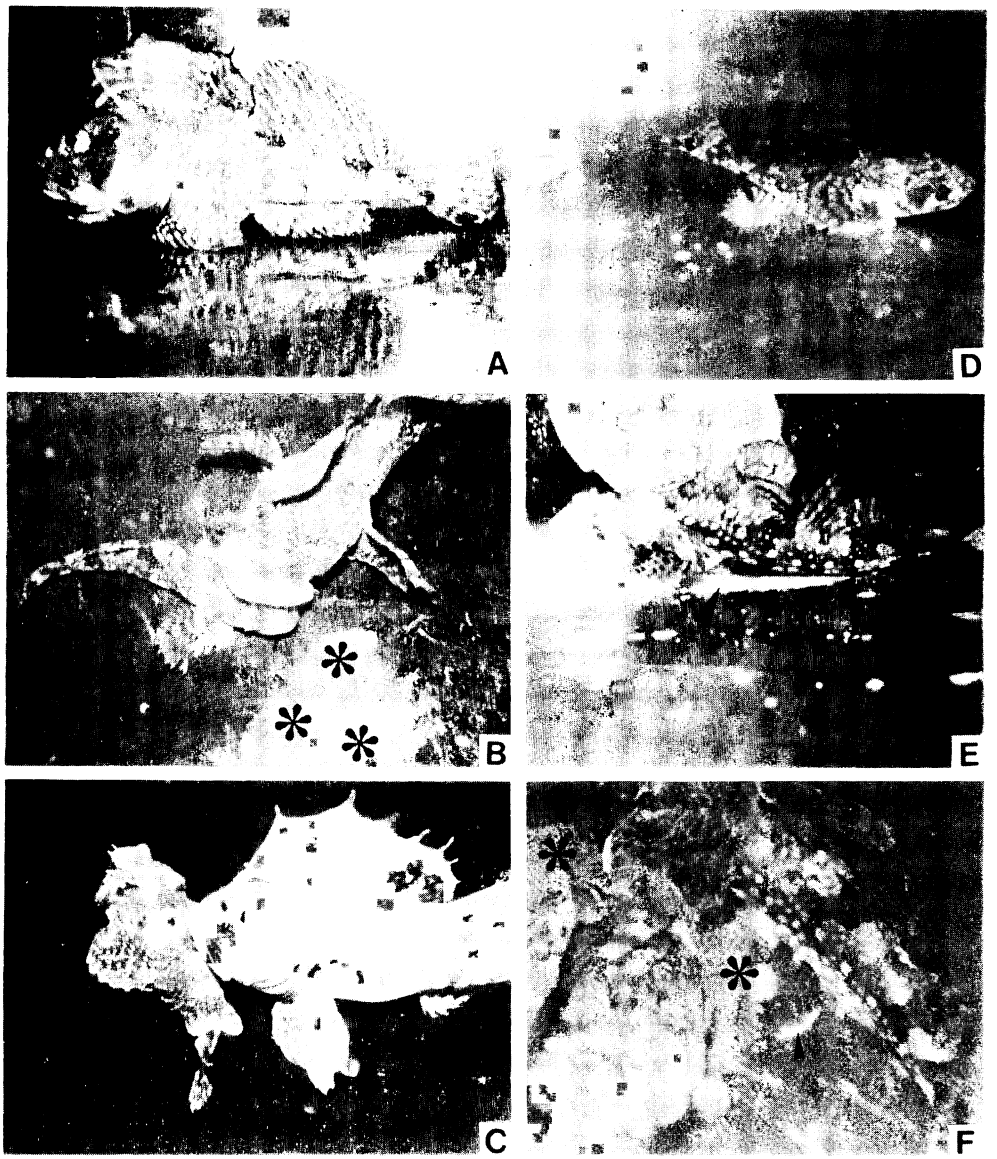


Fig. 2. Reproductive behavior of elkhorn sculpin. The larger fish is the male. A: Male's displaying posture. B: Male biting an unresponsive female. Asterisks represent a thin layer of previously deposited eggs. C: Female's horizontal undulations of the tail in pre-spawning. The tips of the male's first dorsal fin spines are modified as tassels. D: Just after spawning. The eggs are discharged with a viscous ovarian fluid, thus forming a flexible and spherical mass. E: Male copulating, and female flattening the egg mass (arrow). F: Sperm (arrow) emitted from the female genital pore after copulation. Asterisks indicate the eggs which the female spawned.

Mounting and copulation required about 2–5 seconds to complete, and recurred 6–22 times at intervals of 5–60 seconds until the female finished flattening the egg mass and left the spawning site. No further reproductive behavior was then noted.

The male spent most of the time near the eggs except when a female was introduced into the tank. Egg-aeration on the male's part was not observed. The entire reproductive behavior up to the point when the female departed from the

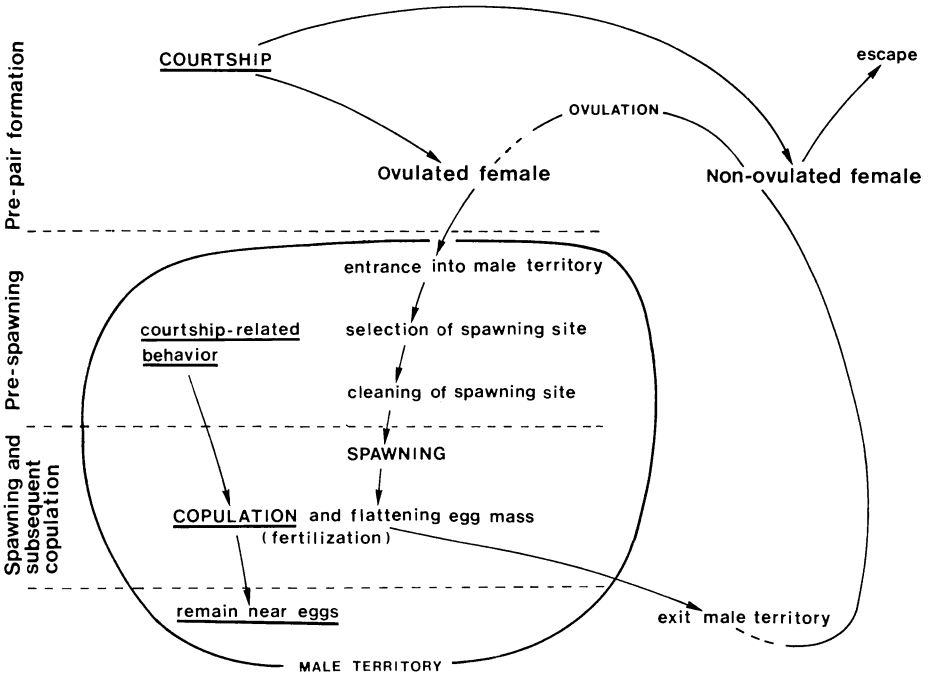


Fig. 3. Ethogram of elkhorn sculpin's reproductive behavior. Arrows indicate the behavior sequence of the male (underlined) and female.

spawning site took 30–238 minutes with a mean of 87 minutes. The mean percentage of fertilization calculated from 100 eggs from each of the 7 experiments was 96 percent (range 85–99 percent), and a student t-test demonstrated no significant difference in the fertilization rate between impregnated (OIF) and nonimpregnated (ONF) females ($p>0.05$).

## Discussion

It is well known that sexual dimorphism of body color, body size, fin length and anal papilla in many cottids is distinct (Vladykov, 1933; Bolin, 1944; Clemens and Wilby, 1949; Watanabe, 1958; Breder and Rosen, 1966; Sandercock and Wilimovsky, 1968; Iwata, 1983). In elkhorn sculpin these differences between sexes have also been clearly recognized. The anal papilla of male elkhorn sculpin functions as a penis at the time of copulation. On the other hand, perch (*Pseudoblennius cottoides*), sunrise (*P. percoides*) and Ishikawa's (*Furcina ishikawae*) sculpins, little-dragon sculpin (*Blepsias cirrhosus*) and sea raven (*Hemitripterus villosus*) have adapted a flexible oviduct for depositing eggs into sea squirts (Shinomiya, unpubl. data), spongy tissues (Munehara, unpubl. data) and polychaete colonies (Munehara, unpubl. data), respectively. Female elkhorn sculpin have no such projectable oviduct, but the area around the genital pore and along both sides near the base of the anal fin becomes notably distended and soft with a serous fluid between the dermis and subcutaneous layer during the breeding season. It is presumed that this edematous modification serves to flatten the egg mass for softness without crushing the eggs, though the exact function has not been elucidated in this study. This modification has not been reported in any other teleosts.

In pre- and post-spawning, the female's action of fluttering the pectoral fins and successively undulating the posterior trunk right and left is a very curious and significant action. In pre-spawning, these actions remove debris which may subsequently foul the eggs, and in post-spawning transform the spherical egg mass into a thin layer so that all eggs can be easily supplied with fresh water. These actions seem to be indispensable for the survival of the eggs because the male does

not ventilate the eggs.

An ethogram of elkhorn sculpin's reproductive behavior observed in an aquarium is shown in Fig. 3. Spawning and subsequent copulation were performed as a series of reproductive actions. Such reproductive behavior has also been reported in bald sculpin (*Clinocottus recalvus*) (Morris, 1952). On the other hand, the copulating season precedes the spawning season by 2–3 months in perch, sunrise and Ishikawa's sculpins (Shinomiya, 1985). Elkhorn sculpin spawn several times in one breeding season like those sculpins but copulation follows egg deposition. Thus, a successful first spawning of a female must depend upon external fertilization by sperm emitted during copulation. In addition, the mode of the fertilization of subsequent spawning has also been made clear. It has been noted through histological observations and conditions of egg activation that sperm has already entered into the micropyle of eggs in the ovarian cavity of the impregnated female (OIF) but the fusion of male and female gametes takes place after spawning (Munehara et al., in press). Accordingly, elkhorn sculpin is considered to be an externally-fertilizing species. Though Morris (1952) did not confirm the lack of copulating experience of the sampled fish, judging from the spawning prior to copulation of bald sculpin, the first spawned eggs of a female may be fertilized in the same manner as elkhorn sculpin.

On the basis of spawning and subsequent copulation and external fertilization, the reproductive mode of elkhorn sculpin seems to be a little advanced from external-fertilization type to internal-fertilization type. Evolution of reproductive behavior and fertilization mode in cottids cannot be satisfactorily discussed because no other copulating types have been clarified concerning the actual point at which fertilization takes place. However, the reproductive mode of elkhorn sculpin can be held as a model example of many reproductive modes in cottids.

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## 水槽内におけるニジカジカの産卵と交尾行動

宗原弘幸

水槽内でニジカジカの繁殖行動を観察した．本種の1回の繁殖行動は，産卵と交尾からなる．雄は雌を発見するとすべての鰭を広げ，一連の求愛行動を行なう．初め，雌は雄の求愛を無視しているが，排卵状態にある時は，最終的に求愛に応じ，産卵とそれに続いて交尾が行なわれる．産卵直前になると，雌は胸鰭と尾部および臀鰭で産卵床を掃き，数回深呼吸の後，尾部を幾分上方に反らせた姿勢で一気に数千粒の卵を産む．雄は産卵するまで，雌の周りを側面誇示しながら旋回遊泳したり，時には咬みついたりするが，産卵前に交尾することはなかった．産卵後，雌は産卵前と類似した行動で尾部と臀鰭により，卵を薄層状に広げて水槽底面に付着させる．卵塊を薄層状にすることは，発生過程で卵に対する酸素供給を容易にするものと推察された．交尾は雌のこの行動の間に繰り返され，その際，精液がペニスあるいは雌の生殖口から漏れ出るのが観察された．実験前まで交尾経験のなかった雌の産出卵は，この漏出した精子によって，体外で受精する．卵塊を広げ終えると，雌は産卵場所を去り1回の繁殖行動が終了する．通常，雄は卵の近傍で過ごすが，ファニング等の保護行動は観察されなかった．また，産卵期間中，雌の生殖口周辺から臀鰭両側の基部にかけて，皮下に漿液が貯留し水腫状を呈する．この水腫様の膨隆部は柔らかく，それ故，卵塊を薄層化する際，卵が潰されないように機能すると推察された．

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