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Transbranchioral spawning: novel reproductive strategy observed for the pirate perch *Aphredoderus sayanus* (Aphredoderidae)

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Spawning period and reproductive behavior of *Aphredoderus sayanus* were investigated between March- June, 2001 and March-May, 2002 in streams of the Cache River basin of southern Illinois, USA and in aquaria. An unexpected and unique method of spawning, which we term transbranchioral, was documented. Gametes of both sexes were passed directly from the urogenital pore through the branchial and buccal (oral) cavities. In aquaria, the female ejected eggs from the orobranchial cavity into a spawning substrate (aquarium filter media, detritus or gravel), and the male dispensed a cloud of sperm over the eggs just as they were injected into the substrate by the female. In streams, eggs were found only in masses of fine tree roots. Amblyopsids, which share with *A. sayanus* an unusual jugular position of the anus and urogenital pore, might exhibit the same or a similar type of reproduction. Transbranchioral spawning could be an evolutionary precursor to branchial brooding of northern cavefish, *Amblyopsis spelaea* (Amblyopsidae), which might pass the eggs directly into the branchial cavity rather than expelling them and retrieving them into the buccal cavity via the mouth. Other aspects of the reproductive biology of *A. sayanus* are discussed.

Introduction

A diverse array of reproductive strategies occurs among the fishes, and a system of 33 reproductive guilds has been established (Balon, 1975, 1981) with one later addition (Kottelat, 1990). The more unusual modes of reproduction include mouth, branchial, and auxiliary brooding, which includes attachment of eggs to the fish's body (Balon, 1975, 1981; Schmidt, 2001). The pirate perch, *Aphredoderus sayanus*, a small freshwater fish endemic to eastern North America, is the only extant representative of the family Aphredoderidae and is considered by some to be the closest living relative of the family Amblyopsidae (Rosen, 1985; Patterson & Rosen, 1989; Jenkins & Burkhead, 1994). *Aphredoderus sayanus*, like its relatives the amblyopsids, has an unusual jugular position of the anus and urogenital pore (Mansueti, 1963; Rosen, 1985; Patterson & Rosen, 1989). The anus and urogenital pore of both sexes of *A. sayanus* start out in a more typical position anterior to the anal fin but migrate forward to a position just behind the isthmus and in front of

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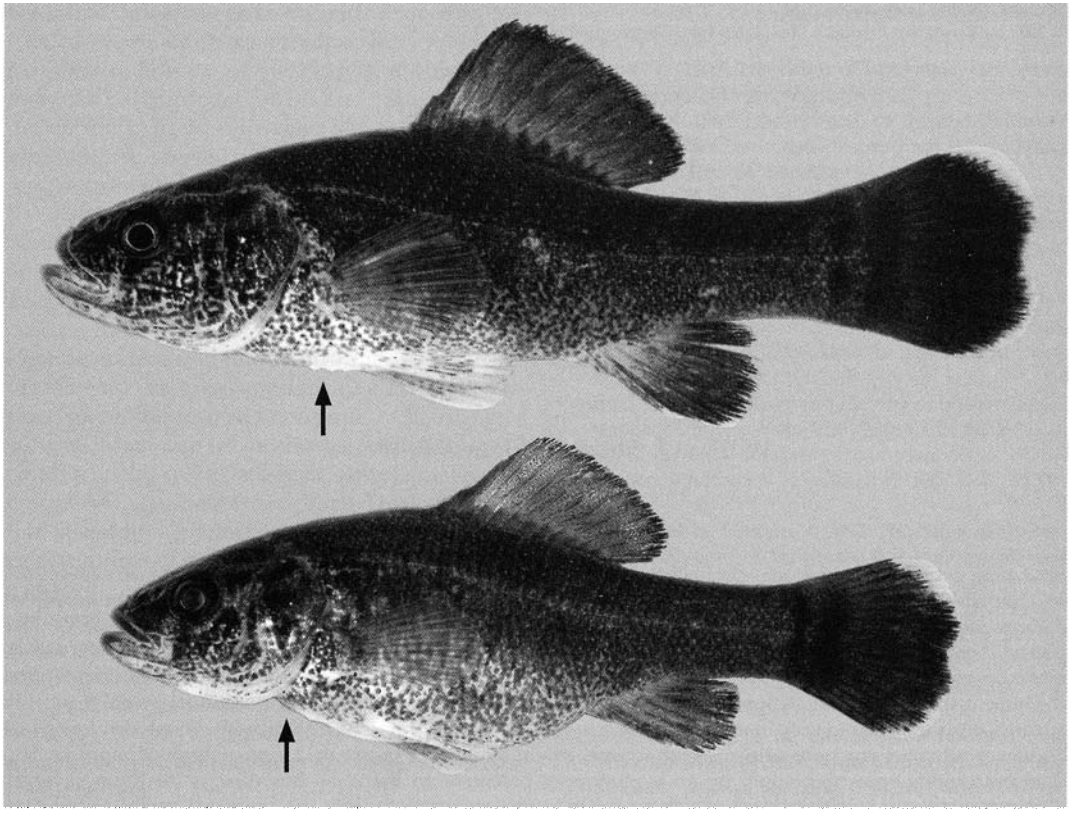

Fig. 1. *Aphredoderus sayanus*, CAS 216605, male 55.2 mm SL (top); female, 47.5 mm SL (bottom). Abdomen of female is distended with eggs (Arrows indicate position of anus and urogenital pore). Photos by Cheryl Broadie and William J. Poly.

the pelvic fins (Jordan, 1878; Mansueti, 1963) (Fig. 1). *Amblyopsis spelaea* is a branchial brooder (Eigenmann, 1900), and its similarity to *A. sayanus* and other amblyopsids has led to speculation that all are branchial brooders (Breder & Rosen, 1966; Martin & Hubbs, 1973; Boltz & Stauffer, 1986). There have been a number of contradictory reports from 1861 to 1999, some of which indicated *A. sayanus* has floating and/or adhesive eggs, is a nest builder (or uses sunfish nests), guards the offspring, or releases eggs over vegetation and detritus (Abbott, 1861, 1870; Breder & Rosen, 1966; Scott & Hall, 1997; Fontenot & Rutherford, 1999). Our observations over two reproductive seasons revealed a novel reproductive method, unknown among fishes. We also describe anatomical modifications in female and male *A. sayanus* that appear relevant to their reproductive biology and discuss other aspects of reproduction and ecology.

Material and methods

Adult male and female *A. sayanus* were collected with nets from streams and ditches in the Cache River drainage of southern Illinois in March-May, 2001 and March-April, 2002 and were transported to the laboratory. Fishes were held in 37.8 l aquaria with undergravel or external filters. Some aquaria had gravel substrates and others lacked gravel, but rocks or notched flowerpot bases were present as cover, and aquarium filter media (empty or filled with crushed oyster shell) or detritus were in some aquaria. Various foods were offered daily or on alternate days. The photoperiod approximated 12-16 hr of light per day. We made visual observations and video recordings of courtship and spawning behavior on more than 10 occasions (duration 1-8 hr) under variable light conditions, including white (varying intensities), red and infrared light, and at various times of day

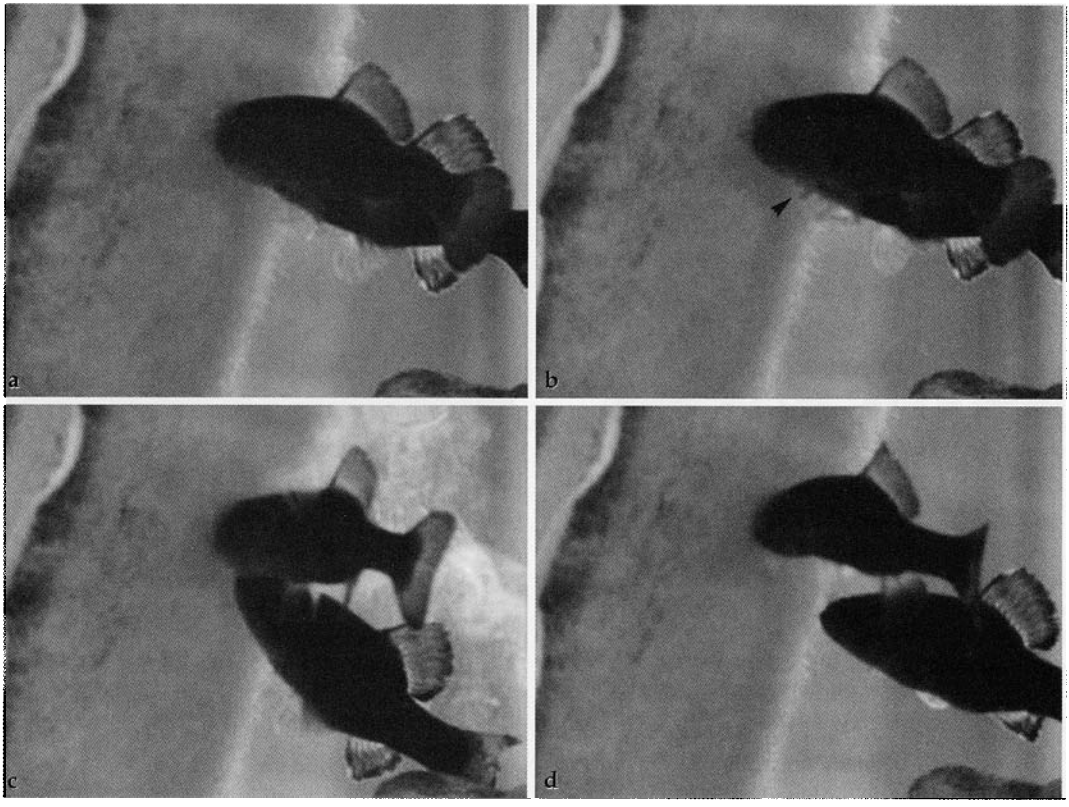

Fig. 2. Spawning sequence of *Aphredoderus sayanus*. **a,** Female, 51.1 mm SL, CAS 216603 (foreground), has penetrated spawning substrate with snout; male, 53.7 mm SL, CAS 216606, has aligned laterally to female. **b,** Female passes eggs forward into orobranchial cavity by rhythmic flaring of opercula. Arrow indicates eggs protruding from posterior of branchial cavity. **c,** Male expels plume of sperm into substrate matrix to fertilize eggs. **d,** Female finishes injecting eggs into substrate matrix. Photos by authors.

or night. Typically, a single male was held with 1-4 females. Aquarium substrates and branchial and buccal cavities of both sexes were examined for presence of eggs. Two clutches of eggs were raised in captivity, and series of larvae were preserved (presently in authors' collection). Searches for eggs, larvae, and juveniles in streams were conducted in May-June 2001 and April-May 2002, and specimens were preserved and stored in 4 % formalin (eggs and larvae) or 7 % formalin (juveniles). Many of the adult fishes used in this study were preserved in 10 % formalin to serve as voucher specimens and to study their morphology and were deposited at the California Academy of Sciences (CAS).

Results

Observations of pre-spawning courtship revealed two basic behaviors exhibited by the males: 1) lateral display with an occasional slow undulation of the body (which might have been agonistic rather than courtship in some cases) and 2) rapid trembling of the male's body with the head oriented toward the spawning substrate. Males and females nip at and push their snouts against the spawning substrate matrix (gravel, detritus, aquarium filter media). When the female is prepared to deposit eggs, she thrusts her head into the substrate, and the male assumes a parallel position (ventral-lateral) (Fig. 2a). The opercula of the female are flared as eggs are extruded from the urogenital pore, then the eggs are directed to the branchial cavity, likely via the

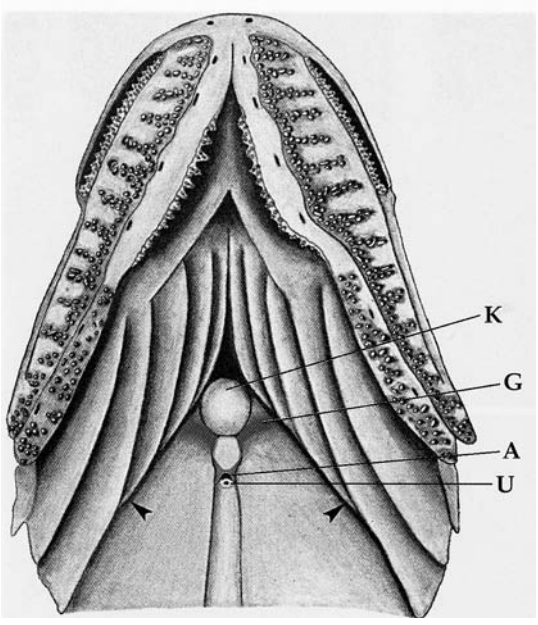

Fig. 3. Ventral view of thoracic (jugular) area of *Aphredoderus sayanus*. **A,** anus; **G,** grooves leading from urogenital pore to branchial cavity on both sides of knob; **K,** knob; **U,** urogenital pore. Arrows indicate location of female branchiostegal-opercular folds that extend into branchial cavity. Modified from Moore & Burris (1956).

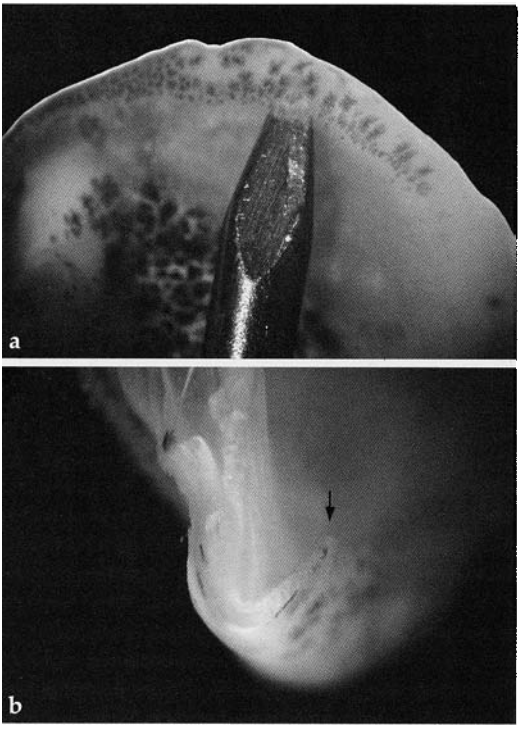

Fig. 4. Inwardly-curled branchiostegal-opercular fold of a gravid female *Aphredoderus sayanus*. **a,** View from inner surface of branchiostegal-opercular complex (Note point of nail extending behind fold). **b,** Cross-sectional view of fold. Arrow indicates free margin of fold. Photos by William Poly and Steve Schmitt.

two grooves leading there from the urogenital pore (Fig. 3). Eggs are passed through the orobranchial cavity during repeated opercular movements, protruding intermittently from the posterior of the branchial cavity (Fig. 2b). Eggs are ejected from the orobranchial cavity through the mouth and into the spawning substrate matrix during the opercular movements (Fig. 2c-d). As the eggs are ejected through the female's mouth, the male expels a large cloud of sperm into the substrate (Fig. 2c). As with the eggs, the sperm are passed through the orobranchial cavity of the male. In the particular spawning sequence shown in Figure 2, the female's abdomen is noticeably deflated following completion of the spawning act (Fig. 2d). During egg extrusion, the female's pelvic fins are extended, rigid, and pointed downward.

One anatomical feature not mentioned previously and that likely facilitates the reproductive strategy of *A. sayanus* is the female's inwardly-curled branchiostegal-opercular folds, presumably helping retain eggs in the orobranchial cavity during passage of eggs from the urogenital pore (Fig. 4a-b). In both sexes there is a groove leading from the urogenital pore toward the branchial cavity. Upon reaching the knob-like protuberance located just posterior to the isthmus, the groove continues to each side of the knob (Fig. 3).

Females were gravid in March, April and early May and aquarium spawnings occurred from mid-March through April. In streams, we collected eggs on 16 and 21 April and 3 May 2002, larvae on 21 April and 3 May 2002 and 13 May 2001, and juveniles on 3 June 2001. On 21 April 2002, we gathered 244 live eggs, 176 dead eggs and 81 larvae (several early larval stages) from a large root mass in a tributary of Bradshaw Creek (Fig. 5). At the same site, on 3 May 2002, we found 227 live eggs, 33 dead eggs and 8 larvae among fine root masses of trees. Number of eggs deposited by 3 females in aquaria were 129, 232 and 290 (normal and abortive spawns). All eggs

Fig. 5. Spawning habitat of *Aphredoderus sayanus* (tributary of Bradshaw Creek). **a,** Tree root mass that contained eggs and early larvae, 21 April 2002 (scale bar: 45 cm). **b,** Same root mass *in situ* along stream bank. Photos by authors.

that we observed (>1,000) in streams and in aquaria, whether live or dead, were demersal and nonadhesive. Eggs were never found in the branchial or buccal cavities of aquarium-held or wild-caught *A. sayanus* (i.e., eggs not retained after spawning). Egg deposition site was chosen before extrusion of eggs began and eggs were never transferred to another site by either sex in aquaria. Males displayed only brief spawning site fidelity (several hours to several days) in aquaria and limited field observations also suggest that no parental care is provided (*A. sayanus* were not collected under the large root mass containing eggs and larvae on 21 April 2002; however, both sexes were associated with root masses containing eggs on 3 May 2002). In two instances, eggs were extruded from the urogenital pore by female *A. sayanus* shortly after placing the fish in preservative.

Habitat of *A. sayanus* varied from low-gradient, muddy areas with much woody debris (ditch near Horseshoe Lake; Tucker Ditch) to small, clear streams with rocky substrates (Bradshaw Creek and tributary; unnamed tributary of Cache River; Hartline Creek). Undercut banks with root masses were typical hiding places, but we also found *A. sayanus* under rocks.

Discussion

We name the mode of reproduction described above transbranchioral spawning; this type of reproductive behavior and egg deposition has not been reported for fishes (Breder & Rosen, 1966; Balon, 1975, 1981; Kottelat, 1990). Transbranchioral refers to the passage of gametes from the urogenital pore through both the branchial and buccal (oral) cavities and finally, out of the mouth and into the substrate matrix. We tentatively place *A. sayanus* in the nonguarding, brood hiding categories of Balon (1981) and name a new guild, phytophils (plant spawners; A.2.6 in Balon's (1981) classification), which is defined by gametes being injected into interstitial spaces of a substrate matrix, including detritus, root masses, and possibly other vegetation. In aquaria, *A. sayanus* demonstrated a preference for soft, fibrous materials, especially aquarium filter media (as shown in Fig. 2) but also deposited eggs in detritus and even in gravel (the latter only when other substrates were not available). In streams, eggs were found only among fine root masses of trees, and spawning reported by Katula (1992) occurred in an aquarium containing sand and gravel with an overlay of sphagnum peat moss.

Branchial brooding is the method by which *Amblyopsis spelaea* care for eggs and larvae (Eigenmann, 1900), but reproductive methods are unknown for the remaining five species of Amblyopsidae. Some authors have speculated that all amblyopsids are branchial brooders based on the observations of a single species in the family. However, considering the reproductive mode of *A. sayanus* and the similarity in position of urogenital pore shared between amblyopsids and *A. sayanus*, at least some of the cavefishes might share the same method of reproduction. The style

of reproduction exhibited by *A. sayanus* may represent the ancestral spawning mode for branchial brooding. Even for northern cavefish, the method by which eggs enter the branchial cavity has been a mystery. Although only direct observations will confirm it, we believe it is possible that the northern cavefish might extrude eggs directly into the branchial cavity as well. All mouth brooding species of fishes, as far as is known, always expel eggs, then gather them with the mouth in contrast with the transbranchioral method of transfer (Breder & Rosen, 1966). Martin & Hubbs (1973) observed that eggs stripped from a ripe female *A. sayanus* traveled through the grooves to the branchial cavity, and Jenkins & Burkhead (1994) thought the knob might act as deflector, diverting the eggs into the branchial cavity. Although we did not observe the gametes traveling through these grooves directly, we believe the grooves and knob must be involved in directing the gametes into the branchial cavity. Egg retention in the orobranchial cavity appears to be facilitated by the inwardly-curled branchiostegal-opercular flaps of females. Considering the unique course of the intestine and oviduct (Mansueti, 1963), gamete extrusion possibly could be assisted by abdominal muscles near the pelvic girdle.

Habitat of *A. sayanus* has been characterized as sluggish, low-gradient areas with undercut banks and abundant detritus, woody debris, or aquatic vegetation (Becker, 1923; Etnier & Starnes, 1993; Jenkins & Burkhead, 1994; Monzyk et al., 1997). In the Cache River basin, we found *A. sayanus* in a variety of habitats that included small to moderate-sized streams with clear water and primarily rocky substrates, but these streams possessed undercut banks and root masses that are used for cover and spawning sites. *Aphredoderus sayanus* was the most abundant fish collected in a spring-fed swamp in southern Illinois (Gunning & Lewis, 1955). In agreement with other studies, *A. sayanus* is most active under low-light or complete darkness (Parker & Simco, 1975) and is a spring spawner through most of its range, with time depending on latitude and seasonal conditions (Forbes & Richardson, 1920; Murdy & Wortham, 1980; Minton et al., 1985). McLane (1955) noted that "Males and females collected during the period October to December appeared to be in breeding condition" in the St. Johns River system, Florida. We found eggs and larvae in April to early May in southern Illinois, and Stegman (1959) briefly noted spawning in late April and early May in Kinkaid Creek, southern Illinois, as determined by his examination of 10 *A. sayanus*. Forbes & Richardson (1920) indicated that spawning occurred during May in northern Illinois. Eggs and early larval stages were found in root masses only, whereas later larval stages and juveniles were found in more open areas over gravel or rock substrates. All eggs that we observed from the stream and in the laboratory (>1,000), including fertilized (live), dead, or non-fertilized/aborted eggs, were non-adhesive and demersal, agreeing with Katula (1992), but in contrast to other reports about eggs of *A. sayanus* being adhesive and/or floating (Breder & Rosen, 1966; Fontenot & Rutherford, 1999). We never encountered eggs in the buccal or branchial cavities of male or female *A. sayanus*, which is consistent with our observations that eggs were passed through the orobranchial cavity quickly, with no long-term retention. Occasional extrusion of eggs from females placed in preservative likely explains the presence of a few eggs in the branchial cavity of a female, which led to further speculation of branchial brooding by *A. sayanus* (Boltz & Stauffer, 1986). Of the earlier reports of reproduction, Katula's (1992) are accurate in some respects (i.e., not a branchial brooder), but his comment that "The female was seen performing some fanning of the substrate in order to form this pit" disagrees with our observations. We observed spawning of *A. sayanus* in gravel substrates in aquaria; the pits (or furrows) are formed by the female positioning her head against the substrate and swimming vigorously into it (as done with other substrates); thereafter the female expels eggs into the substrate, and the male fertilizes the eggs from a ventral-lateral position (as in Fig. 2). Adults do not build nests and do not appear to provide parental care. Reports of parental care could be due to observations of adult *A. sayanus* residing in typical daytime habitats (i.e., sheltered, low-light areas, such as undercut banks with root masses), which also serve as egg deposition sites during the spring spawning season. Also, male site fidelity could be linked to the presence of gravid females and potential spawning opportunities. In addition to our fecundity information, Gunning & Lewis (1955) counted 769 ova in a 57 mm TL fish, and McLane (1955) reported a range of 129-160 ova.

Further study of reproduction by *A. sayanus* should address fecundity, courtship, social be-

havior, and potential alternative reproductive strategies (see Chan & Ribbink, 1990).

Material examined. USA: Illinois: CAS 216605, 2, 47.5-55.2 mm SL; tributary of Cache River at U.S. Rt. 51 bridge, Union Co.; 3 Apr 2002. – CAS 216599, 2, 54.2-65.8 mm SL; same locality; 28 Mar 2001. – CAS 216600, 4, 51.0-57.6 mm SL; same locality; 19 Apr 2001. – CAS 216666, 3, 56.9-66.5 mm SL; same locality; 14 Mar 2002. – CAS 216603, 1, 51.1 mm SL; same locality; 7 Apr 2002. – CAS 216652, 1, 47.5 mm SL; same locality; 16 Apr 2002. – CAS 216598, 1, 54.9 mm SL; Bradshaw Creek upstream of Wing Hill Rd. bridge, Union Co.; 30 Mar 2002. – CAS 216606, 1, 53.7 mm SL; same locality; 3 Apr 2002. – CAS 216651, 12, 44.0-61.2 mm SL; mixture from Bradshaw Creek upstream of Wing Hill Rd. bridge and tributary of Cache River at U.S. Rt. 51 bridge, Union Co.; 30 Mar-16 Apr 2002. – CAS 216655, 3, 66.5-74.4 mm SL; Tucker Ditch at U.S. Rt. 45 bridge, Massac Co.; 1 Apr 2001. – CAS 216604, 1, 50.7 mm SL; tributary of Bradshaw Creek at Bennett Lane, Union Co.; 14 Apr 2002. – CAS 216669, 1, 60.8 mm SL; ditch along Promised Land Rd. near Horseshoe Lake spillway, Alexander Co.; 3 Mar 2001. – CAS 216668, 1, 54.2 mm SL; same locality; 14 Mar 2001. – CAS 216667, 1, 41.4 mm SL; Sandy Creek at Tamms-Olive Branch Rd., Alexander Co.; 3 May 2001.

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