1992

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Mark Henry Sabaj

SPAWNING CLASPS AND GAMETE DEPOSITION IN PEBBLE NEST-BUILDING MINNOWS (PISCES: CYPRINIDAE)

by

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B. S., University of Richmond, 1990

A Thesis
Submitted to the Graduate Faculty
of the University of Richmond
in Candidacy
for the degree of
MASTER OF SCIENCE
in
Biology

July, 1992

Richmond, Virginia

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ACKNOWLEDGEMENTS

I extend my sincere thanks to Dr. William S. Woolcott, my advisor, for his assistance with the research and preparation of the thesis. I also thank the members of my commitee, Dr. Eugene G. Maurakis for his guidance and fellowship in the field and laboratory over the course of this study, and Dr. Joseph C. Mitchell for his critical and prompt review of the manuscript.

I thank Dr. Wilton R. Tenney for his assistance with the photographic preparation of presentations related to my thesis; and William R. McGuire, a fellow graduate student, for pertinent information on the epidermal morphology of fishes and camaraderie during our graduate school tenure.

I am indebted to the Graduate School of Arts and Sciences for funding my graduate education at the University of Richmond. I am especially grateful to the Smart and Dickinson families whose awards made my summer research possible.

I give my sincerest thanks to my parents, Mr. and Mrs. Henry Sabaj, whose love and support fostered my endeavor to get a good education.

ABSTRACT

Spawning in five species of North American cyprinid minnows (*Rhinichthys a. atratulus, Exoglossum laurae, Semotilus atromaculatus, Nocomis leptocephalus,* and *N. micropogon*) are analyzed on videotape filmed in Virginia and Maryland streams. Behaviors are chronologically resolved into a sequence of categories (Interim, Approach, Alignment, Run, Clasp and Dissociation) exemplary of a successful spawn as defined by the clasp. The categories are used to describe spawning behaviors in each species. In all species studied, a successful spawn is a female initiated event effecting the clasping response of a male.

The species-specific pattern of male-female interactions not only reflects the coordination of the spawning clasp, but the architecture of the spawning substrate. In the pebble nest-building cyprinids (i. e., species of *Exoglossum, Semotilus* and *Nocomis*) a successful spawn is the end result of a topographically fixed sequence of behaviors that place the clasped pair over a discrete area of the nest specifically composed for gamete deposition and retention.

INTRODUCTION

A successful spawn in pebble nest-building minnows (Pisces: Cyprinidae) is defined as a clasp (Reighard, 1910; Maurakis et al., 1991a). The clasp is a momentary flexure of the male's body about an axis determined by the appositioned female. Reighard believed this maneuver to coordinate the simultaneous release of milt from the male and eggs from the female. This concurrence is verified by video-tape analysis of spawning clasps of *Semotilus atromaculatus* (creek chub), a pebble nest-builder.

The clasp is the end product of genetically choreographed behaviors of species-specific male-female interactions that are responsible for aligning the spawning pair. Deviation from the course of interaction leading to a clasp, whether intrinsic or extrinsic (e. g. confrontation with an intruding male), affects an abrupt separation of the spawning pair.

Male and female behavioral patterns not only reflect the coordination of the spawning clasp, but the architecture of the nest itself. The pebble nest represents a substrate specifically modified to accomodate the clasping fishes, promote the fertilization of gametes, and retain the developing eggs and larvae. A successful spawn is the result of an interplay between the topography of the substrate and the sequence of behaviors that place a clasped pair over a discrete area of the nest that has been prepared for gamete deposition.

Unlike previous spawning accounts based on field observations and captive breeding, this study uses behaviors on videotape filmed in the field. Focus is on the spawning clasp and egg deposition in four representatives of the 14 species (three genera) of pebble nest-building cyprinids endemic to North America: *Exoglossum laurae* (tonguetied minnow), *Nocomis leptocephalus* (bluehead chub), *Nocomis micropogon* (river chub), and

S. atromaculatus. *Rhinichthys atratulus atratulus* (eastern blacknose dace), a non-nest building substrate spawner, was included for comparison to demonstrate the significance of the modification of substrate to reproductive activities of nest-building species. My objective is threefold: 1. to describe and illustrate the spawning clasp in each species; 2. to identify and catagorize male-female interactions that both contribute to and disrupt the coordination of the spawning clasp; and 3. to relate the reproductive behaviors to the composition and topography of the spawning substrate.

MATERIALS AND METHODS

Reproductive behaviors and spawning substrates of fishes were filmed in streams in Maryland and Virginia from 1986 to 1990 (Appendix, Tables 1 and 2). Behaviors were observed and videotaped from above the surface of the water in the field during daylight hours. Fishes were filmed from a site on the bank that afforded the clearest view and did not appear to disrupt spawning. Measurements and compositions of spawning substrates and their associated water current velocities are based on direct field observations, personal communications, and literature accounts.

Two types of color video cameras were used to record events onto different tape formats. A Panasonic industrial camera (model NV-8420) and portable VHS videocassette recorder (model WV-8420) were used to record behaviors onto 1/2 inch tape. A Sony video camera (model DXC-3000) and portable videocassette recorder (model VO-6800) recorded behaviors onto 3/4 inch tape. Lenses of both cameras were fitted with polarizing filters to minimize interference from light reflected from the water's surface. Zoom lenses on cameras allowed the field of vision to be manually adjusted and focused for close-up and wide angle views.

In the laboratory, tapes recorded in the field were played on a Panasonic VHS videocassette recorder (model AG-2400) with functions of normal speed (30 frames/second), slow motion, frame pause and single frame advance. Tapes were viewed on a 25 inch screen color television monitor. Laboratory analysis consisted of a four part review. (1) Activites associated with a particular nest were viewed in their entirety at normal tape speed. Subjects and behaviors were identified at this time. (2) A tape was replayed with pauses for note taking. Activities of fishes were described and divided into a chronological sequence of episodes. A single episode consisted of a particular behavior (e. g. nest-reworking) expressed by a single fish (e. g. dominant male). (3) Episodes directly related to spawning were replayed at normal speed, in slow motion and frame by frame. Orientations of fishes to each other and the spawning substrate were determined and pencil-sketched at this time. (4) The entire tape was reviewed again at normal speed to confirm previous observations.

Illustrations of fishes and nests supplement text descriptions of behaviors observed on tape. Illustrations were traced from pencil sketches taken from frame by frame analysis of videotapes. Museum specimens were used to supplement morphological details (e. g. scales and fin rays) not clearly visible in tape recordings. Final illustrations were drawn in indelible black ink with Koh-I-Noor, Rapidograph mechanical pens on bond white typing paper.

ANALYSIS

Behaviors exhibited by fishes actively spawning were analyzed in each of the pebble-nest building species and *R. a. atratulus*. Descriptions of male behaviors focused on those of the single dominant male that constructed the nest. Except when specified, descriptions of female behaviors were based on those exhibited by several females as individuals were difficult to identify. Analysis of behaviors in *R. a. atratulus* were either of a single male and female or of several males and females active over a particular area of streambed.

Six spawning behavioral categories were created *de novo*: Interim, Approach, Alignment, Run, Clasp, and Dissociation. The categories, arranged chronologically, reflect a sequence of intraspecific male-female interactions characteristic of a successful spawn regardless of duration. Each behavioral category is defined accordingly:

Interim: (male only) The behavior of a male (e. g. posturing) that precedes a female's approach.

Approach: (female only) The behavior associated with movement of a female towards a male in Interim.

Alignment: Behavior that affects the precise orientation of a male and female with respect to each other over a discrete area of a spawning substrate.

Run: (female initiated) Behavior associated with a synchronized movement of an apposed male and female during a spawning run over a particular distance.

Clasp: Behavior that affects the flexure of the male's body about an axis determined by the female's position at the end of her run.

Dissociation: Behaviors of a male and a female that result in their separation following the clasp.

Male and female behaviors other than those described in the successful spawning sequence of a particular species are considered disruptive. Intraspecific and interspecific behaviors (e. g. predator species) may disrupt a spawning sequence in any category except Dissociation.

RESULTS AND DISCUSSION

Rhinichthys atratulus atratulus

(eastern blacknose dace)

<u>Literature</u>

Traver (1929) provided the first detailed report of spawning behaviors in *R. a. atratulus* based on his observations of the species in Cascadilla Creek, New York. Since then there have been numerous references to spawning behaviors in all three subspecies of *R. atratulus* (*R. a. atratulus*, *R. a. meleagris*, and *R. a. obtusus*). Shontz (1962) gave a general account of spawning in *R. a. atratulus* in Virginia and Maine. Raney (1940) compared the breeding habits of *R. a. atratulus* to those of *R. a. meleagris* (western blacknosed dace). Captive spawning behaviors in *R. a. meleagris* were described in detail by Bartnick (1970). Conflicting accounts of reproductive behaviors have been provided for *R. a. obtusus* (southern blacknosed dace) by Schwartz (1958) and Jenkins in Matthews et al. (1982).

Additional studies of *R. atratulus* ssp. have focused on systematic variation (Matthews et al., 1982), early development (Fuiman and Loos, 1977), age and growth (Tarter, 1968), fecundity (Tarter, 1969), feeding habits (Tarter, 1970), behavioral and developmental plasticity (Gee, 1974), and life history and ecology (Bragg, 1978; Noble, 1965).

<u>Spawning Substrate</u>

Unlike males of species of *Exoglossum*, *Semotilus*, and *Nocomis*, *R. atratulus* ssp. males do not use their jaws to physically modify the substrate for spawning (e. g. build a nest). *Rhinichthys atratulus* ssp., however, establish and guard a discrete area of streambed for spawning. Although successful clasps of *R. a. atratulus* were not topographically fixed, they always occurred within or near a male's territory over a substrate of sand or fine gravel. In the following description *R. atratulus* refers to *Rhinichthys atratulus atratulus*.

Sequence of Spawning Behaviors

Interim: Between spawning episodes, a male *R. atratulus* established and attended a territory over a particular area of the streambed. Interim activities are described separately for two locations included in this study.

In one study site (WSW-VA-382; Appendix, Table 1), a lone male attended a substrate of sand and small pebbles that extended about 40 cm above the head of a riffle and was the width of the stream (approximately 60 cm). For up to two minutes, he either hovered or remained motionless on the substrate with head faced upstream, body procumbent and parallel to the current, and paired fins extended (Fig. 1). At times, he randomly surveyed but never strayed from his territory for about an hour prior to a female's approach. Occasionally, he dipped his snout into the substrate and displaced sand; however, the activity was not in preparation of a particular spawning site.

In the second study site (EGM-MD-201; Appendix, Table 1), two males established and guarded separate territories over abandoned concavities excavated by *S. atromaculatus.* The territories, each roughly 50 cm in diameter, were spaced about 1.5 m apart near the center of the stream. Interim behaviors (i. e., procumbency, surveillance, and snout dipping) of the two males were similar. At times, each male remained motionless on the substrate in the concavity with head facing downstream or at an angle to the current. Reduced flow in *S. atromaculatus* pits (Maurakis et al., 1990) might allow procumbency to occur irrespective of the stream current. The males occasionally strayed from their holdings and engaged each other in agonistic behaviors between female approaches.

Approach: A female approached a male's territory from any one of several directions. Unlike females of pebble nest-building species, her approach was not directed towards a topographical feature of the spawning substrate (e. g. pit or trough) or a male positioned over such a feature. A female's entrance into a territory immediately attracted the attending male (Fig. 2).

Alignment: Alignment in *R. atratulus* refers to a suite of courting maneuvers performed by a male and responses typical of a female prior to her initiation of a spawning run. Courting maneuvers, which include paralleling, tacking, yawing and corralling may occur in any order. If the female was in the water column, the male closely paralleled her motion and frequently swam either over or beneath her body (Fig. 3). If the female either paused on the substrate or hovered, the male tacked or yawed ahead of her. When tacking (Fig. 4), the male swam perpendicular to the current in one direction for up to 20 cm. He then turned and swam past the female in the opposite direction. With each pass, the male's flank was exposed 1 to 4 cm in front of her snout. The male made from one to seven passes and aligned parallel to the female after the final pass. When yawing, the male's head faced upstream while he moved from side to side ahead of the female. His caudal region occassionally passed over or beneath her snout. Corralling swims occurred when the female swam away from the male or his holding. If, for instance, the female turned away from the tacking male and swam downstream, he quickly swam in a semicircle ahead of her and redirected her motion upstream.

During these maneuvers, the male continually aligned parallel and adjacent to the female on the substrate. While aligned he often nuzzled either her head or anterior flank with his snout. If the female did not initiate a run

(e. g. rose from the substrate into the water column) the male resumed his maneuvers (e. g. tacking or yawing).

Run: The spawning run was initiated by the female (Fig. 5). The run began with the female positioned on the substrate. Following the contour of the substrate, she swam up to 6 cm upstream with 5 to 10 rapid tail beats. The accelerated tail beats signaled the initiation of a run. A female might begin a run as the male came to occupy a position at her side and nudged her head. She might return the gesture and begin moving upstream. In this case, the male accompanied her for the length of the run, his tail beating in sync and his snout even with or slightly behind that of the female's. If the male was either upstream or downstream of the female, he quickly aligned with her once she began accelerated tail beating. The male and female became tightly appressed anteriorly at the end of a run.

Clasp: The clasp began as the male pressed his head and caudal peduncle to the aligned female. His caudal peduncle slipped over that of the female's and forced her vibrating tail downward into the substrate (Fig. 6). The flexure continued as he arched his body sagittally over her dorsal fin. This action drove her vent and pelvic fins into the substrate while her head and pectoral region arched upwards. As the female elevated her head, the male's head either remained appressed or turned beneath hers. When pressed towards the female, the male's head might brace her anteriorly and provide leverage while his body arched over her back. His vent and pelvic fins were placed on the opposite side of the female near her dorsal fin. The clasped pair appeared as the Greek letter "psi", that is, the sagittal plane of the female bissected the crescent figure assumed by the superimposed male (Fig. 7).

Spawning clasps lasted from 1 to almost 3 seconds. During this time the male and female vibrated violently as the female furrowed into the substrate. Her tail and caudal peduncle acted like a plowshare as the clasped male applied pressure to her midbody. The vibratory motion of the clasped pair created a cloud of sand from the clasping site.

Dissociation: The clasp was completed as the male relaxed parallel to the direction of current and the female ceased vibration. The male often immediately resumed courting maneuvers as the female moved randomly about his territory. At times, the male and female remained prostrate in contact or close proximity for over a minute after the clasp (Fig. 8).

Behaviors Disruptive of the Spawning Sequence

Interim: At times, after several clasps, the male broke off courting maneuvers and forcibly evicted the female from his territory with either harry or swivel swims. Harry swims occurred as the male, with his snout pressed towards the female's anterior flank, aggressively goaded her in a direction away from his territory. Swivel swims began when the harried female turned sharply away from the appressed male. Swinging her body through a complete circle, her head became repositioned towards his tail. The male quickly accompanied her revolution and with heads apposed the pair swivelled their bodies through up to four rotations before separating (Fig. 9). Aggressive behaviors subsided as the female retreated from the area and the male resumed interim behavior within his holding.

Alignment: Courting maneuvers were characteristic of alignment between a single male and female. Alignment behavior was disrupted if additional males (i. e., males from adjacent holdings or males with no apparent holdings) intruded upon a spawning pair. The attending male and intruding males continually displaced each other from a position adjacent to that of the female as she moved randomly either over the substrate or in the water column. The intense competition prevented any one male from exclusively aligning with a female (Fig. 10). At times a female became pinned to the substrate beneath a school of up to seven males. She promptly wriggled free of such bodily entrapments and was generally unresponsive to the shoaling males (i. e., she did not initiate a run). As a female vacated the territory, the aroused intruders either disbanded or continued their fervent pursuit. At times the attending male accompanied the shoal for a short distance before returning to his territory to resume interim behaviors.

Clasp: Successful spawns presumably occurred as a female's caudal peduncle furrowed into the substrate of sand or fine gravel beneath a clasped male. On one occasion a female paused over a substrate of small pebbles at the end of her run. Though the appressed male arched his caudal peduncle over her back, she held her body without vibration. The male clasped her midbody for nearly a second before relaxing. It is uncertain whether the spawn was successful as the female's vent remained above the surface of the substrate. If gametes were released they would have washed downstream as the pebble substrate prevented the female from furrowing posteriorly.

Location of Fertilized Eggs

Gametes are presumably extruded directly into the substrate as the female furrows beneath the "psi" clasped male and the cloud of sand, upon settling, facilitates the burial of fertilized eggs. The location of fertilized eggs, collected from discrete areas just beneath the surface of the substrate (Maurakis, pers. comm.), coincided with sites over which "psi" clasps were observed. Eggs are demersal, adhesive and noncohesive.

Figure 1. Procumbent male *Rhinichthys a. atratulus* in territory.

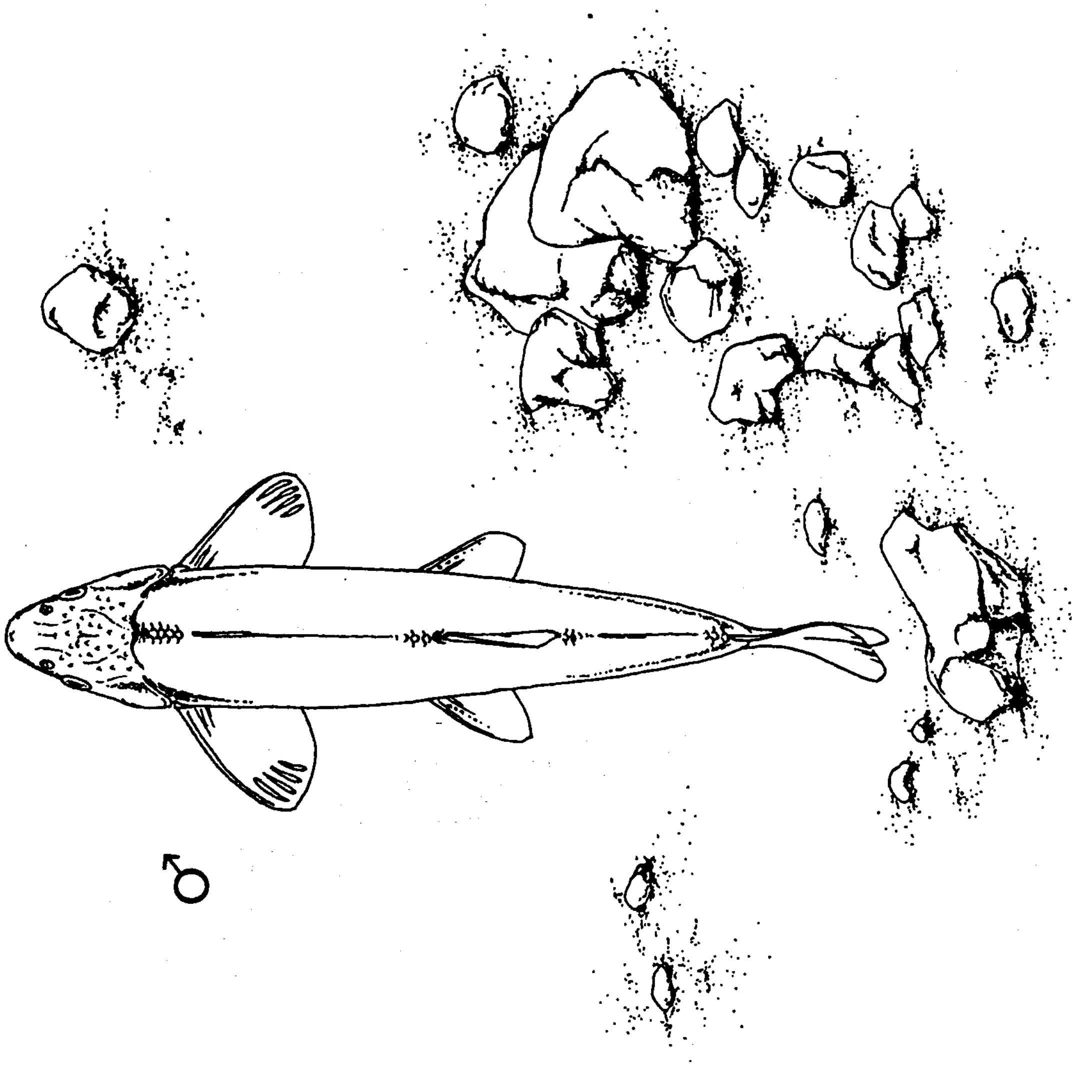

Figure 2. Female *Rhinichthys a. atratulus* entering male's territory.

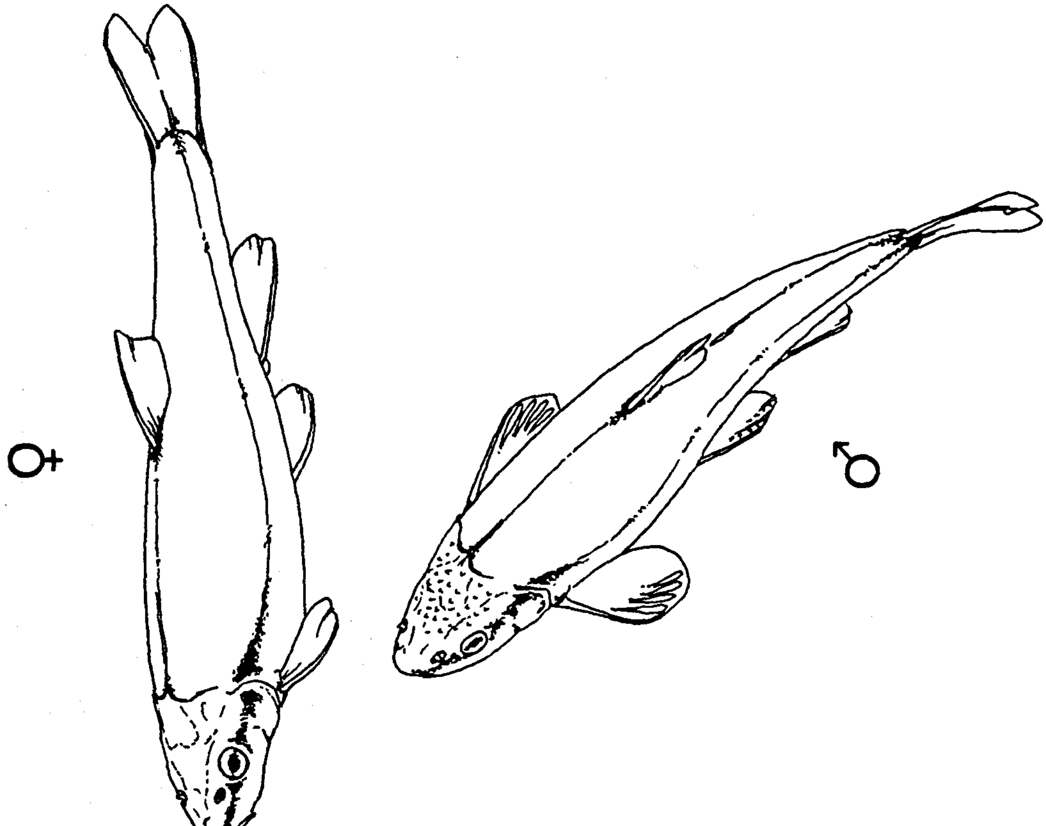

Figure 3. Male *Rhinichthys a. atratulus* paralleling female in water column.

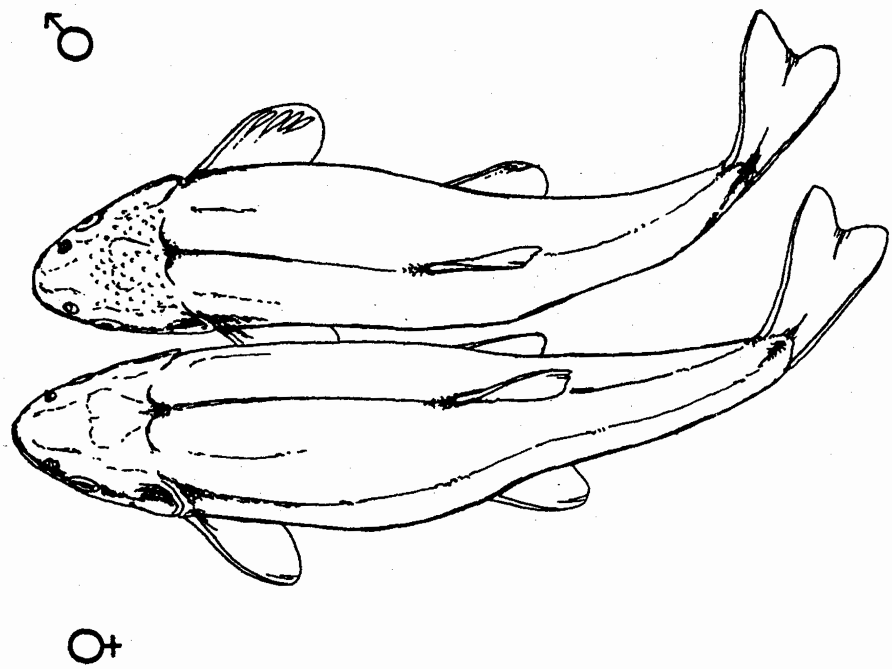

Figure 4. Male *Rhinichthys a. atratulus* tacking before female on substrate.

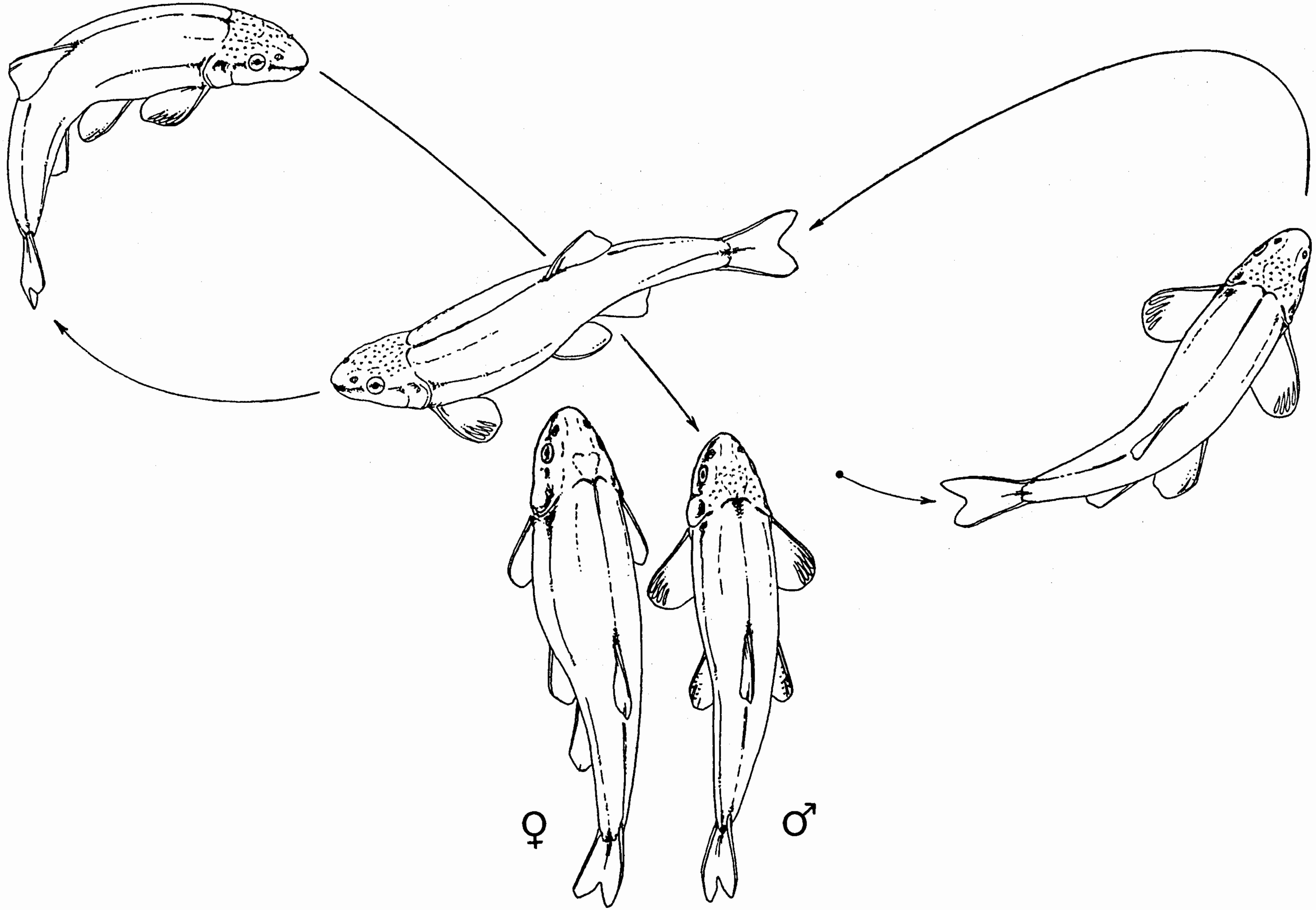

Figure 5. Female *Rhinichthys a. atratulus* initiating spawning run.

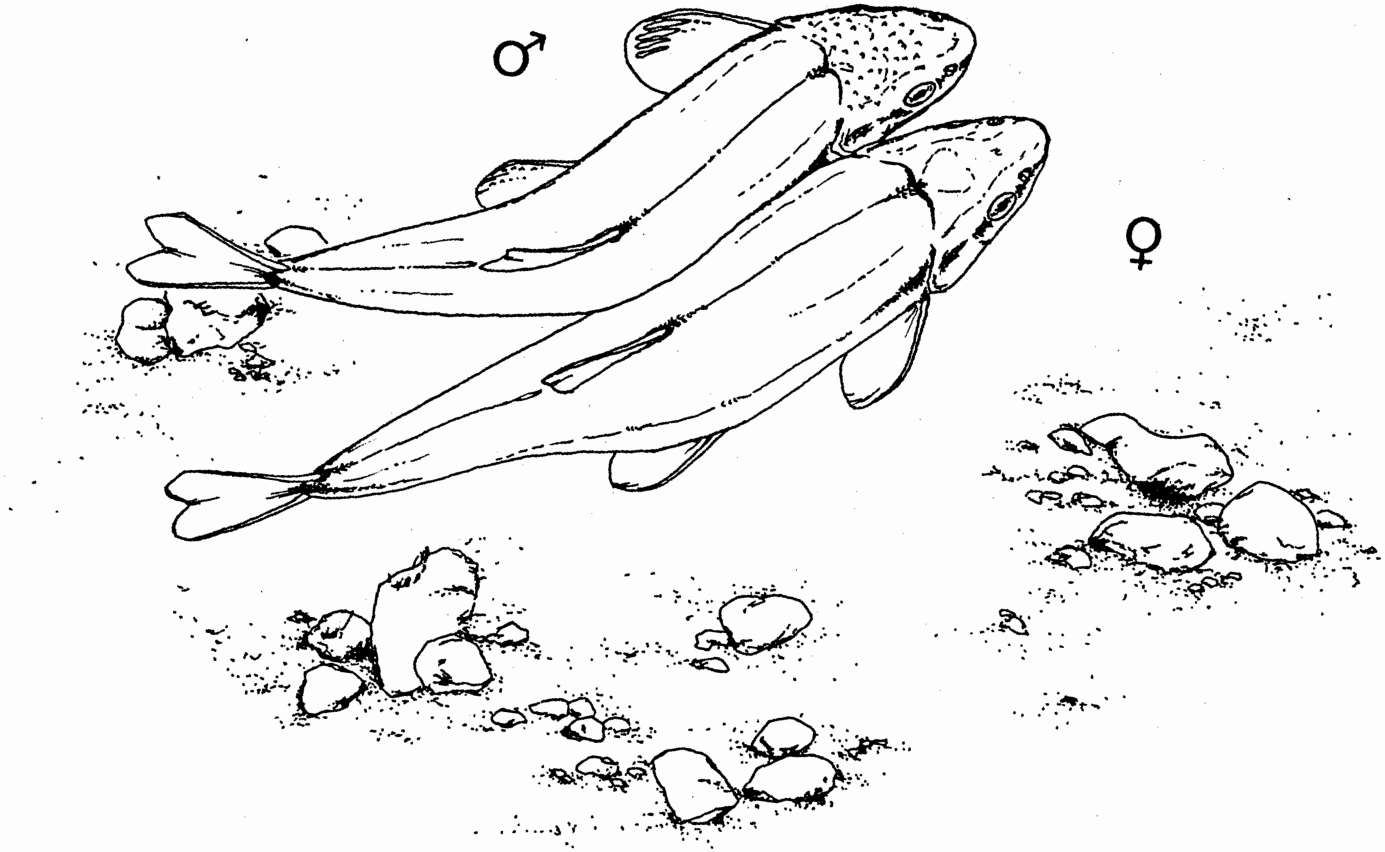

Figure 6. Male *Rhinichthys a. atratulus* initiating spawning clasp.

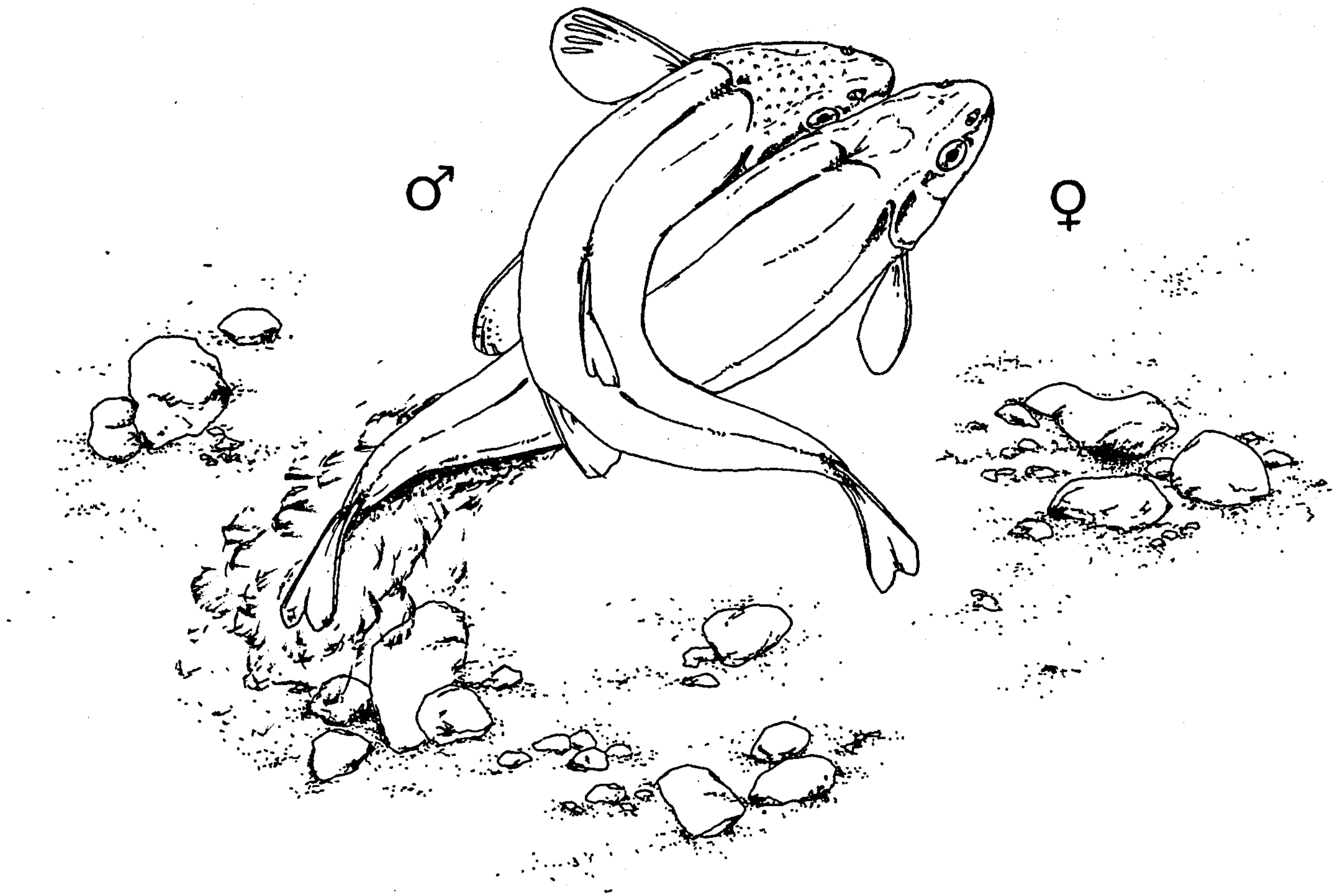

Figure 7. Male *Rhinichthys a. atratulus* applying "psi" clasp to furrowing female.

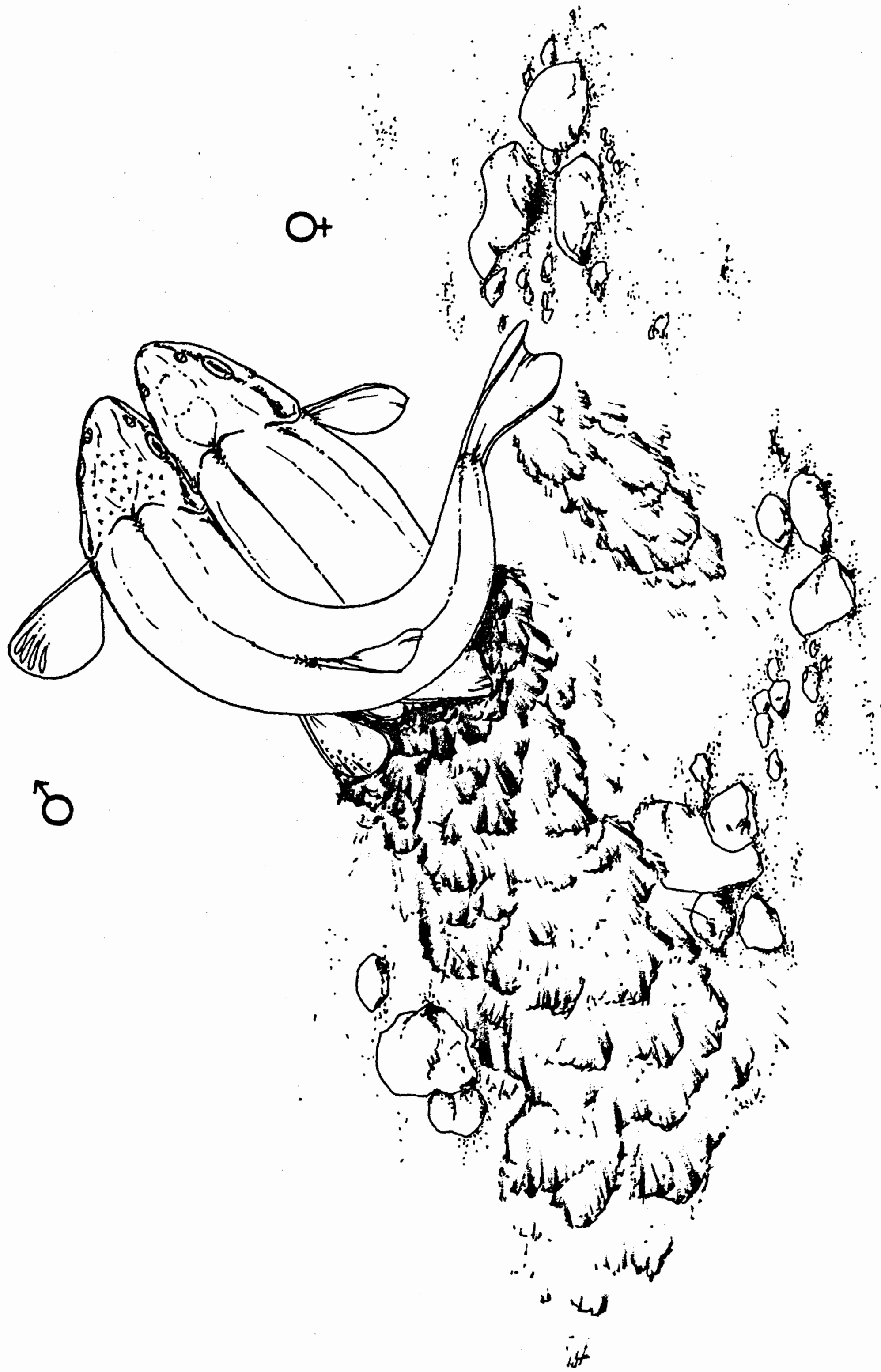

Figure 8. Male and female *Rhinichthys a. atratulus* prostate on substrate.

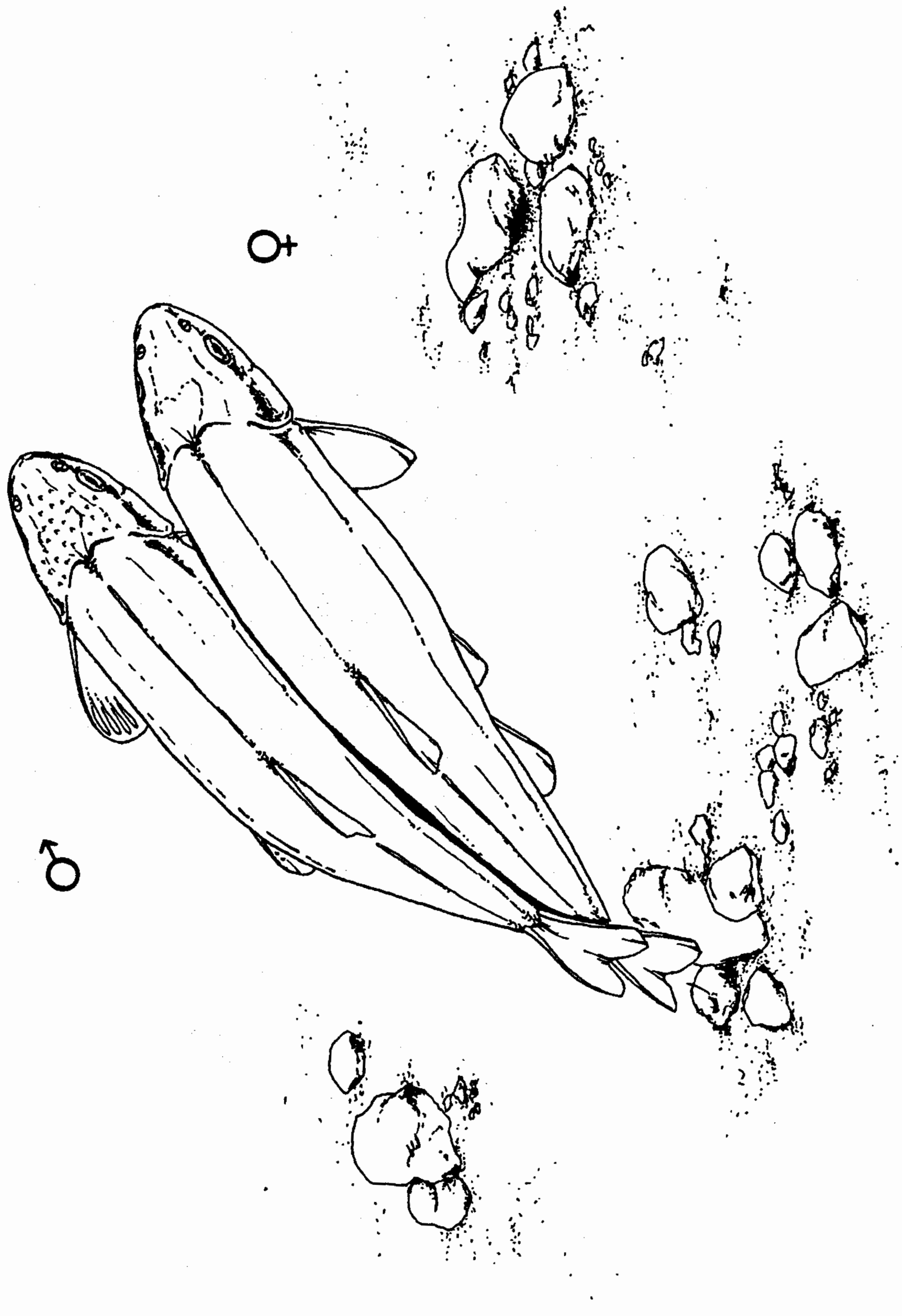

Figure 9. Male *Rhinichthys a. atratulus* evicting female from territory with swivel swim.

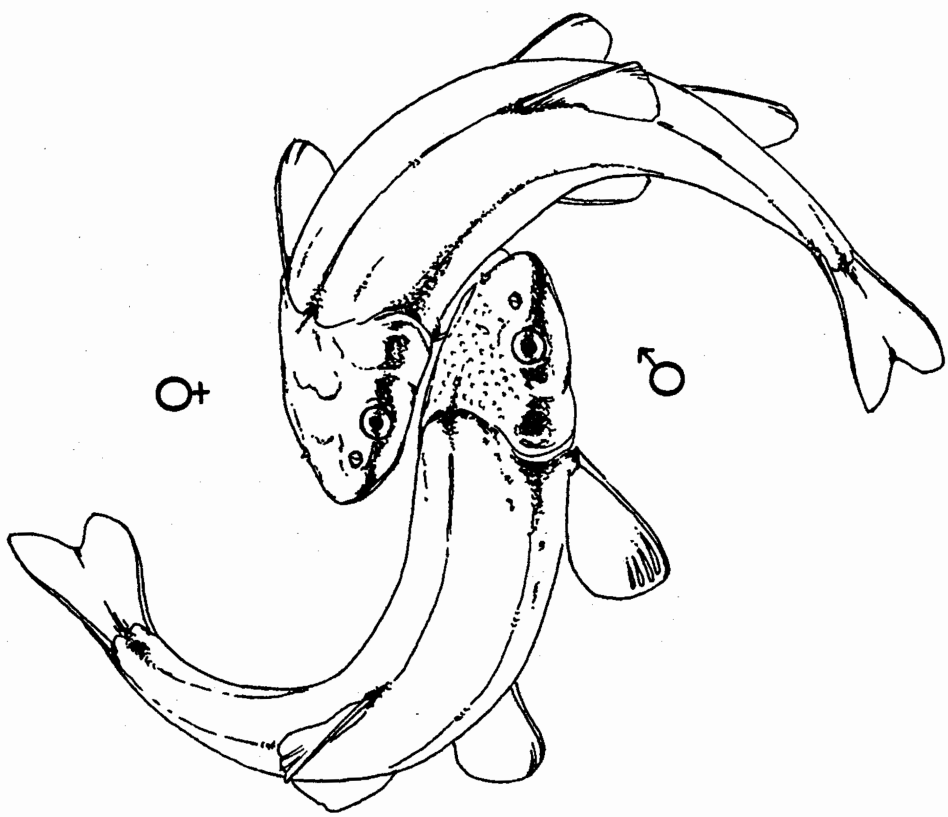

Figure 10. Male *Rhinichthys a. atratulus* shoaling a female.

Exoglossum laurae

(tonguetied minnow)

Literature

Reproductive behaviors of *E. laurae* were first described by Maurakis et al. (1991b). The study compared spawning habits of *E. laurae* in Sinking Creek, Virginia, with those of *Exoglossum maxillingua* (cutlips minnow) in Jackson River, Johns Creek, and the North Fork Roanoke River, Virginia. Reproductive behaviors of *E. maxillingua* were previously described by Van Duzer (1939) based on her observations made in New York. Nests of *E. laurae* in Pennsylvania were described by Raney (1939) and those of *E. maxillingua* in New York by Hankinson (1922).

Spawning Substrate

Pebble nests of *E. laurae*, composed of stones between 0.8 and 6.0 mm in diameter, are either circular or oblong mounds of pebbles (Maurakis et al., 1991b). Upon completion the nest observed in this study (EGM-VA-226; Appendix, Table 1) was an oblong mound of pebbles approximately 30 cm long and 50 cm wide. A central crest rising about 8 cm above the streambed divided the nest into upstream (45 degrees) and downstream (30 degrees) slopes (Fig. 11). Unlike the pebble nest of *Nocomis* and *Semotilus* species, the topography of an *Exoglossum* mound nest does not include a spawning concavity (i. e. pit or trough). According to Maurakis et al. (1991b), the nest functions as a breakwater by reducing water currents to 0.04 m/second on its upstream slope.

Sequence of Spawning Behaviors

Interim: The attending male continually swam about the nest between female approaches. Much of the movement involved displacement of stones from the periphery of the nest to the crest. Picking up a pebble with his jaws, the male swam to the crest of the mound and oriented his body parallel to the current before depositing the pebble. At times, the male swam down the upstream slope of the nest, turned laterally at the base, and circled back to the crest without retrieving a stone. Positioned over the crest, the male paused with head facing upstream, occasionally yawing from side to side along the crest (Fig. 12).

Rarely, did the male leave the nest to collect pebbles from the surrounding substrate. He never strayed more than 3 meters from the nest to collect stones between spawning episodes and always promptly returned to the nest to continue reworking activities.

Approach: Females moved randomly about the stream or hovered either upstream, downstream, or to the side of the nest before committing to an approach. As the female moved to within approximately 1 m of the nest, the male often darted in the direction of her approach. As the male quickly returned to the nest, the female continued towards the nest to assume a position over the downstream slope.

Alignment: The male immediately assumed a posture position over the crest of the nest as the female moved onto the downstream slope. Hovering about 2 cm above the nest, the male straddled the crest of the mound with head facing upstream and body parallel to the current. While crest postured, the male's pectoral, pelvic and dorsal fins remained fully extended (i. e., the first fin ray of each fin was at a right angle to his body).

As the male postured over the crest, the female moved over the downstream slope to a position beneath his caudal peduncle, her snout at times touching his vent (Fig. 13). The male remained poised as the female paused or passed from 1 to 3 times beneath his tail. He occasionally yawed from side to side over the length of the crest but did not move onto the upstream slope until the female initiated the spawning run.

Run: The spawning run down the upstream slope of the nest was always initiated by the female. A female crossing the plane of the crest from a downstream position stimulated the postured male's run down the upstream slope. After passing beneath his tail, she moved forward to a position either adjacent to or about 4 cm to the side of the poised male. As the female swam over the crest, the male immediately responded with strong tail beats and accompanied her run upstream with his snout either even with or slightly ahead of hers. With four to five strong tail beats, the pair moved past the crest and followed the contour of the mound approximately 5 cm down the upstream slope (Fig. 14).

Clasp: The spawning clasp began as the male tilted his body, turned his head towards the female and curved his caudal peduncle over her tail. Simultaneously, the female pressed the dorsal half of her body towards the concave side of the male. Her head became slightly elevated (< 30 degrees) but remained appressed to the male just above his operculum. The male's caudal peduncle arched sagitally over that of the female and rotated about 45 degrees towards the substrate in the direction of his turned head. With his caudal fin draped over the female's caudal peduncle, the male vigorously vibrated up to nine times during the clasp.

Spawning clasps lasted from 1 to 2 seconds. During this time the clasped pair moved a short distance (< 8 cm) from the midpoint to the base of the nest (Fig. 15). Their bodies, directly above the upstream slope, at times dipped into the surface and displaced pebbles from the nest. At the base of the upstream slope the male turned laterally and rounded off the pair's forward motion as the female remained appressed to the concave side of his clasped body. Upon completing the clasp the spawning pair approximated a perpendicular direction to the current. Before releasing the female, the male might momentarily constrict his body as if to push off the appressed female.

Dissociation: The pair quickly separated at the base of the upstream slope. The female turned sharply away from the male and swam downstream. She either circled to a position over the downstream slope from which she might initiate another spawning sequence or depart from the vicinity. Regardless of the female's disposition, the male circled to a posture position over the crest of the nest. If the female withdrew from the crest postured male, he often darted after her but quickly returned and resumed interim acitivities over the pebble nest.

Behaviors Disruptive of the Spawning Sequence

Interim: Between spawning sequences, the dominant male seldom ventured from the nest. When collecting material from the streambed for addition to the nest, he never strayed for more than 10 seconds. Although intruding fishes might momentarily distract the male from interim activities, he promptly returned to the nest and was thereby regularly available to receive a female's approach.

Nest associate species (i. e., cyprinids that use nests for spawning and feeding but do not contribute to its construction) did not congregate either over or near the *E. laurae* pebble nest. Associate species of nearby *N. leptocephalus* nests (e. g. *Campostoma anomalum*, stoneroller minnow) infrequently wandered towards the nest but were viciously driven away by the attending *E. laurae* male. While reshaping the mound, the male also dismissed bullfrog tadpoles (*Rana* sp.) that occasionally stumbled upon the nest.

Approach: An approaching female was occasionally driven away from the nest by the attending male. As she neared the nest, the male aggressively darted towards her. The female aborted her approach and quickly retreated from the vicinity as the male returned to his nest.

Alignment: At times a female moved onto the downstream slope of the nest, paused or passed beneath the male's tail, and swam downstream without initiating the run. Once aware of the female's departure, the crest postured male darted from the nest in momentary pursuit.

On two occasions, two females contended for position beneath the postured male's caudal peduncle. The first competition ended as the females departed downstream while the male remained crest postured. The second competition ended as a larger female chased a smaller one from the downstream slope over the crest of the nest (Fig. 16). Although neither female aligned with the male, their movement over the crest stimulated the male to swim down the upstream slope.

Run: A successful spawning run depended on the male's ability to gain a positon adjacent to the female whereby he could initiate the clasp. Runs failed if as the male turned towards the female, she broke away and either circled downstream or lingered over the upstream slope apart from the male. In either case, the male continued swimming to the base of the nest, turned, and circled to a crest posture.

On one occasion a male aborted the spawning run. As the male and a large female began swimming down the upstream slope, a smaller female moved onto the downstream slope. Midway down the upstream slope, the male stopped and circled back to a crest posture as the larger female continued the run to the base of the nest.

Clasp: A male appeared to have difficultly when attempting to clasp a female larger than himself. As the male and larger female ran down the upstream slope, the pair would quiver their bodies as they converged over its midpoint. The male did not arch his caudal peduncle, however, and their bodies remained appressed above the surface of the slope. The pair continued to the base of the slope where the female either broke away or lingered over the base of the upstream slope as the male circled to a crest posture. It is uncertain whether a successful spawn was completed.

Location of Fertilized Eggs

Presumably gametes were released as the clasped pair moved down the lower half of the upstream slope of the nest. Eggs were collected from the pebble interstices of the nest's upstream slope where water current velocities were greatly reduced. The greatest concentration of eggs occurred between the midpoint and the base of the slope. Eggs are demersal, nonadhesive and noncohesive.

Figure 11. Pebble mound nest of *Exoglossum laurae*.

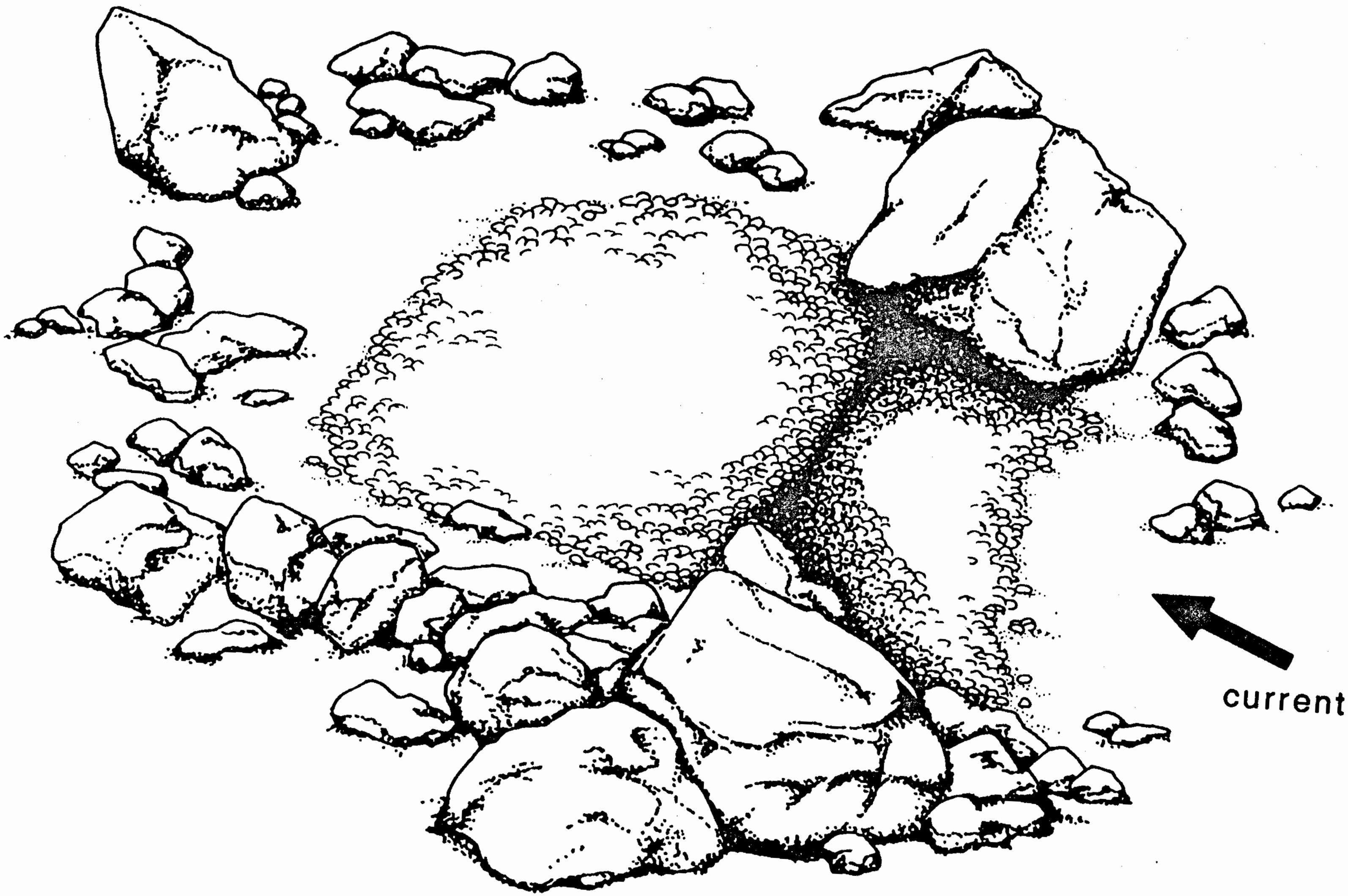
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Figure 12. Male *Exoglossum laurae* hovering over crest.

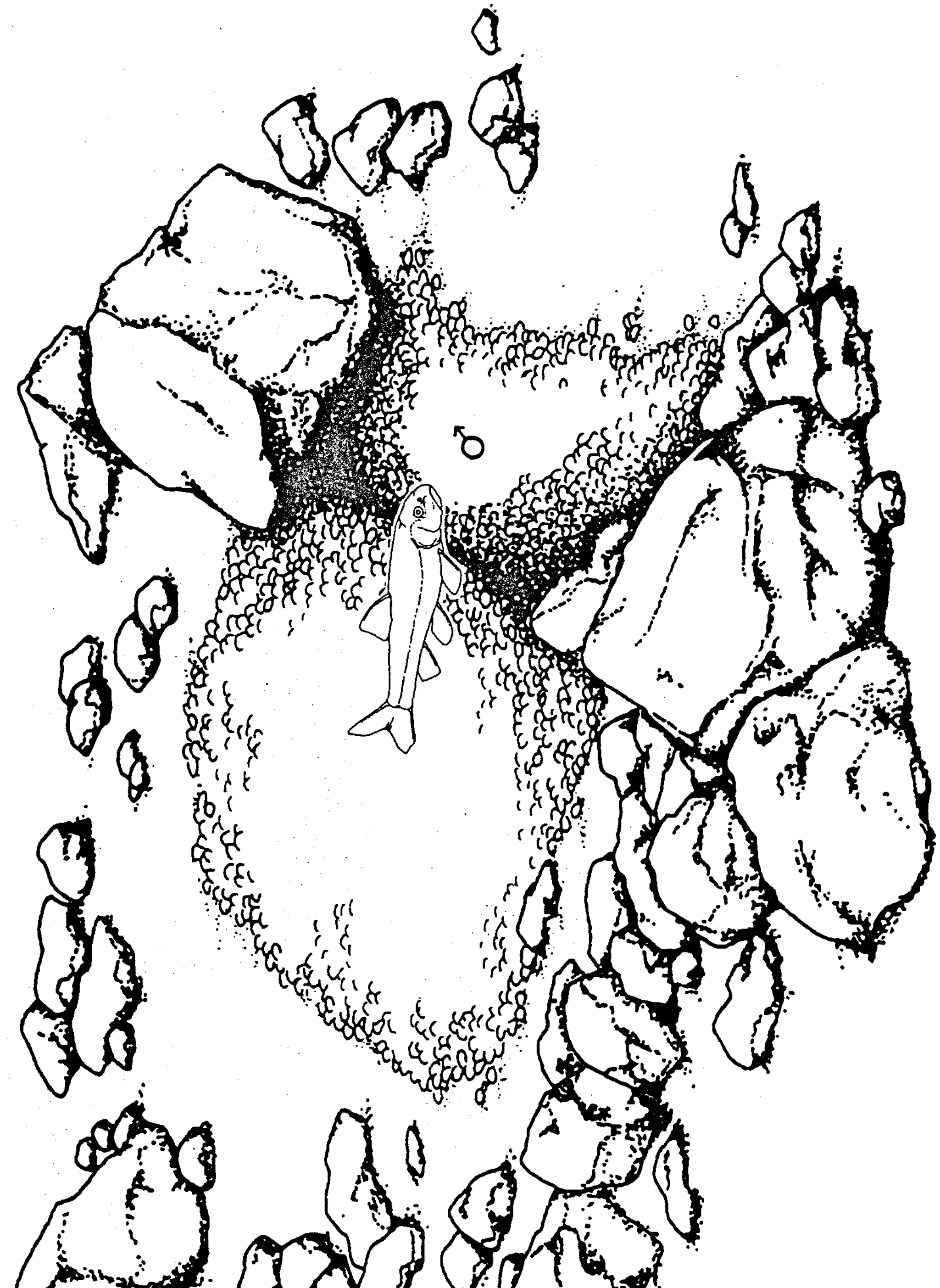


Figure 13. Female *Exoglossum laurae* passing beneath caudal peduncle of crest postured male.

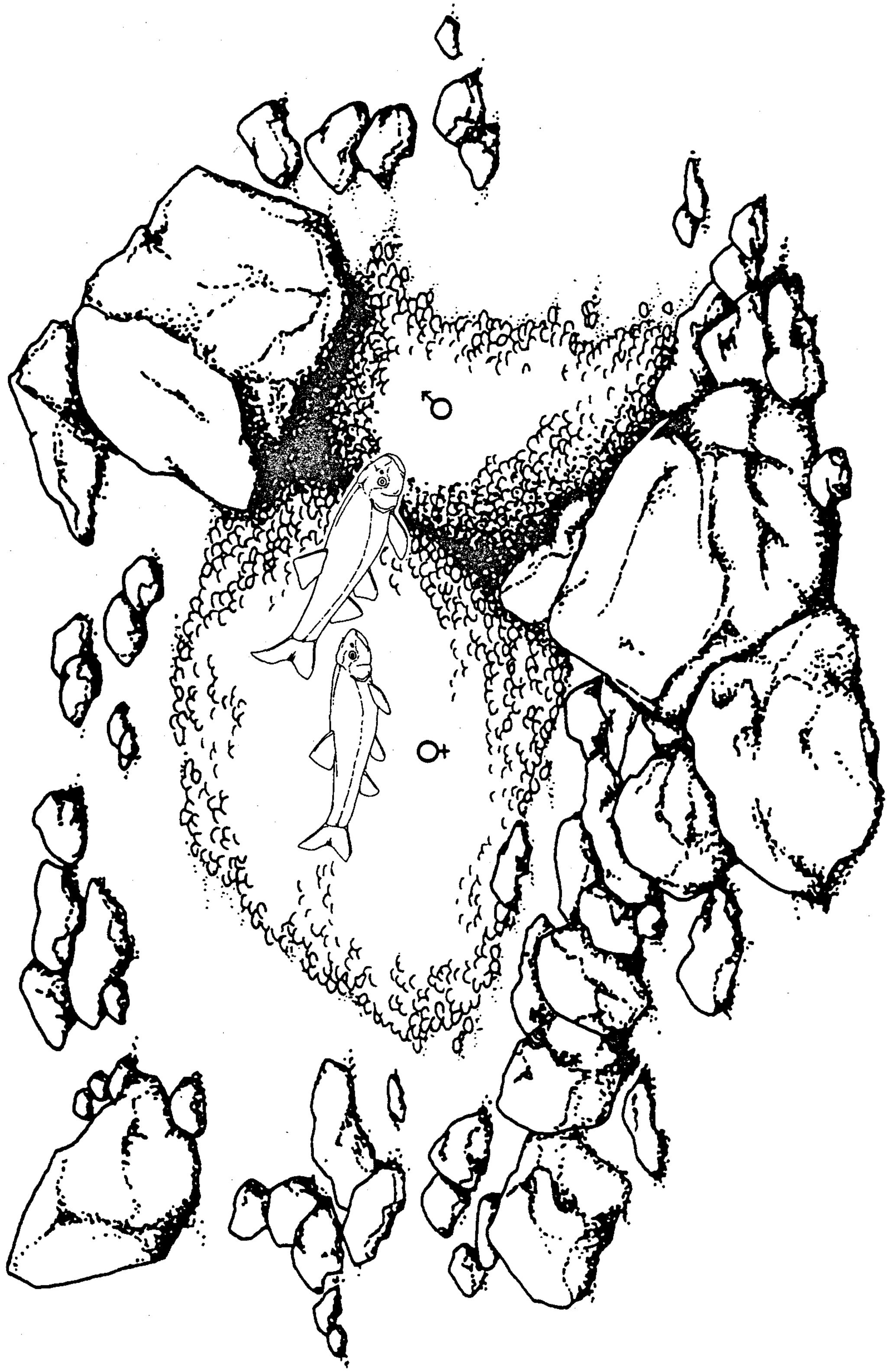


Figure 14. Spawning run of *Exoglossum laurae* down upstream slope of nest.

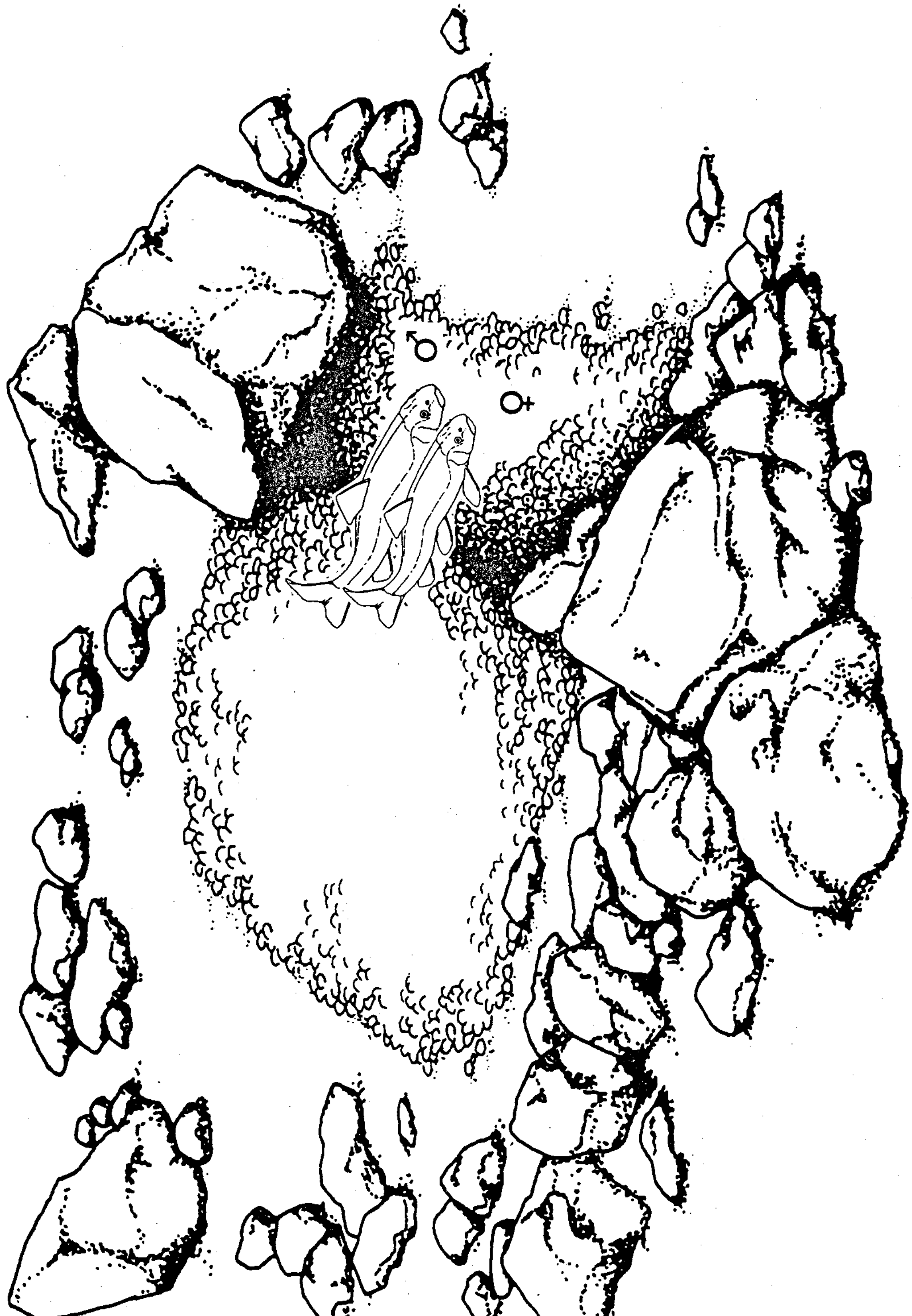


Figure 15. Spawning clasp of *Exoglossum laurae*.

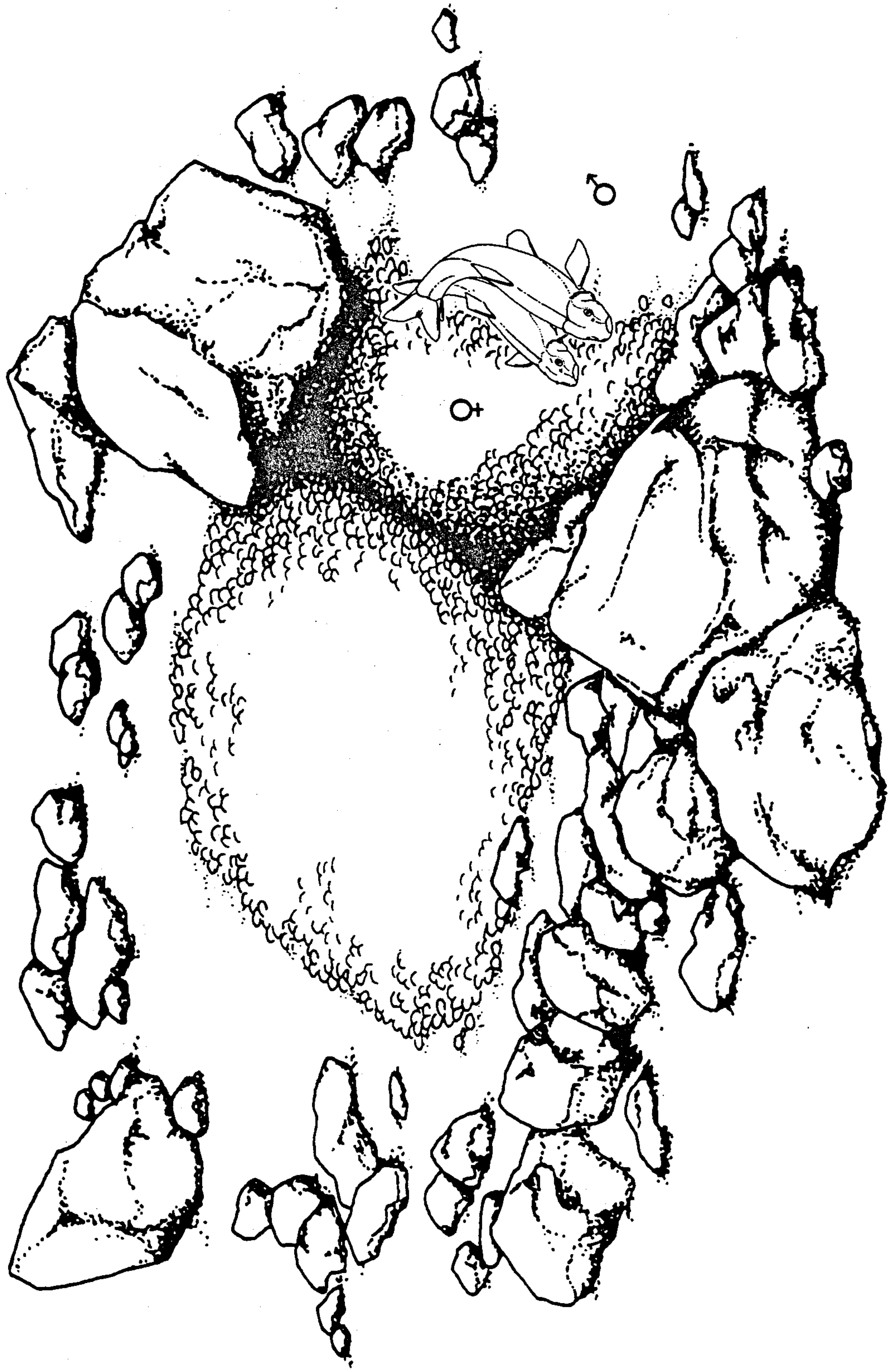
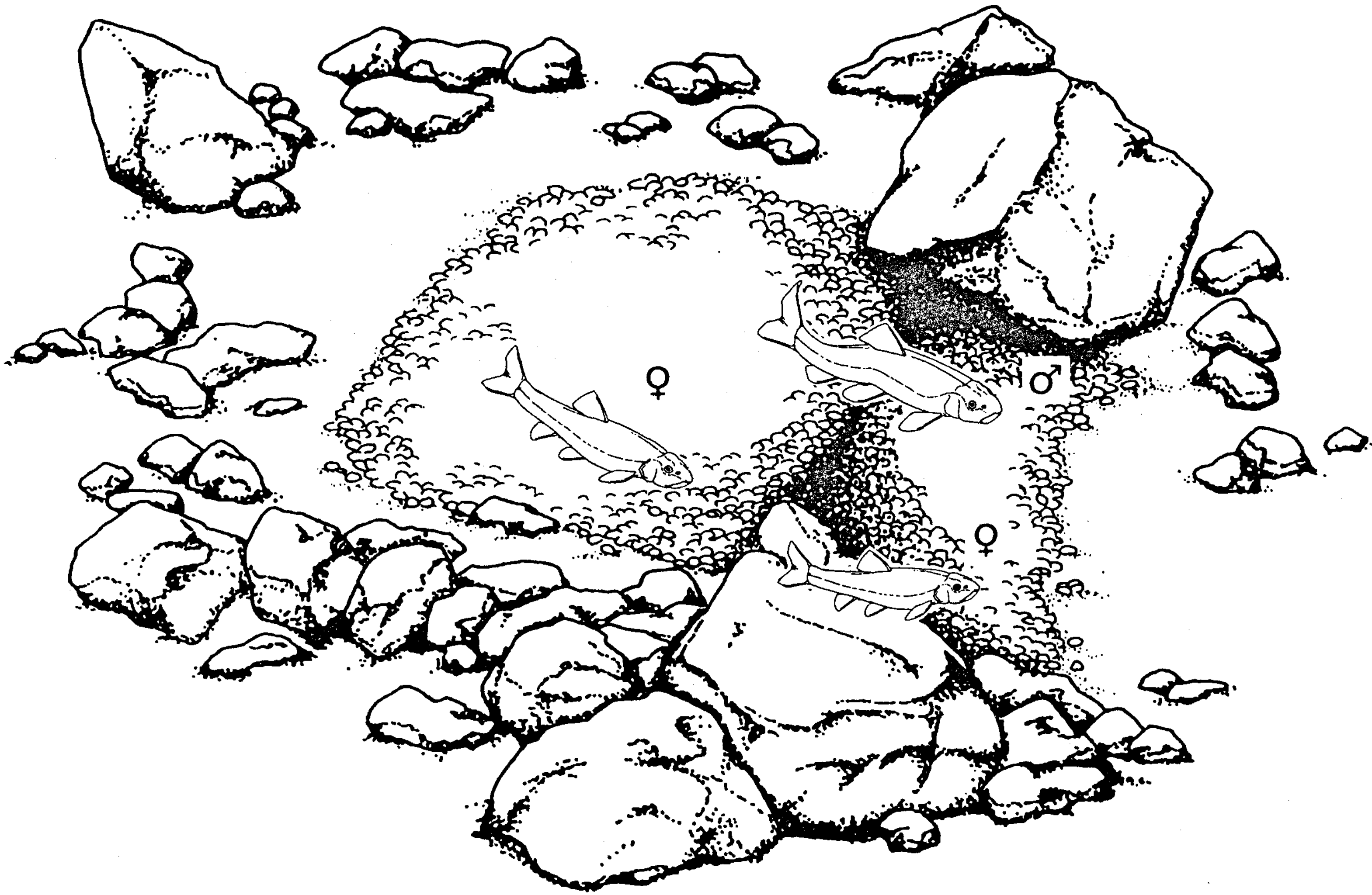


Figure 16. Female *Exoglossum laurae* competing for alignment with male.



Semotilus atromaculatus

(creek chub)

Literature

Nest-building, spawning and agonisitc behaviors of *S. atromaculatus* were originally described by Reighard (1910). Subsequent reports of *S. atromaculatus* spawning behaviors have been published by Hankinson (1932), Miller (1964, 1967), Sisk (1966), Raney (1969), and Moshenko and Gee (1973). Other studies on the creek chub have described propagation in artificial streams (Washburn, 1948), nest entry of females (Ross, 1976), male aggression (Ross, 1977; Miller, 1964), and life history and ecology (Greeley, 1930; Copes, 1978). In 1990, Maurakis et al. compared the pebble nest microhabitats of four species of *Semotilus*.

Spawning Substrate

The pebble nest of *S. atromaculatus* exemplified a dynamic morphology. With the onset of spawning, the basic topography of the nest studied (EGM-VA-221; Appendix, Table 1) was that of an elliptic spawning pit bordered upstream by a ridge of pebbles (Fig. 17). The pit was approximately 10 cm wide, 4 cm deep and its long axis extended about 15 cm downstream from the ridge which paralleled the current (Maurakis, pers. comm.). The ridge, composed largely of stones between 6.0 and 23.0 mm (Maurakis et al., 1990), was appoximately 15 cm wide and rose about 4 cm above the streambed. Its downstream slope descended approximately 8 cm into the pit at about a 60 degree angle.

Stones excavated from the downstream margin of the pit were deposited on the downstream slope of the ridge. In a period of about 2 hours the length of the ridge was extended approximately 0.4 m downstream. Through this activity, the basin of the spawning pit and pit-ridge interface were continually displaced downstream. Dimensions of the pit remained relatively constant. Water current velocities in the spawning pits of nests of *S. atromaculatus* are significantly less (about 0.04 m/sec) than those measured either upstream of the ridge, over the ridge, or downstream of the pit (Maurakis et al., 1990).

Sequence of Spawning Behaviors

Interim: Between spawning episodes, the male *S. atromaculatus* actively extended the ridge downstream. Using his jaws, he removed pebbles from the downstream margin of the pit and deposited them upstream on the interface of the ridge and concavity. Excavation occurred as he dipped his head, beat his tail vigorously and drove his snout into the substrate. The rooting action displaced sand downstream. Gaining a purchase on a pebble, he lifted his head, swam quickly upstream into the pit and up the downstream slope of the ridge. After depositing the stone on the ridge, he drifted downstream tail first to repeat the procedure. The male paused for up to 1 minute between excavations and hovered either in the pit or with his snout over its downstream rim.

The male rarely ventured from the spawning pit between female approaches. Unlike males of *Exoglossum* and *Nocomis* species, the male *S. atromaculatus* did not collect substrate material from the surrounding streambed for deposition on the nest. The ridge was compiled entirely from pebbles removed from the rear margin of the pit.

Approach: A female's approach usually originated from a position downstream of the nest. While the male excavated, five females hovered over a discrete area from 0.5 to 1 m downstream of the pit-ridge nest. From this position, from one to three females swam upstream either directly towards or to the side of the pit. Movement of one female upstream often stimulated others to follow.

A successful approach was completed once the female moved into the spawning pit and posted herself at the base of the ridge's downstream slope. Although a typical approach of a female was from downstream of the nest, she might enter the pit from any direction. When entering from downstream, she quickly darted over the rear margin to the upstream end of the pit. Alternately, she might swim upstream to the side of the pit and glide laterally to the base of the pit-ridge interface. Finally, a female from downsteam might swim past the pit to a position either over or upstream of the ridge. When entering from upstream, the female always drifted tail first into the pit.

A female's movement into the spawning pit appeared opportunistic. That is, her entrance depended on the relative position of the male and the accessibility of the pit. A female frequently entered over the downstream rim as the male was positioned either over the ridge or the upstream end of the pit. If, for instance, the male deposited a pebble on the ridge, a female might dart beneath him and post herself in the upstream end of the pit as he drifted downstream.

Upstream and lateral entries often occurred as the male occupied the downstream rim of the pit. For example, if the male rooted at the rear margin, a female approaching him from downstream might swim in a semicircle upstream either to a position in the pit or over the ridge.

If the male, hovering directly above the pit, discouraged a female from entering over the downstream rim, she reacted by circumventing him and swimming either to the ridge or the side of the pit. The poised male might then proffer access to the pit by drifting to the downstream margin. If he held his position, the male conceded access to the pit only if the female motioned towards him. As she either drifted downstream or glided laterally toward the pit, the male rose into the water column allowing her to assume a position at the base of the pit-ridge interface.

Alignment: As the female moved into the upstream end of the pit, the male quickly aligned directly above and parallel to her body. Lifting his head he rose into the water column, and with from one to four tail beats mounted the posted female. His snout was either even with or slightly behind hers. His pectoral fins remained fully extended, straddling her dorsum. While the male remained poised in this position for up to 2 seconds, the female remained appressed to the upstream floor of the pit, her snout touching the downstream base of the ridge.

Run: The spawning run was initiated by the female. With from four to six tail beats, she swam upstream from beneath the male following the contour of the pit-ridge interface. The male quickly responded to her forward motion, and with from two to three strong tail beats positioned himself even with and to one side of the female. As he began moving upstream, he folded his pectoral fin nearest the female and then extended it beneath her head.

Before reaching the apex of the ridge's downstream slope, the female abruptly pitched her head upwards as her tail and caudal peduncle remained appressed to the slope. Her mouth opened as her vent remained arched towards the pebble ridge. At the height of the frontal retroflexure, the anterior region of the female (i. e., from her snout to her pelvic girdle) became vertical and approximated a 90 degree angle with her caudal peduncle.

It is uncertain what triggers the female to vigorously throw her head into the water column. It has been proposed that the movement of the male's tuberculated pectoral fin from a postion extended over the female's back to a position beneath her head stimulates her to arch anteriorly. More likely, however, the occurrence and timing of a female's retroflexure is a function of pebble nest topography and its effects on water currents at the pit-ridge interface. For example, female *Semotilus thoreauianus* (Dixie chub), whose spawning activites are like those of *S. atromaculatus*, were observed on video-tape to perform solo retroflexures independent of a male *S. thoreauianus.* As the male relaxed from clasping one female, two unaccompanied females quickly ran up the downstream slope of the ridge and pitched their heads upwards as their tails became even with the base of the pit-ridge interface. Although unaccompanied retroflexures have not been observed in *S. atromaculatus*, females may be similarly stimulated as the topography of the nests of the two species is identical.

Clasp: As the female began arching her head upwards, the male contorted his body into a sigmoid configuration (Fig. 18). The male simultaneously turned his head sharply before the female and drove his posterior flank towards her epaxial region just below the dorsal fin. His tail and caudal peduncle faced away from the female in a direction opposing that of his head. At times the male's posterior flank appeared to momentarily anchor the female's caudal peduncle to the pit-ridge interface as she arched anteriorly into the water column.

The sigmoid contortion progressed to a full spawning clasp with the male's body completely encircling the uplifted female (Fig. 19). At the height of the constriction, the male faced downstream with his caudal peduncle either perpendicular to the current or arched upstream up to 45 degrees beyond perpendicular. Turning downstream, his head displaced the vertical female towards the concave side of his body as his caudal peduncle prevented her from drifting downstream. At the focal point of the clasp, the female was compressed laterally and dorso-ventrally between her pectoral and pelvic fins.

Dissociation: The female was released in the vertical position as the male turned his head upstream in the opposite direction and swung his body parallel to the direction of current. She either immediately rolled upright with head facing downstream or drifted vertically up to 20 cm from the ridge before falling forward.

Once balanced the female either retreated downstream or remained in the vicinity of the pebble nest from where she might initiate another spawning sequence. The male either resumed excavation or remained poised either in the spawning pit or over the downstream rim.

Behaviors Disruptive of the Spawning Sequence

Interim: The appearance of an intruding male *S. atromaculatus* in the vicinity of the pebble nest distracted the attending male from interim activities. If the intruder vacated the vicinity of the pebble nest, the attending male resumed normal interim behaviors. If the intruder did not vacate the area, one of three forms of intraspecific agonistic behaviors were observed: parallel swims, head butting, and serpentine displays. Parallel swims occurred as the two males aligned eye to eye with their dorsal and paired fins extended. Beating their tails vigorously, the pair either drifted downstream or moved slowly upstream. While aligned they might exchange as many as 14 violent head butts before separating (Fig. 20). Lateral head butts occurred as the aligned males moved in close, their proximal flanks touching at times. Each male then forcefully arched his midbody driving his tuberculate head and tail into the those of his adversary. They appeared to push off of each other as their bodies contracted into opposing crescent shaped figures. Serpentine displays were occasionally performed by the attending male when positioned upstream of the intruder. Displays occurred as he deliberately threw his head from side to side and undulated his body up to six times while moving slowly upstream.

Approach: A female might hover for up to 9 seconds either upstream, downstream, or to the side of the spawning pit without moving into the pit to assume a post position. The male dismissed the tarrying female if she failed to motion towards the pit.

Alignment: Alignment failed if a female entered and then withdrew from the spawning pit without initiating a run. She either quickly exited as the male rose in the water column to mount her or remained posted beneath the aligned male and rooted in the pit-ridge interface for up to 4 seconds before exiting. At times, the male remained poised over the pit as she exited. Alternately, he either displaced the stalled female with a head butt or momentarily chased her from the pit.

Run: To reiterate, the spawning run is a female initiated event. If the aligned male moved forward up the downstream slope of the ridge before the female initiated her run, she quickly exited the pit.

At times a female appeared crowded to the upstream floor of the concavity beneath the aligned male. When unable to initiate her spawning run up the pit-ridge interface, she turned laterally, and exited either downstream or to the side of the pit.

On occasion, a female failed to retroflex at the end of her run. As she moved upstream on the pit-ridge interface the male turned his head and began arching his caudal peduncle over her back in an attemp to clasp her. The clasp was not completed, however, as she continued upstream over the ridge.

Clasp: Spawning clasps failed if the male was improperly positioned as the female arched to a vertical position. For example, if the male's arched caudal peduncle failed to cradle the vertical female posteriorly, the current quickly carried her downstream. At times the male's head failed to position the female within his constricted body. As he swung his head downstream his snout was driven into the abdominal region of the vertical female. It is uncertain whether this represents a successful spawn as the female did not occupy the focal point of the male's clasp.

Location of Fertilized Eggs

Eggs and sperm were extruded from the spawning pair during the clasp which occurred just below the apex of the downstream slope of the ridge where the current velocity was reduced. Gametes settled into the pebble interstices of the pit-ridge interface and were later covered by the male with material excavated from the downstream margin of the spawning pit. Fertilized eggs were collected throughout the pebble ridge from its upstream end to the base of its downstream slope (Maurakis, pers. comm.). Eggs are demersal, nonadhesive and noncohesive.

Figure 17. Pit-ridge pebble nest of *Semotilus atromaculatus*.

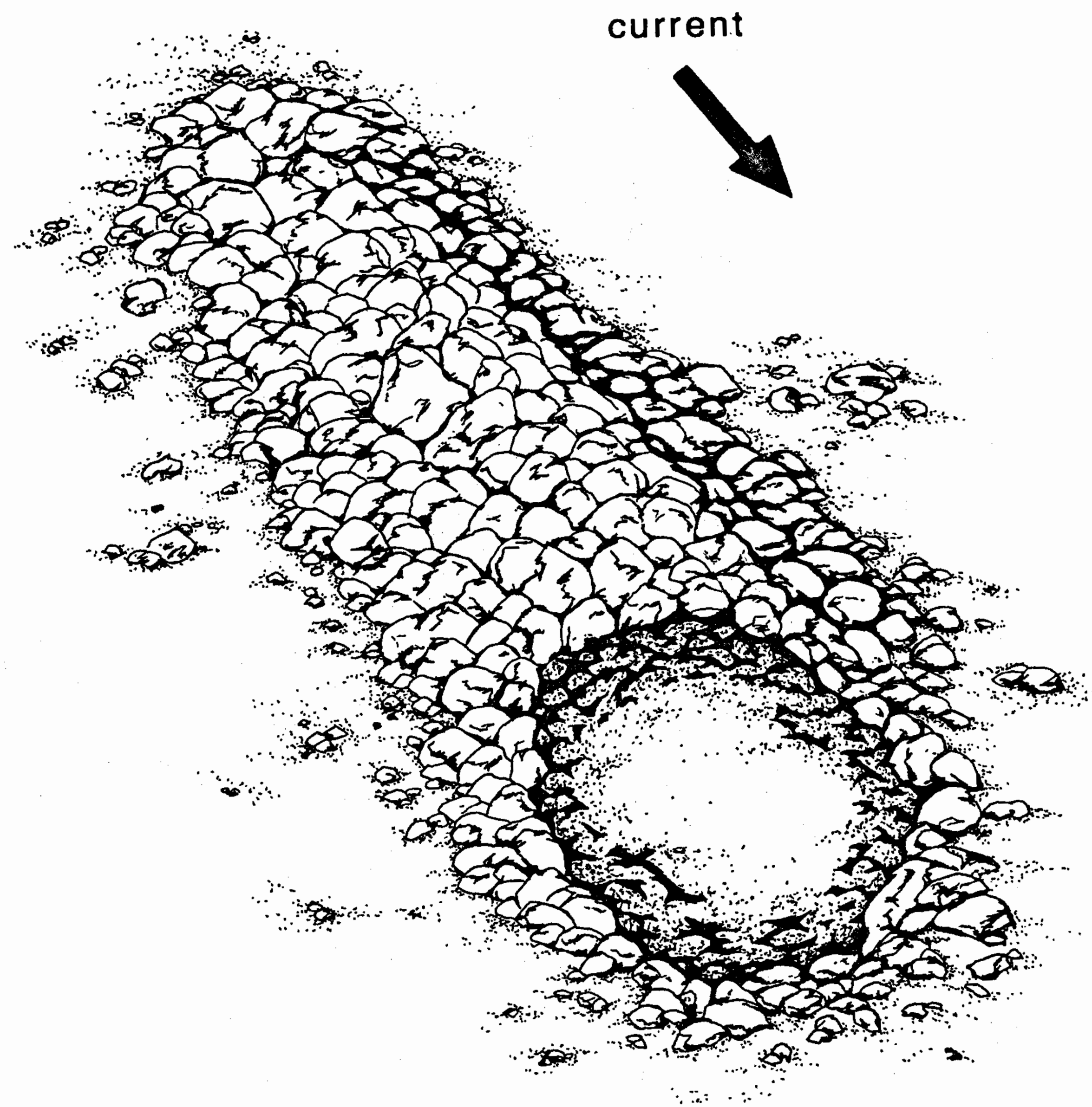

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Figure 18. Female *Semotilus atromaculatus* initiating retroflexure as male assumes sigmoid configuration.

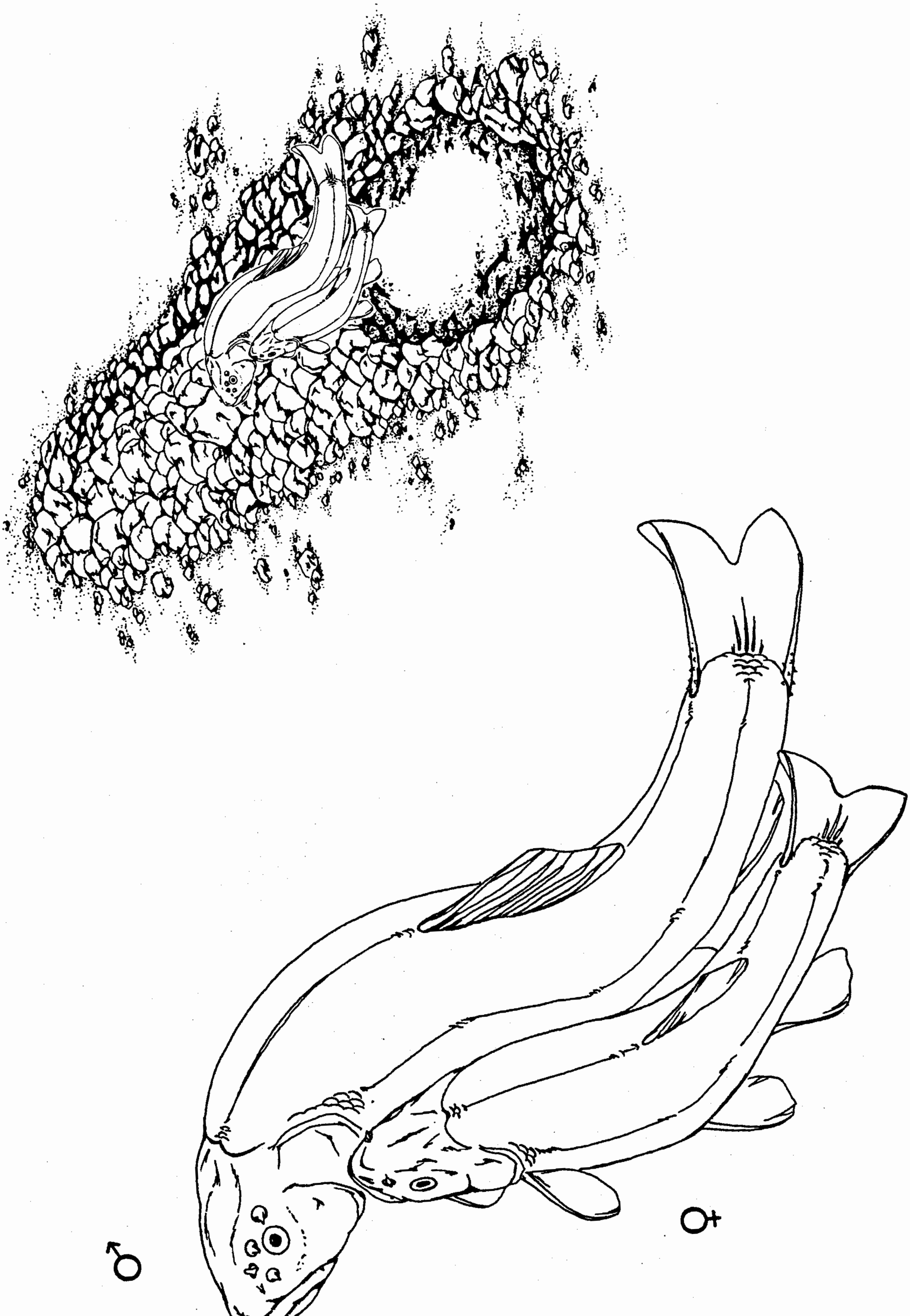


Figure 19. Spawning clasp of *Semotilus atromaculatus*.

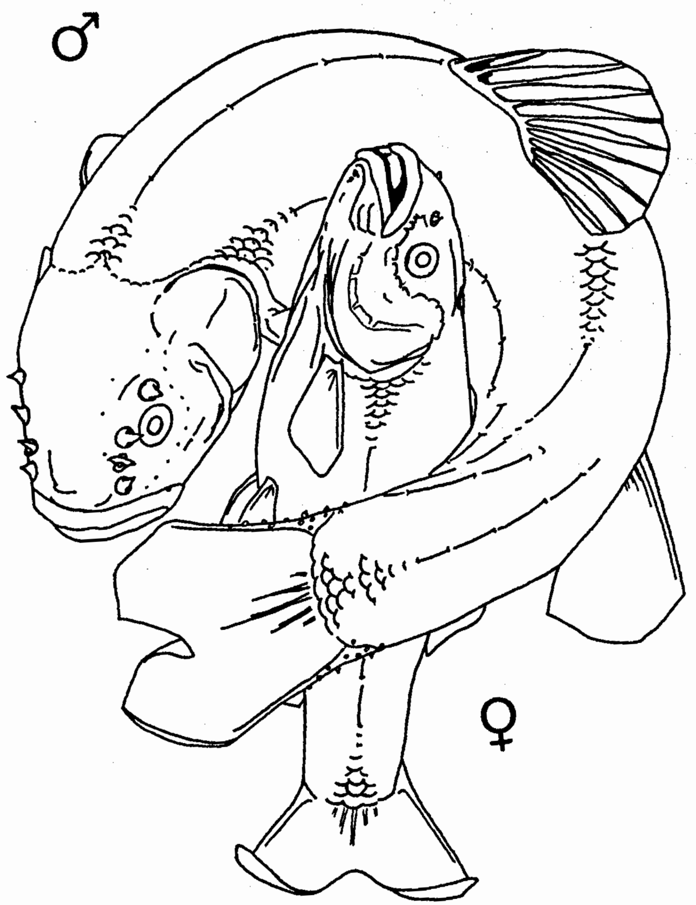


Figure 20. Male *Semotilus atromaculatus* exchanging lateral head butt during parallel swim.

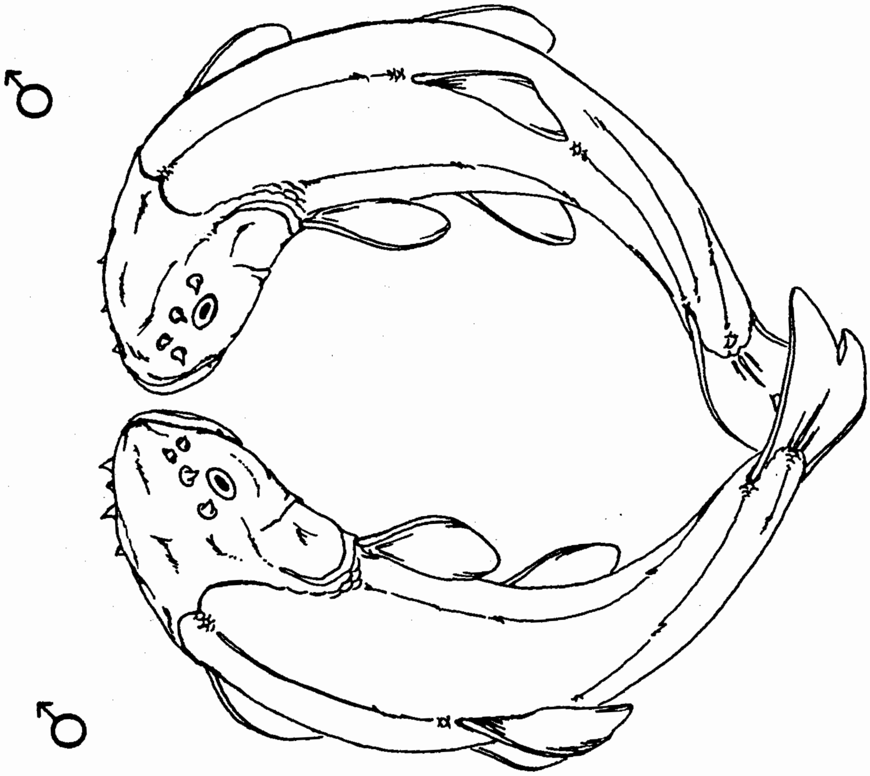
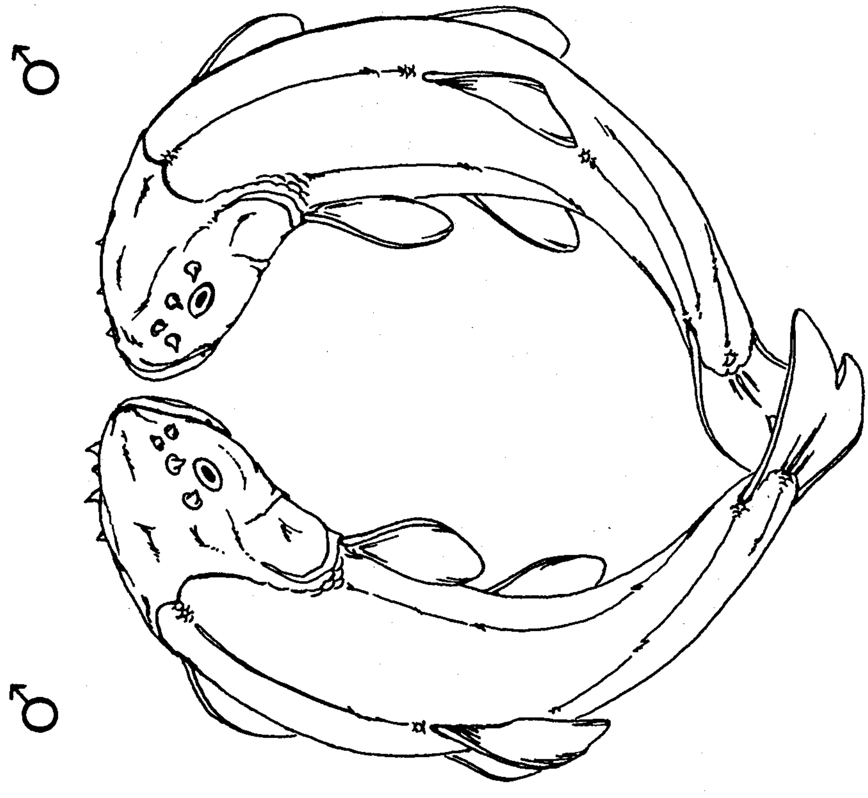


Figure 20. Male *Semotilus atromaculatus* exchanging lateral head butt during parallel swim.



Nocomis leptocephalus

(bluehead chub)

Literature

Raney (1947) reported nests of *N. leptocephalus* in the Roanoke River, Virginia. Concurrently, he described hybrids of *N. leptocephalus* X *Campostoma anomalum*. Lachner (1946) described the morphology, feeding and reproductive behaviors of three *Nocomis* species (*N. biguttatus, N. leptocephalus, and N. micropogon*) and later reported on their age and nuptial characteristics (Lachner, 1952). An extensive investigation of the systematics, distribution , evolution and ecology of the chub genus *Nocomis* was conducted by Lachner and Jenkins (1967, 1971a, 1971b). Maurakis et al. (1991a) were the first to describe the spawning clasp in *N. leptocephalus*. Their phylogenetic analysis based on reproductive behaviors supported the monophyly of the genus *Nocomis* proposed by Lachner and Jenkins (1971a); however, species groupings within the genus differed between the two studies. Studies on pebble nests of the bluehead chub have described its composition (Doyle et al., 1989), its modification of water currents (Maurakis et al., 1992), and its use by *Notropis lutipinnis*, yellowfin shiner (Wallin, 1989), and by *Hybopsis rubrifrons*, rosyface chub (Maurakis and Loos, 1986).

Spawning Substrate

The topography of nests of *N. leptocephalus* is that of a shallow dome-shaped structure (Fig. 21). Upon completion the nest observed in this study (EGM-VA-225; Appendix, Table 1) was a circular mound of pebbles approximately 0.7 m in diameter with a central apex rising about 13 cm above the surrounding streambed (pers. obs.). According to Doyle et al. (1989), nests are composed of stones of moderate size (from 2.5 to 23.0 mm); however, the greatest portion (about 67%) of stones by weight are between 6.0 and 11.3 mm.

Two elliptic spawning pits were on the mound's upstream slope. Each pit was about 4 cm wide, 6 cm long and 2.5 cm deep. The upstream rim of each pit was poorly defined as it did not rise above the contour of the upstream slope. One pit was just below the midpoint of the nest's upstream slope; second was spaced about 10 cm downstream and to the side of the first. Water current velocities in spawnings pits of *N. leptocephalus* are less than 0.01 m/second, significantly less than current velocities over the nest and in the surrounding stream (Maurakis et al., 1992).

Sequence of Spawning Behaviors

Interim: In *N. leptocephalus*, spawning sequences were prefaced with the pit postured male. Head facing upstream, he hovered with his head and pelvic girdle positioned from 2 to 4 cm above the floor of the pit. In this position, his pectoral, pelvic and dorsal fins remained fully extended. The anterior portion of the male was angled slightly downwards such that the frontal plane of his body paralleled the upstream slope of the nest (approximately 10 degrees).

The male routinely reshaped and fanned the spawning pit with his anal fin between postures. Reshaping occurred as the male dipped his head and drove his snout into the floor of the pit while beating his tail vigorously. Seizing a stone with his jaws, he lifted his head and swam forward with tail quivering. As he deposited the material upstream, he precisely positioned his anal fin over the pit. Lowering his caudal peduncle, he vibrated his extended anal fin in the pit for up to 2 seconds (Fig. 22). Pit fanning may clear sand and loose debris from the recently excavated area. At times, the male either shaped or fanned the pit without removing substrate material. Rooting with his snout, he displaced pebbles to the sides of the pit. When fanning without prior excavation, he first dipped his head into the pit before moving forward to coordinate the positioning of his anal fin with the pit.

Approach: Between spawning episodes, as many as 10 females either hovered or moved randomly over the rear slope of the nest and the area immediately downstream of its base. Although a female might swim towards the nest from any direction, she always approached the postured male from downstream.

A successful approach was completed as a female moved into the pit beneath the postured male. She entered the pit from a position either directly behind his tail or to one side of his body. Swimming over the apex of the nest onto the upstream slope she darted beneath his elevated tail over the downstream rim of the pit. If she swam onto the upstream slope to the side of the postured male she darted laterally into the pit.

Alignment: Alignment refers to the position assumed by a female upon entering the pit and the male's response to her intrusion. After entering the pit over the downstream rim, a female moved forward between the male's extended pelvic fins and placed her snout either directly beneath or slightly ahead of his pectoral girdle. When entering from the side, she similarly positioned her snout beneath his extended pectoral fins. In the pit her body aligned parallel to its long axis as her ventral surface became appressed to its floor. Upon gaining this position she either remained posted in the pit for up to a second beneath the male or immediately initiated the spawning run.

As the female's snout moved beneath his pectoral girdle, the male responded by tilting the dorsal half of his body to one side (Fig. 23). The female's movement into the pit determined the direction of his sagittal tilt. If she darted laterally into the pit, his dorsal surface tilted in the direction of her entry.

If she moved over the downstream rim directly beneath his tail, he might angle his body in either direction.

Run: The spawning run was initiated by the female. The run began as she moved from directly beneath to one side of the male's tilted body. With her body appressed to the substrate she swam upstream quivering her tail and caudal peduncle. With body tilted, the male quickly accompanied her forward movement with rapid tail beats.

As the run progressed, the ventral surface of the female conformed to the to the topography of the spawning pit. As a result, her head became elevated relative to the position of her tail as she moved up the upstream slope of the pit. Continuing her motion forward, she gaped and pitched her head vertically into the water column in a manner similar to that described for female *S. atromaculatus*. Unlike *S. atromaculatus*, retroflexures of female *N. leptocephalus* included a twisting motion that began anteriorly and continued over the entire length of the her body. She initiated her upward pitch with body upright and the ventral surface of her caudal peduncle appressed to the upstream slope of the pit. Her snout traversed a 45 degree arc as the side of her head apposed either the male's opercle or his anterior flank just above the pectoral fin (Fig. 24). As her snout became vertical she rolled the anterior portion of her body in such a way that her dorsum became appressed to the male's anterior flank (Fig. 25). The twisting motion continued posteriorly and appressed the lateral surface of her caudal peduncle to the substrate. Although her retroflexure initiated in the frontal plane of her body, it concluded in the sagittal plane. The sagittal component of the retroflexure might stop her forward motion thereby positioning her vent below the upstream rim of the pit. At the height of the flexure, the anterior portion of the female (from snout to pelvic girdle) became vertical and approximated a 90 degree angle with her caudal peduncle.

It is uncertain as to what stimulates the *N. leptocephalus* female's retroflexure, but like *S. atromaculatus*, her retroflexure may be topographically cued by the spawning substrate (i. e., upstream slope of the pit) and its effect on water currents.

Clasp: The male initiated the clasp as the female pitched vertically into the water column. As she arched frontally, he turned his head towards hers and began to curve his posterior flank over her back (Fig. 24). As the arch of her body twisted sagittally, the male drove his posterior flank into her side between her pectoral and pelvic girdles. His body (from snout to anal fin) contracted into a semicircle and appeared as a "horsehoe" about the vertically pitched female (Fig. 25). At the height of the clasp, the male's vent became appressed to the dorsolateral surface of the female's caudal peduncle.

The sagittal twist to the female's retroflexure directed the ventral surface of her vertical body away from the concave side of the male's body. Positioned accordingly, her abdominal region was compressed laterally as the male constricted his body. While compressed, her vent remained in contact with the upstream rim of the pit.

Dissociation: After the clasp, the male's body relaxed parallel to the current as he drifted downstream to a posture position. Upon release, the female continued to rise into the water column with her body vertically oriented. She immediately rolled to an upright position as her tail became level with her head. Once balanced she either retreated downstream or drifted to a position behind the postured male from which she might initiate another spawning sequence.

Behaviors Disruptive of Spawning Sequence

Interim: Between spawning episodes, rather than pit posturing, the dominant male engaged in stone carrying activities (e. g. nest addition and reworking). He left the nest momentarily, collecting pebbles from the surrounding streambed to place on the mound nest. The male also reworked the nest by displacing pebbles from the periphery of the nest to its apex. Although constructive to the nest, such activities reduced the availability of the male to approaching females as spawning sequences always began with the pit postured male.

The attending male tolerated the presence of slightly smaller subordinate males. One small subordinate male exhibited interim behavior (e. g. posturing, excavation) and successfully spawned females over the pit that was not used by the nest-building male. However, an intruding male similar in size to the nest-builder elicited intraspecfic agonistic behaviors characteristic of *Nocomis* species (e. g. head butts, parallel and circle swims). Head butts occurred as the attending male drove his tuberculate head into either the head or body of the intruding male. Aligned eye to eye two males might parallel each other's motion, locking cephalic tubercles while moving upstream. When performing circle swims (Fig. 26), males swim head to tail in a tight circle for as long as 10 seconds (Maurakis et al., 1991). Contests ended when the attending male expelled the intruder from the vicinity of the nest.

At times the presence and activities of other fishes (i. e., nest associates) distracted the interim male. From 10 to 15 *Campostoma anomalum* males held positions scattered about the nest and vigorously rooted in the pebble nest with their snouts and mouths. If a *C. anomalum* encroached on the spawning pit of the *N. leptocephalus* male, he promptly diplaced the interloper either with his body or with sharp head butts. The male also performed dismissal swims to "clear" the spawning pit of intrusive nest associates. From a posture position he quickly turned his head laterally and swung his body through a complete circle downstream of the pit. The motion momentarily displaced fishes occupying positions near the pit.

Approach: Females actively competed for access to the spawning pit. Their jockeying for position downstream of the pit occasionally elicited dismissal swims by the postured male.

Female approaches appeared disrupted by the presence of the *C. anomalum* males. When crowded downstream and to the sides of the postured male, they physically obstructed a female's movement into the spawning pit.

Alignment: Alignment failed if a female either exited or became displaced from the spawning pit prior to her initiation of the run. She might remain posted in the spawning pit beneath the postured male for up to 4 seconds before turning laterally and retreating downstream. On two occasions a female was diplaced from her position in the pit. The first disruption occurred as an invading female moved laterally into the spawning pit that was already occupied by a female. Both females quickly exited dowstream as the male began his tilt. The second disruption was caused by a small *C. anomalum* male that attempted to align in the pit. As the postured male *N. leptocephalus* became inclined, the *C. anomalum* male darted laterally into the pit beside the female. As though frustrated, the male chub immediately exacted a powerful head butt downwards, expelling both fishes from the spawning pit.

Run: The same small *C. anomalum* male accompanied a total of nine spawning runs, disrupting six. He frequently held a position slightly upstream and to the side of the spawning pit. As the female initiated her run with tail quivering, he quickly darted towards her and became appressed to her flank opposite the *N. leptocephalus* male. Positioned accordingly, the stoneroller physically obstructed both the female's retroflexure and the male's clasping motion.

Infrequently, runs were disrupted as the female failed to retroflex at the end of her run. As she exited the pit upstream the male either pursued her momentarily or drifted back to a posture position.

Clasp: On three occasions the retroflexed female and the concomitant *C. anomalum* male were clasped simultaneously by the *N. leptocephalus* male (Fig. 27). The female did not appear laterally compressed during the clasp as the male's posterior flank impacted the stoneroller's dorsal surface. It is uncertain whether any or all three of the fishes released gametes during interspecific clasps.

Three infrequent variations of the clasp might be considered disruptive of a successful spawn. On two occasions, the female pitched her snout through a 45 degree arc as she arched entirely in the frontal plane (Fig. 28a). The retroflexure failed to appress her dorsum to the concave side of the constricted male. In the absence of the sagittal twist, the male's posterior flank impacted the dorsal surface of the female during the clasp. On two occasions, the sagittal twist of the female's body was reversed appressing her breast and abdominal region to the concave side of the male (Fig. 28b). While the first two variations were female induced, a third was an inappropriate response of the male to the normal retroflexure of the female resulting in an uncompleted clasp (Fig. 28c).

Location of Fertilized Eggs

After up to 30 clasps over a single spawning pit, the male fills the depression with material removed from the pebble nest. As fertilized eggs were collected from the basins of active pits and sites on the upstream slope previously occupied by spawning pits, gametes may have been released just below the upstream margin of the spawning pit where the retroflexed female was laterally compressed by the male. As current velocity in the pit was reduced, the gametes settled into the pebble interstices of the pit floor. Eggs are demersal, nonadhesive and noncohesive.

Figure 21. Pebble mound nest of *Nocomis leptocephalus* with single spawning pit on upstream slope.

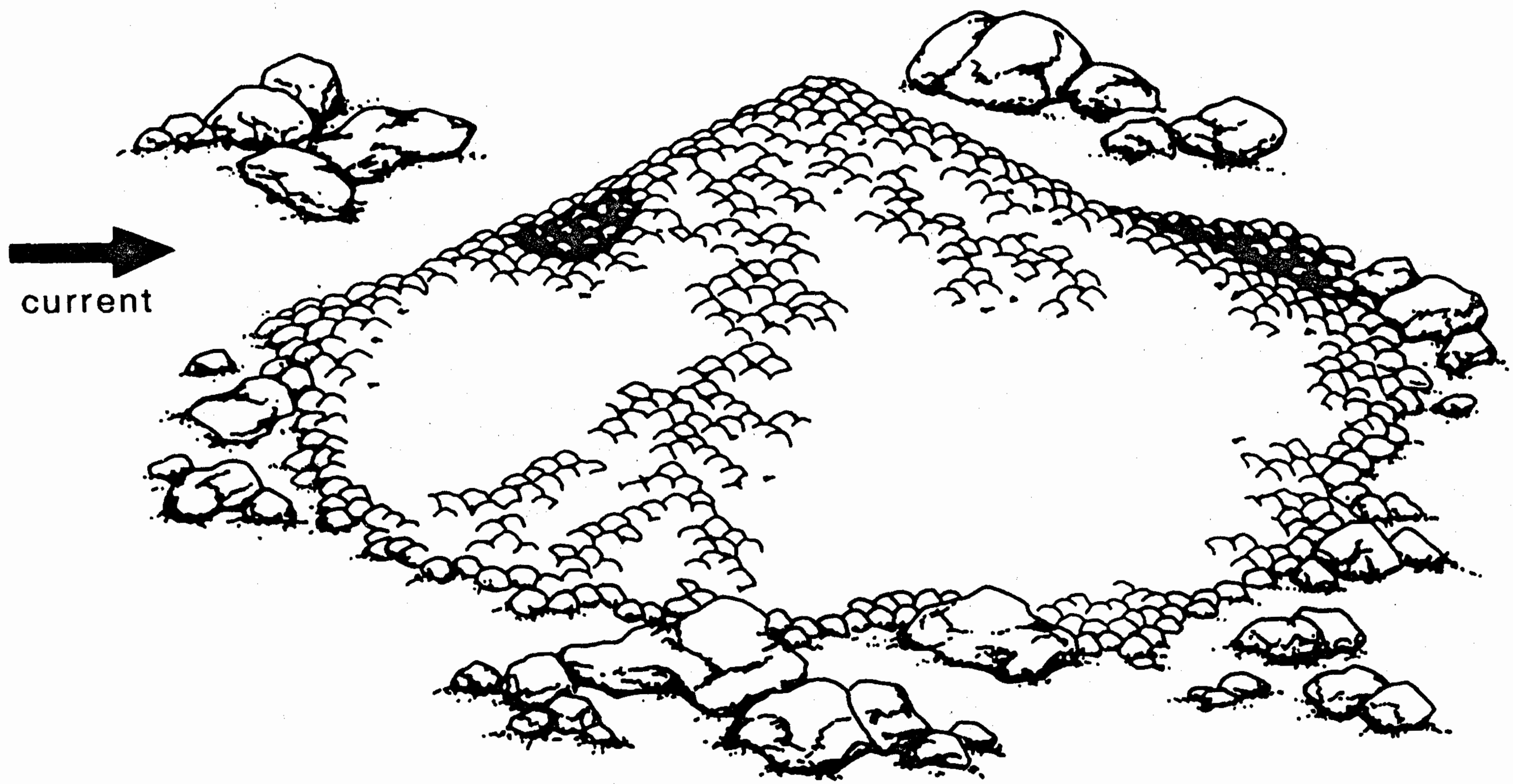
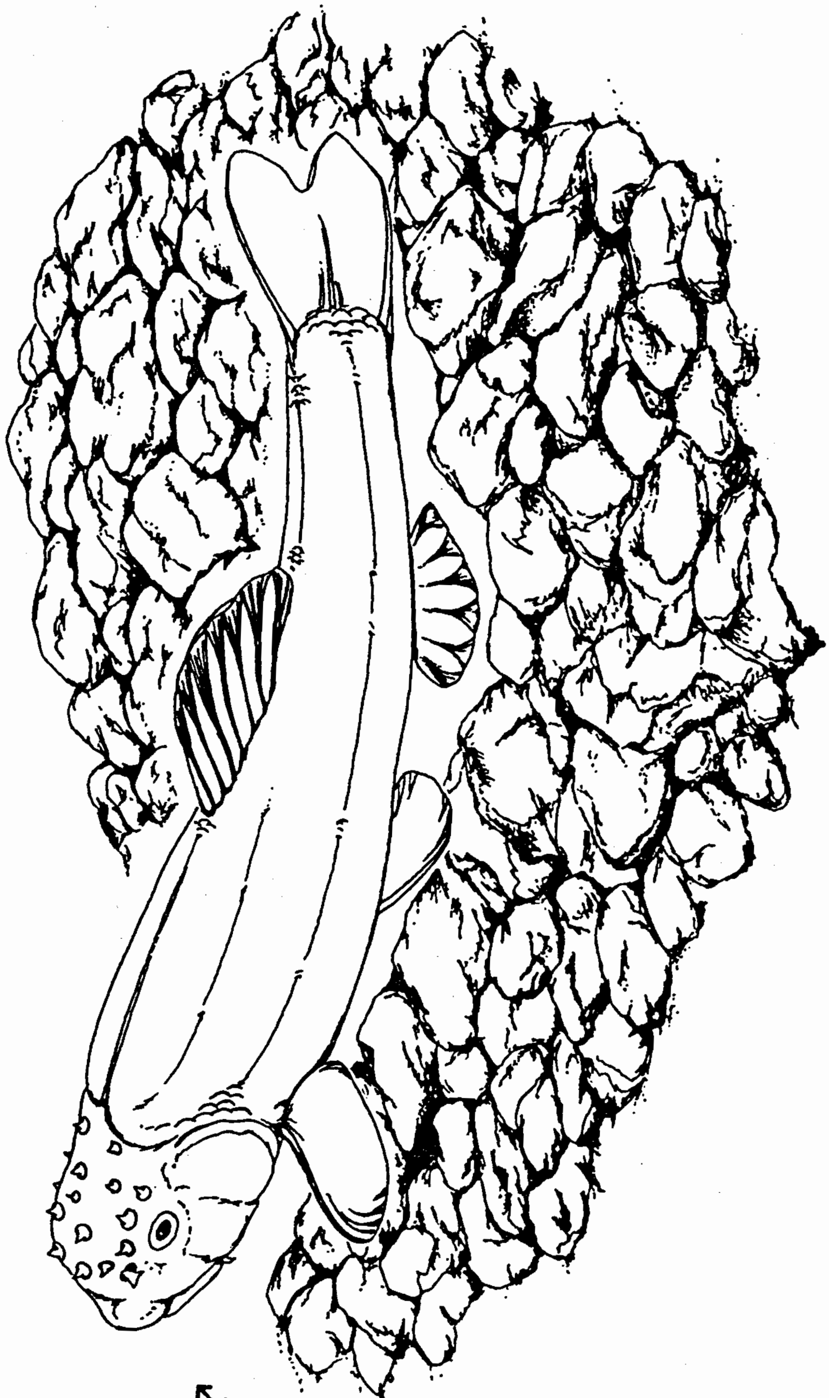

current

Figure 22. Male *Nocomis leptocephalus* fanning spawning pit with anal fin.



♂

Figure 23. Male *Nocomis leptocephalus* tilting body towards female posted in spawning pit.

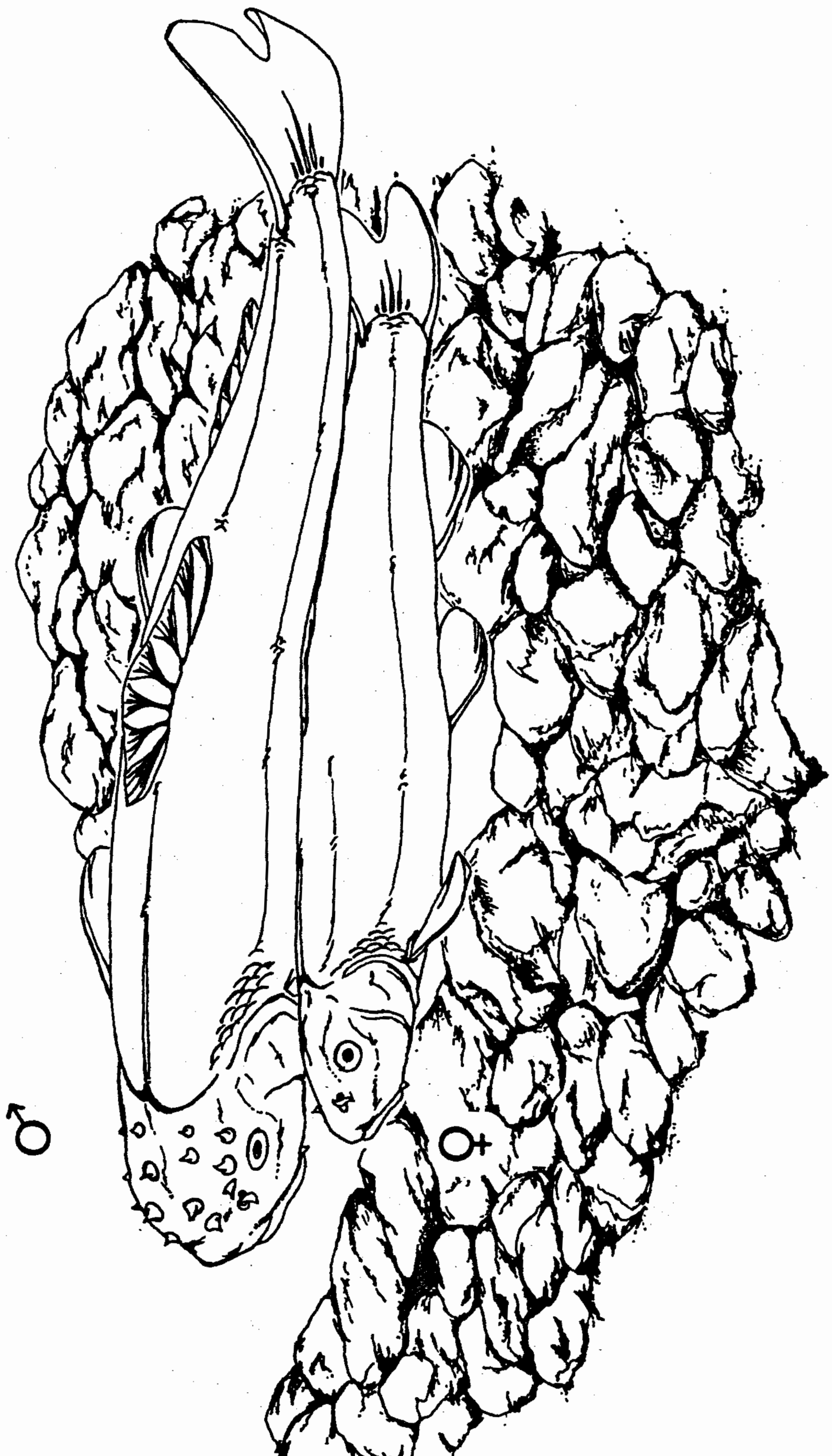


Figure 24. Female *Nocomis leptocephalus* initiating retroflexure in frontal plane of her body.

Figure 25. Female *Nocomis leptocephalus* completing retroflexure in sagittal plane of her body as male applies "horseshoe" clasp.

Figure 26. Male *Nocomis leptocephalus* engaged in circle swim.

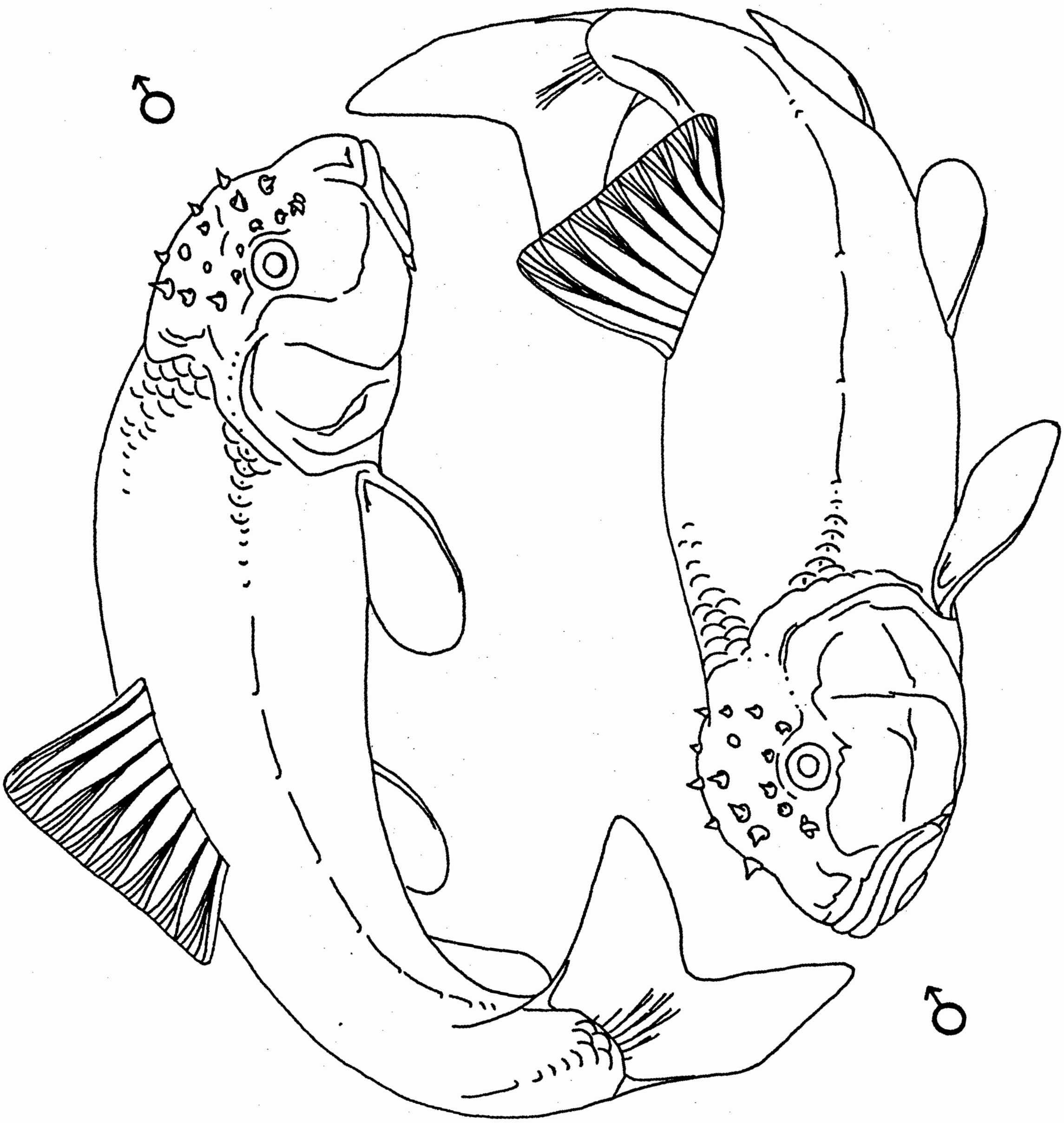
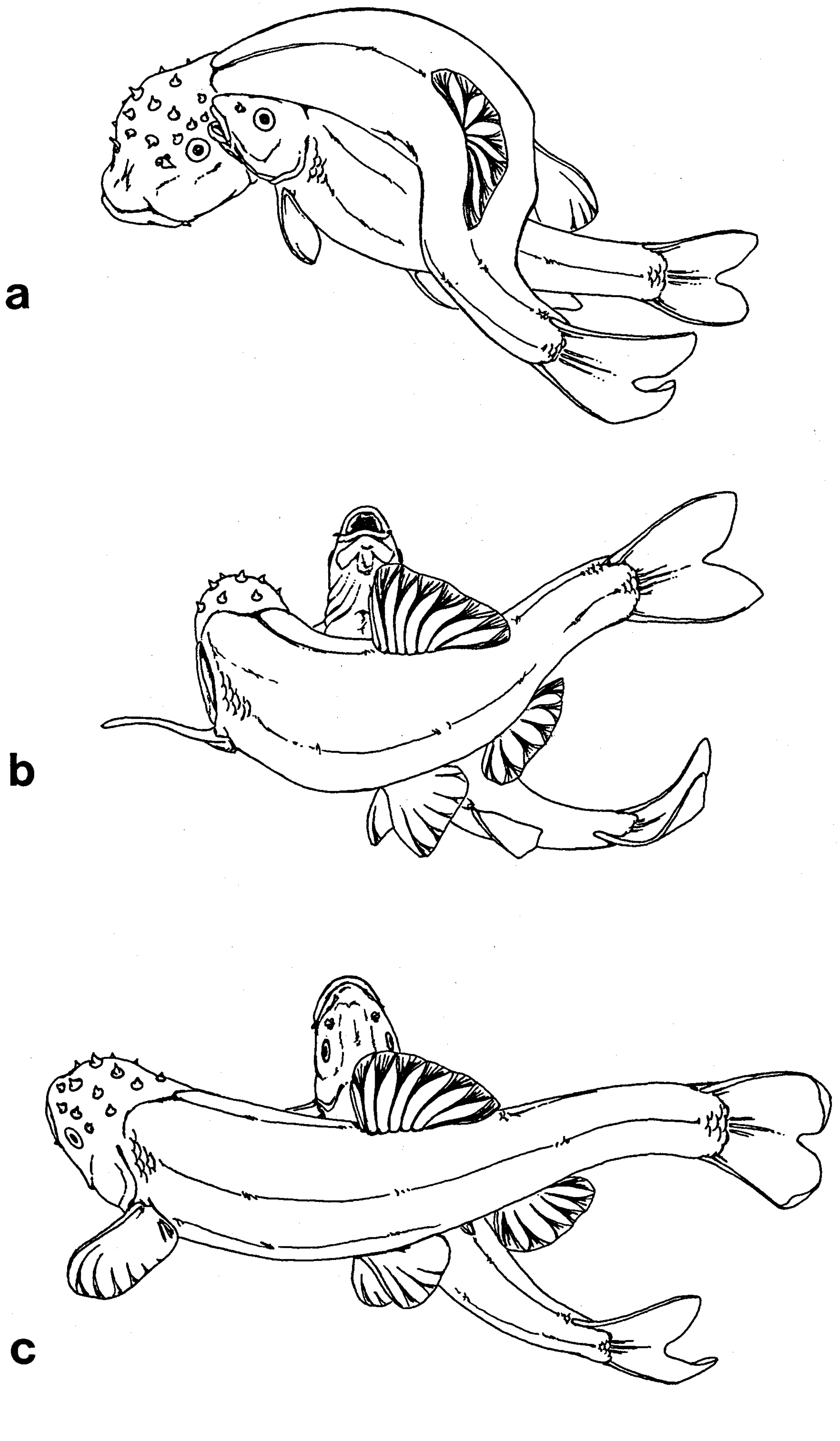


Figure 27. Interspecific clasp of female *Nocomis leptocephalus* and male *Campostoma anomalum* by male *Nocomis leptocephalus*.

Figure 28. Infrequent variations of the spawning clasp in *Nocomis leptocephalus*: a) uncompleted retroflexure, b) reversed retroflexure and c) uncompleted clasp.



a

b

c

Nocomis micropogon

(river chub)

Literature

Reighard (1943) provided a comprehensive description of the breeding habits of *N. micropogon* based on his observations of the species in Mill Creek, Michigan. Spawning behaviors in *N. micropogon* also were described and applied to a phylogenetic analysis of genus *Nocomis* by Maurakis et al. (1991a).

Spawning Substrate

The composition of *N. micropogon* pebble nests are similar to that of *N. leptocephalus* nests (Maurakis et al., 1991a). Upon completion the *N. micropogon* nest studied (EGM-VA- 254; Appendix, Table 1) was a roughly circular mound of pebbles approximately 50 cm in diameter with a central apex rising about 10 cm above the streambed (Maurakis, pers. comm.). A single spawning trough approximately 4 cm deep and 10 cm wide extended the length of the nest's upstream slope parallel to the stream's current (Fig. 29). Although structurally different from the spawning pits of *N. leptocephalus*, the trough on the upstream slope of the *N. micropogon* nest provided a similar region of slack water (Maurakis, pers. comm.).

Sequence of Spawning Behaviors

Interim: As in *N. leptocephalus*, the behavioral sequence preceding successful clasps in *N. micropogon* began with the postured male. Facing upstream, he held a position just above the apex of the spawning trough with the long axis of his body parallel to the direction of current. With paired and dorsal fins extended, he placed his pectoral girdle over either the apex of the nest or the downstream end of the trough. Positioned accordingly, the male was available to receive a female's approach.

Also like *N. leptocephalus*, the *N. micropogon* male routinely reshaped and fanned the spawning trough during interim. He reshaped the trough by rooting with his snout into its floor and moving pebbles with his jaws to its lateral margins. When fanning, the postured male dipped his anal fin into the trough before swimming forward with tail quivering. As he lowered his caudal peduncle, he vibrated the extended anal fin for up to 2 seconds in the upstream end of the trough. After these activities, the male drifted tail first to the apex of the nest and resumed a trough posture.

Approach: As many as three females either hovered or moved randomly over the rear slope of the nest and the area immediately downstream. As in female *N. leptocephalus*, a successful approach originated from a position downstream of the interim male. As he postured over the apex of the nest, she swam upstream either directly towards or to one side of his tail. She darted into the trough either beneath his caudal region or over the lateral margin of the trough. If the trough was inaccessible, she delayed entry until the male is correctly postured and the space becomes available. If a female initiated an approach when the male was trough rooting or fanning, she similarly delayed her entry until the male began to drift downstream.

Alignment: In the trough the female aligned parallel either directly beneath (i. e., her snout straddled by his extended pectoral fins) or to one side (her snout even with and just above the base of his pectoral fin) of the postured male. As she aligned in the trough, the male imparted a sideways tilt to his body with one or two strong beats of his tail. The male and female became appressed as he inclined the dorsal half of his body toward her and extended his pectoral fin beneath her head.

The direction of the male's tilt was determined by the female's alignment in the trough. For example, on one occasion a female entered the trough from a position slightly to the right of the postured male. As she moved laterally into the trough, he began tilting right in the direction of her entry. The female passed beneath the inclined male and came to occupy a position in the trough to his left. He quickly became upright and tilted left according to the final alignment of the female. Evidently, the final direction of the male's sagittal tilt determines to which side the clasp will be directed.

Run: As the male tilted, the female moved from beneath his abdominal region and initiated the spawning run with several quick beats of her tail. Still inclined the male promptly accompanied her forward movement with three to seven rapid tail beats. As the pair moved upstream, he remained slightly ahead with either his eye or the posterior margin of his opercle even with the female's snout.

The female followed the contour of the substrate 3 to 6 cm upstream with her dorsal surface apposing the male's flank and her side crowded to the lateral face of the trough. Upon reaching the upstream rim of the trough she pitched her snout upwards into the water column in a manner similarly described for *N. leptocephalus* females. Her vent and the ventral surface of her caudal peduncle remained appressed to the upstream slope of the trough near the perimeter of the pebble nest.

Unlike *N. leptocephalus*, retroflexures of *N. micropogon* females were generally less acute and occurred entirely in the frontal plane (i. e., the sagittal twist characteristic of *N. leptocephalus* was not present in *N. micropogon*). At the height of the flexure, the anterior portion of the female *N. micropogon* (i. e., from snout to pelvic girdle) approximated a 45 degree angle with her caudal peduncle (Fig. 30). A small female during her retoflexure arched the anterior region of her body 90 degrees.

Also unlike the consistent spawning run of *N. leptocephalus*, the length of the *N. micropogon* run was variable. As the female's retroflexure was topographically fixed to the upstream slope of the trough, the length of the run was determined by the location of alignment relative to the trough. For instance, if alignment occurred in the downstream end of the trough, five to seven tail beats propelled the fish to its upstream slope. If alignment occured at mid-trough, the run was abbreviated and only three to five tail beats positioned the spawning pair accordingly.

Clasp: The male initiated the clasp as the female arched anteriorly. The clasp began as he turned his head towards her and sagitally arched his body over her back. His posterior flank appeared to anchor the female posteriorly to the substrate as it slid over her tail and then the caudal peduncle. His posterior flank was then driven into the epaxial region of the female just posterior to the dorsal fin at the height of her retroflexure (Fig. 31). Her vent was thereby pressed to the upstream slope of the trough as the male's vent was positioned slightly to one side.

In *N. micropogon* the male's tail often faced downstream during the clasp as in *N. leptocephalus*, however, the curvature of the male's body at the height of constriction was not as great as *N. leptocephalus*. Whereas in *N. leptocephalus*, the male's body (i. e., from snout to anal fin) contracted into a semicircular arch at the height of the clasp, in *N. micropogon* the male's body assumed a semi-elliptical crescent from snout to anal fin during the clasp.

Dissociation: The male and female quickly separated following the clasp. The male's body relaxed parallel to the direction of current as he drifted dowstream tail first to a trough posture. Rising into the water column, the female levelled and either disappeared downstream or returned to the downstream slope of the nest from where she might initiate another spawning sequence.

Behaviors Disruptive of Spawning Sequence

Interim: Spawning sequences of *N. micropogon* always began with the trough postured male. As in *N. leptocephalus*, posturing in *N. micropogon* male was similarly interrupted by stone carrying activities (e. g. nest building and reworking), dismissal swims, and agonistic behaviors with intruding males similar in size.

Unlike *N. leptocephalus*, the dominant *N. micropogon* male was often distracted from a trough posture by a smaller subordinate male. Whereas subordinate males over the downstream slope of the nest did not interfere with interim activites of the dominant male, movement of a subordinate either towards the trough or onto the upstream slope often provoked head butting and chasing.

Approach: Intraspecific behaviors disruptive of an approach included dismissal swims and competition among females for access to the postured male. A female's approach also was disrupted if the trough became inaccessible beneath the postured male (e. g. his abdominal region was flush with the rim of the trough).

Interspecific behaviors disruptive of a female's approach included the activities of nest associates and predatory species. The presence of nest associates (e. g. *Luxilis cornutus*, common shiner) over the downstream slope of the nest physically obstructed a female's approach. On several occasions a predatory species, *Micropterus salmoides* (largemouth bass), actuated the immediate disappearance of females from the vicinity of the nest.

Alignment: Alignment was unsuccessful if the female withdrew from the trough without initiating a spawning run. The female exited downstream or to the side of the trough as the male initiated his tilt.

Run: During a successful spawning run, the male remained slightly ahead of the appositioned female. Runs failed when the female overran the male (i. e., her snout became even with or ahead of the male's). As their snouts became even, the male turned his head and displaced the female from the trough with a lateral head butt. Alternately, the female retroflexed from the upstream slope of the trough and swam downstream before the lagging male was able to initiate a clasp.

Clasp: The subordinate male occasionally accompanied a run of the spawning pair and either stole or affected a mechanical breakdown of their clasp. The subordinate male approached the spawning pair during alignment and tilted towards the female opposite to tilt of the dominant male. With a male on either side, the female began her run towards the upstream slope of the trough. On three occasions, the subordinate male was first to arch his posterior flank over the female's back as she began her retroflexure. He subsequently displaced the dominant male and stole a clasp (Fig. 32). On one occasion, the female was apparently unable to pitch her snout upwards from the floor of the trough as she remained buried beneath the tilted males. Unable to constrict his body, the dominant male promptly dismissed the stalled female and the subordinate male with a single head butt.

<u>Location of Fertilized Eggs</u>

Gametes were most likely released onto the upstream slope of the trough where the retroflexed female was posteriorly depressed by the male's constricted body. Because water current velocities in the trough were reduced, the gametes settled into the interstices of the floor of the upstream trough. Fertilized eggs were collected from the floor of the trough between its midpoint and upstream margin (Maurakis, pers. comm.). Eggs are demersal, nonadhesive and noncohesive.

Figure 29. Spawning trough extending length of upstream slope of pebble mound nest of *Nocomis micropogon*.

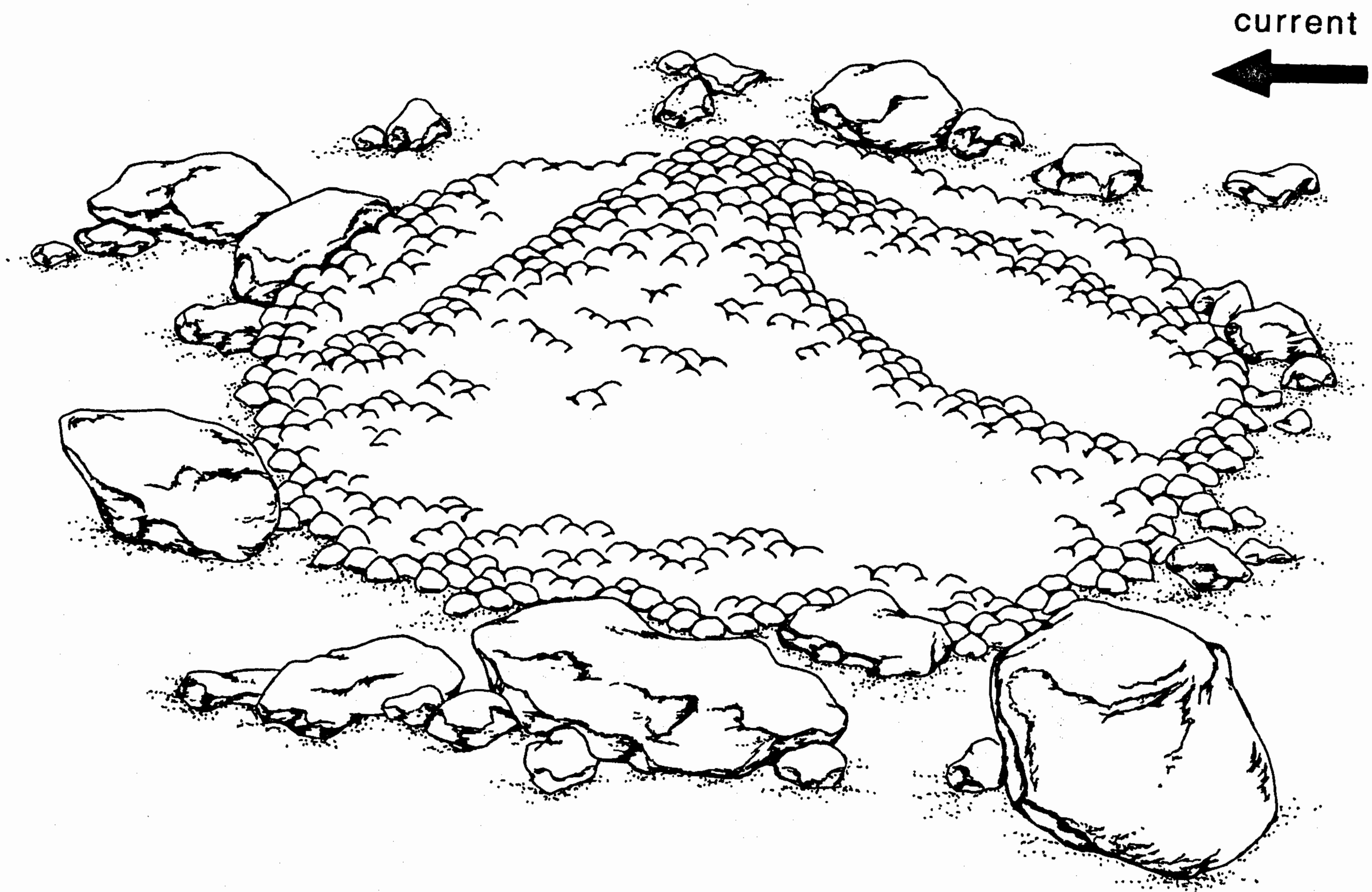

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Figure 30. Female *Nocomis micropogon* initiating frontal retroflexure.

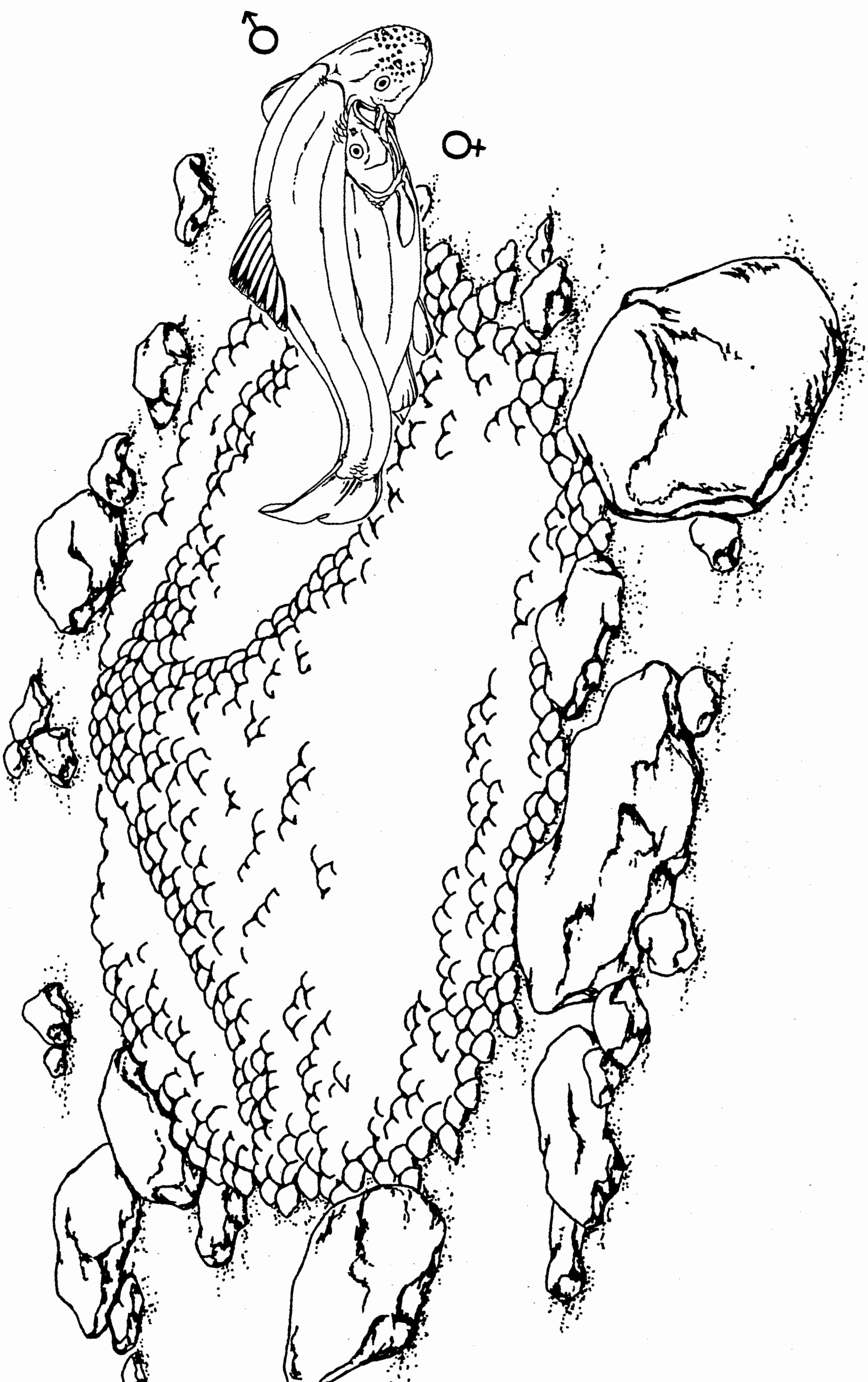

Figure 31. Spawning clasp of *Nocomis micropogon.*

Figure 32. Subordinate male *Nocomis micropogon* stealing clasp from dominant male.

CONCLUSIONS

Categories established in this study may be applicable for analyzing spawning behaviors in other fishes that spawn over the substrate. The behavioral preface to a successful spawn in species of *Rhinichthys*, *Exoglossum*, *Semotilus*, and *Nocomis* reflects a chronological sequence of male-female interactions coordinative of the spawning clasp. For the first time, the progression of events effecting the clasp is comprehensively resolved into behavioral categories that provide a format for comparative analysis of reproductive behavior. For a spawning pair, each category may represent a suite of behaviors (e. g. Interim, Approach, Alignment, and Dissociation) or may be a singular expression of a particular stage (e. g. Run and Clasp) of the spawning sequence. In each species, extrinsic or intrinsic disruption of behaviors described for any category except dissociation necessitates the re-initiation of the spawning sequence for a successful clasp to occur. Variation exists among species in their execution of each stage of the spawning sequence (Table 1).

Videotape analysis of reproductive behaviors displayed the relative contributions of male and female to the execution of a successful clasp. In all species, the female intiated the spawning run. A female's run conformed to, and may actually be directed by, the contour of the spawning substrate. Inherent to the completion of a run, retroflexures described for females of *S. atromaculatus*, *N. leptocephalus* and *N. micropogon* exemplifly a behavior induced by the upward sloping surface of pebble substrate. In all species, the male initated the spawning clasp in response to the female's relative positioning at the end of her run. Behavior leading to spawning clasps in *R. a. atratulus* may generally occur within or near a male's territory as the clasp itself is restricted to a substrate of either sand or fine gravel. In pebble nest-builders the entire sequence of spawning behaviors is topographically fixed to a substrate of a species-specific design and composition. As a result, the female's run and the male's clasp coordinate the simultaneous release of eggs and sperm over a discrete area of the pebble nest suited for their deposition and subsequent fertilization.

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Table 1. Categorical comparison of sequences of spawning behaviors in species of *Rhinichthys, Exoglossum, Semotilus, and Nocomis.*

Behavioral Categories					
Interim (♂)	Approach (♀)	Alignment (♂♀)	Run (♂♀)	Clasp (♂♀)	Dissociation (♂♀)
Rhinichthys a. atratulus					
surveyed or remained procumbent in territory; dipped snout into substrate	swam into ♂ 's territory from any one of several directions	♂ performed courting maneuvers as ♀ moved randomly about territory	♀ accelerated tail beating; ♂ accompanied her forward movement over substrate; pair became tightly appressed anteriorly	vibrating ♀ furrowed into sand or fine gravel as "psi" clasped ♂ applied downward pressure to her midbody	♂ resumed courting maneuvers as ♀ moved about territory; pair remained prostrate on substrate
Exoglossum laurae					
regularly re-shaped nest; occasionally hovered over crest	swam onto downstream slope from any one of several directions	♀ paused or passed beneath caudal peduncle of ♂ postured over crest	♀ initiated movement over crest onto up-stream slope; ♂ and ♀ converged midway down slope	♂ draped vibrating caudal peduncle over that of appressed ♀ ; clasped pair moved from midpoint to base of upstream slope	♀ circled to down-stream slope as ♂ circled to a crest posture position
Semotilus atromaculatus					
excavated spawning pit depositing material on ridge; hovered in or over downstream margin of pit	swam into pit laterally or from down-stream; drifted from ridge tail first into pit	♀ remained posted in pit as ♂ moved forward to mount her	♀ swam forward from beneath poised ♂ and retroflexed at pit-ridge interface	♂ 's body contracted through sigmoid configuration to encircle vertical ♀ ; ♀ 's body compressed below apex of ridge	♀ rolled to horizontal or drifted down-stream in vertical position as ♂ swung body parallel to current over pit

Table 1 (cont.). Categorical comparison of sequences of spawning behaviors in species of *Rhinichthys, Exoglossum, Semotilus, and Nocomis.*

Behavioral Categories					
Interim (♂)	Approach (♀)	Alignment (♂♀)	Run (♂♀)	Clasp (♂♀)	Dissociation (♂♀)
Nocomis leptocephalus					
excavated spawning pit on upstream slope of nest; postured over and fanned pit with anal fin	swam upstream towards or to one side of postured ♂'s tail and darted beneath ♂ into pit	♂ tilts dorsal half of body towards ♀ as she became posted in pit	♀ swam forward to one side of tilted ♂ and retroflexed from upstream slope of pit, twisting sagittaly	♂ contracted body into semicircle, laterally compressing vertical ♀ over pit	♀ rose vertically into water column, levellud and swam or drifted downstream as ♂ drifted tail first to pit posture position
Nocomis micropogon					
reshaped spawning trough on upstream slope of nest; postured over apex and fanned trough with anal fin	swam upstream towards or to one side of postured ♂'s tail and darted beneath ♂ into downstream trough	♂ tilts dorsal half of body towards ♀ as she became posted in trough	♀ swam forward to one side of tilted ♂ and retroflexed from upstream slope of trough without twisting sagittally	♂ contracted body into semi-elliptical crescent depressing epaxial region of ♀ near her dorsal fin to floor of trough	♀ levelled in water column and swam downstream as ♂ drifted tail first to a trough posture position

APPENDIX

Collection, Locality, Stream, and Film Footage Data
Pertinent to Spawning Behavioral Analyses

Table 1. Collection and locality data for reproductive behavioral studies of species of *Rhinichthys, Exoglossum, Semotilus, and Nocomis.*

Species	Collection No.	State (County)	Location	Collector	Date
R. atratulus	WSW-VA-382	Virginia (Spotsylvania)	unnamed tributary of Matta R., 0.5 km W of Co. Rt. 617, 1.0 km NW of jct. Co. Rt. 617 and Co. Rt. 605, approx. 3 km W of jct. Co. Rt. 605 and St. Rt. 1	Woolcott Maurakis	15 May 1988
R. atratulus	EGM-MD-201	Maryland (Montgomery)	Cabin John Run, Tuckerman Lane bridge, approx. 2 km E of jct. with St. Rt. 189 in Rockville	Maurakis	3 May 1986
E. laurae	EGM-VA-226	Virginia (Craig)	Sinking Creek, St. Rt. 42, 0.8 km S of jct. with Co. Rt. 226	Maurakis Woolcott Sabaj	29 May 1989
S. atromaculatus	EGM-VA-221	Virginia (Goochland)	E. Fork Genito Creek, Co. Rt. 644 bridge, 0.6 km W of jct. with Co. Rt. 654, approx. 6 km N of St. Rt. 6	Maurakis	17 Apr 1989
N. l. leptocephalus	EGM-VA-225	Virginia (Craig)	Sinking Creek, St. Rt. 42, 0.8 km S of jct. with Co. Rt. 226	Maurakis Woolcott Sabaj	26 May 1989
N. micropogon	EGM-VA-254	Virginia (Loudon)	Catoctin Creek, Co. Rt. 663 0.2 km W of jct. with Co. Rt. 665, approx. 8.4 km ESE of Lovettsville	Maurakis	25 May 1990

Table 2. Stream and film footage data for reproductive behavioral study of species of *Rhinichthys, Exoglossum, Semotilus, and Nocomis*. (current: * < 0.1, ** > 0.1)

Species	Physical Characteristics of Stream: name (drainage)	width (m)	depth (cm)	current (m/second)	temperature (degrees C)	Film Footage: no. spawns observed	tape duration (catalog no.)
R. a. atratulus	unnamed trib. of Matta R. (York R.)	0.6	10	slow	18.9	4	44 minutes (003)
R. a. atratulus	Cabin John Run (Potomac R.)	13.7	20	moderate	9.0 - 12.5	9	61 minutes (056)
E. laurae	Sinking Creek (New-Ohio R.)	9	52	slow *	15.5	5	68 minutes (023)
S. atromaculatus	East Fork Genito Creek (James R.)	3	12.6	moderate **	19.3 - 20.3	50	203 minutes (059 & 060)
N. l. leptocephalus	Sinking Creek (New-Ohio R.)	7.5	31	moderate **	15.5	70	64 minutes (026)
N. micropogon	Catoctin Creek (Potomac R.)	30	20	moderate	15 - 17	19	158 minutes (055)

VITA

Mark Henry Sabaj was born in the Chicago suburb of Hinsdale, Illinois on February 11, 1969. He attended Lincoln Elementary School in Brookfield, and graduated from Lyons Township High School in LaGrange, Illinois in 1987. He received a Bachelor of Science degree in Biology from the University of Richmond, Virginia in May 1990. The following year he enrolled in the Graduate School of Arts and Sciences at the University of Richmond where he is currently a candidate for the Master of Science degree.

As a undergraduate junior, he began assisting Drs. William S. Woolcott and Eugene G. Maurakis in their field and laboratory studies of the pebble nest-building cyprinids of eastern North America. He has presented results of their research at scientific meetings and contributed to publications and video documentaries of the breeding activities of nest-building minnows.

In 1992 he was awarded a full scholarship with stipend from the University of Illinois at Urbana-Champaign where he will continue piscatorial studies as a candidate for the Doctorate of Philosophy degree in Biology.

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