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Spawning Behavior and Genetic Parentage in the Pirate Perch (*Aphredoderus sayanus*), a Fish with an Enigmatic Reproductive Morphology

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We describe for the first time reproductive behaviors in the Pirate Perch (*Aphredoderus sayanus*), a secretive nocturnal fish whose urogenital opening is positioned far anteriorally, under its throat. Some naturalists had speculated that this peculiar morphological condition might serve to promote egg transfer to the fish's branchial chamber for gill-brooding; others hypothesized that Pirate Perch spawn in the substrate of streams but offered no adaptive rationale for the odd placement of the fish's urogenital pore. Here we solve the conundrum through a combination of intensive field investigations, underwater filming, and molecular parentage analyses. We show that Pirate Perch spawn in underwater root masses, the first documentation of such nesting behavior in any species of North American fish. Female Pirate Perch thrust their heads and release their eggs into sheltered canals of these masses. Males congregate at these sites and likewise enter the narrow canals headfirst, to release sperm. Thus, the forward-shifted urogenital pore may facilitate spawning under this special nesting circumstance. We found no evidence of extended parental care. Fish formed their own canals or used burrows made by aquatic macro-invertebrates and salamanders. Genetic analyses based on three polymorphic microsatellite loci demonstrate that a total of at least five to 11 sires and dams were the parents of embryos within each of three assayed root-mass nests (of a total of 23 nests found). Males defended the oviposition sites by body-plugging canal entrances after spawning. This and more direct aggressive behaviors by males probably relate to selection pressures imposed by intense competition for fertilization success under these group-spawning conditions.

FOR almost two centuries, naturalists have been puzzled by a peculiar morphological feature of the Pirate Perch (*Aphredoderus sayanus*): the jugular position of its urogenital opening (LeSueur, 1833; see Fig. 1). In the larvae, this joint terminus of the excretory and reproductive tracts occurs in the conventional position for fishes—just anterior to the anal fin—but as Pirate Perch grow to adulthood the vent migrates forward to a position just behind the gill chamber, near a knoblike mass of thoracic muscle (Jordan, 1878; Mansueti, 1963). Martin and Hubbs (1973) were the first to hypothesize that this extraordinary anterior placement of the urogenital pore might facilitate gill-brooding of eggs. Their suspicions of gill-brooding originated from an observation that eggs artificially stripped from a female Pirate Perch can follow a groove from the vent to the branchial chamber (Martin and Hubbs, 1973). Although Jenkins and Burkhead (1994) did not support gill-brooding as the norm, they speculated that the muscle knob in an ovipositing female might function to split and direct the torrent of ova from the vent into the left and right gill chambers via the grooves described by Martin and Hubbs. Furthermore, gill-brooding has been reported in the Northern Cavefish *Amblyopsis spelaea* (Breder and Rosen, 1966), a representative of the related family Amblyopsidae, whose six member species all also display a forward shifted urogenital pore (Nelson, 1994). However, no direct evidence for gill-brooding exists in Pirate Perch, the closest suggestion perhaps being a single observation of three eggs inside the branchial cavity of one museum-preserved female (Boltz and Stauffer, 1986). One reason to doubt gill-brooding was noted by Katula (1992): space within the branchial cavity of this species is insufficient to hold an entire clutch of eggs.

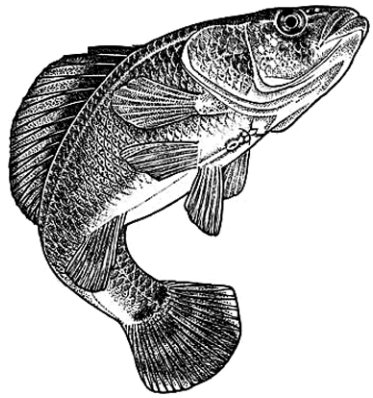

Fig. 1. An adult Pirate Perch (*Aphredoderus sayanus*) and the peculiar forward position of its urogenital opening. Drawing by Trudy Nicholson.

Other naturalists have speculated that Pirate Perch are substrate spawners (although the relevance, if any, of the anterior urogenital pore to this reproductive mode remains unclear). In aquarium observations, Brill (1977) and Fontenot and Rutherford (1999) noticed that Pirate Perch broadcast apparently aborted eggs on tank bottoms, and Katula (1987, 1992) described the deposition of viable eggs in or near a shallow depression swept by a female in the streambed. From field observations, Abbott (1861, 1868) also posited that Pirate Perch construct saucer-shaped nests but later reinterpreted this as possible spawning in abandoned sunfish nests (Abbott, 1870). Based on their aquarium observations Fontenot and Rutherford (1999) suggested that Pirate Perch may broadcast eggs over leaf litter and woody debris.

Despite all of the hypotheses listed above, documentation of the spawning of the Pirate Perch in nature remained elusive. Although this species is widespread and often common in the central and eastern United States (Lee et al., 1980; Boltz and Stauffer, 1993), Pirate Perch are nocturnal and generally difficult to observe (Abbott, 1861; Parker and Simco, 1975). Additionally, these fish inhabit dense vegetation, woody debris, root masses, and undercut banks of freshwater streams and swamps (Abbott, 1868; Becker, 1923; Monzyk et al., 1997) and have been thought to be rather solitary and sedentary (Pflieger, 1975; Whitehurst, 1981; Robison and Buchanan, 1988).

The objectives of our study were to (1) critically identify and describe the site(s) of oviposition for Pirate Perch in the wild, (2) document reproductive behaviors associated with spawning, (3) use molecular-genetic markers (from nuclear microsatellites) to evaluate biological parentage and the genetic mating system, and (4) interpret results in the context of the remarkable anterior location of the Pirate Perch's urogenital opening.

Materials and Methods

Field observations.—Using a backpack electroshocker, fish were surveyed on numerous occasions from Fourmile Branch, a Savannah River tributary located on the Department of Energy's Savannah River Site in South Carolina. This sandy-bottomed, braided lowland stream often flows under an open canopy, and has an abundance of aquatic macrophytes (Fletcher et al., 2000).

During the spawning season (Murdy and Wortham, 1980; Fontenot and Rutherford, 1999), wild-caught females were examined for reproductive condition by gauging abdominal distention and assessing gonadal colors as seen through the ventral abdominal wall (orange turning yellow as the ova mature). For example, more than 350 females from 50 field collections were scrutinized from 4 February through 7 April 2001. Females that were not gravid or did not appear to be recently spent were released after examination. Every captured adult was also examined for presence of eggs in the branchial chamber.

When females were discovered to be in reproductive condition, or when aggregations of males were encountered, we searched for embryos and larvae nearby. When located, these offspring as well as the adults were preserved in a buffer solution (20% DMSO and 5M NaCl). In the laboratory, offspring were counted and classified according to stage of development. We collected samples from each of 23 discovered ''nest sites'' (Pirate Perch do not necessarily construct a nest per se, but for simplicity, we will refer to oviposition sites as nests) and recorded water depth and velocity, as well as additional features of the stream, sediments, and vegetation. The root-mass nests were collected and dissected, initially with pruning shears, and characterized for distributions of eggs and canals.

At one root-mass nest (see below), we deployed a field camera system (Fuhrman Diversified, Inc., Field Cam WCMS type6/V801, equipped with two Sony HVM-332 external cameras and infrared lighting) to film underwater spawning activity. Camera 1 was focused on the lower lobe of the root mass, penetrated by multiple entrances, and an upper lobe of denser roots with fewer entrances. Camera 2 focused on the lower edge of coarse roots in another

lobe of the mass, and on the sandy stream substrate directly below. A total of 15.8 h of nest-associated activity was taped on the nights of 2, 3, 5, 6, and 14 March 2001.

To learn about the structure and origin of the canals, nine additional nests were examined in spring 2002. To determine whether organisms regularly observed in the canals in both 2001 and 2002 could create the root-mass burrows where Pirate Perch spawned, canal-free cubes of root mass (approximately 15 cm^3, with fine dense rootlets) were placed in screened compartments of an artificial raceway during the spring of 2002. We then introduced two primary organisms that we often observed in association with root burrows in the field: large dobsonfly larvae (insect family Corydalidae), and aquatic salamanders (*Desmognathus* sp). During subsequent weeks, root masses were monitored for canal formation.

Genetic analyses.—Genomic DNA from a single Pirate Perch was digested with *Mbo*I and subjected to gel electrophoresis. Fragments 200–700 bp in length were ligated into *Bam*HI-digested, dephosphorylated pBluescript (Stratagene). The ligations then were heat-shock transformed into competent XL1-Blue *Eschericia coli* (Stratagene) and the resulting genomic library was screened with ^{32}P-labeled oligonucleotides complementary to all possible 2–4 base repeat motifs.

From positive clones, DNA was isolated and then sequenced (at the Molecular Genetics Instrumentation Facility at the University of Georgia) using T3 and T7 primers. Three primer pairs (GenBank accession numbers AY225445–7) were designed from the sequenced clones and subsequently used to amplify three microsatellite loci whose levels of polymorphism were suitably high for genetic parentage analyses.

From each adult, DNA was extracted from a small tissue sample using a standard proteinase K, phenol-chloroform protocol (Maniatis et al., 1982). From Stage A embryos (those not yet displaying visible tissue under a dissecting microscope), DNA was isolated from the whole egg. From more advanced-stage embryos (those with visible tissue formation), egg membrane and yolk sac were removed before DNA isolations. In some cases, a standard proteinase K phenol-chloroform protocol (Maniatis et al., 1982) was employed; otherwise, embryos simply were digested in a buffer solution following DeWoody et al. (2000).

Amplification of each microsatellite locus was carried out separately in a total volume of 12 μL containing 1U of *Taq* DNA polymerase, 0.2 mM of each dNTP, 1X Promega buffer, 0.5 mM of each primer, and 1 μL of DNA. Final concentrations of $MgCl_2$ were 2.5 mM for loci AS13 and AS72, and 2 mM for locus AS16.2. Cycling conditions in all cases consisted of an initial denaturing step of 95 C for 2 min, followed by 30 cycles of 95 C for 30 sec, annealing at 51 C for 30 sec, extension at 72 C for 1 min, and a final extension step of 72 C for 2 min.

Microsatellites were electrophoresed on an ABI 377 genotyping system, after labeling one of each primer pair with a fluorescent dye. Fragment sizes were visualized and scored using the GENESCAN and GENOTYPER programs. Fifty-two adults were assayed to estimate population allele frequencies, and a total of 351 embryos from three nests were scored for parentage assessments. The three nests genetically surveyed were selected to represent a large nest (nest 10), medium-sized nest (nest 7), and small nest (nest 5). All specimens were deposited in the Georgia Museum of Natural History (GMNH; catalog numbers to be assigned). For the adult sample, genotypic disequilibria between loci and exact tests for Hardy-Weinberg equilibrium were conducted using Genepop (Raymond and Rousset, 1995).

Because of the poor preservation of many adult samples, we were unable to directly match embryos with their parents. Instead, the total number of alleles present at each locus was counted within each age-class of embryos in each nest, and also for all embryos in each entire nest. Following Kellogg et al. (1998), the minimum number of parents contributing to each age class and to a whole nest were conservatively estimated as the numbers of different alleles at the most polymorphic locus, divided by two.

RESULTS

Spawning habitat and oviposition substrate.—Twenty-three nests (each a root mass containing fertilized eggs or yolksac larvae) were discovered in spring 2001. Water velocities at the exact nest sites averaged 15 cm/sec (range 0–39). Most nests were widely dispersed; exceptions involved nests 5 and 6 that were only 2 m apart (but nonetheless on separate root masses), and nests 21 and 23 that were only 7 m apart. No other nests had known spawning sites within 20 m.

Underwater root masses of primarily woody riparian plants and occasionally of aquatic macrophytes were the identified sites within which Pirate Perch deposited and fertilized their eggs (Figs. 2–3). These root masses were anchored to the bank, stumps, and logs and can be cate-

A

B

C

Fig. 2. Photographs regarding Pirate Perch spawning: (A) habitat scene showing stream bank and underwater flowing roots; (B) opening to Pirate Perch nest canal in a root mass with a single egg near the entrance; and (C) distribution of Pirate Perch eggs in a dissected root mass, egg diameter 1.5 mm. Color versions of these and other photographs, as well as videotape excerpts of Pirate Perch spawning, can be viewed at website www.genetics.uga.edu/Asayanus/.

gorized as ''flowing,'' ''hanging,'' or ''bank.'' Flowing root masses, the type most commonly used by the fish, were anchored at the upstream end and oriented parallel to water flow, either laying on the bottom or suspended horizontally in the water column in a flattened shape tapered toward the downstream edge (Figs. 2A, 3). They ranged from small ellipses (20 × 15 cm in length and upstream width) to large mats (100 × 20 cm). Hanging root masses were blocks of compact rootlets suspended from larger stems. Bank roots existed as less distinct clumps, with less dense rootlets hanging down almost vertically into the water.

Fig. 3. Schematic composite showing a typical flowing rootmass nesting site with multiple canal entrances and illustrating various behaviors displayed by Pirate Perch in the immediate vicinity (see text). Drawing by Trudy Nicholson.

Although it was sometimes difficult to characterize the exact natural positions of Pirate Perch eggs from dissected root masses, eggs often appeared to occur in one or several foci. Most such clusters were less than 10 cm in diameter, but one (probably deposited by multiple females from different access points) was 20 × 25 cm. Offspring within an assemblage often were of distinct developmental stages, strongly implying that the eggs had been deposited and/or fertilized during multiple spawning events. In the largest nests, more than 2000 total offspring were present (Table 1). In comparison, the clutch size of a single female Pirate Perch ranges from about 100–400 depending upon body size (DEF, unpubl. data).

Each egg cluster was accessed by one or more canals tracing to the surface of the root mass. These canals were about 1–2 cm in diameter, with walls ranging from rigid and relatively smooth to pliable and ill-defined, depending in

TABLE 1. NUMBERS OF EMBRYOS (IN EACH OF SIX DEVELOPMENTAL STAGES[a]) AND ADULT MALES AND FEMALES ASSOCIATED WITH 23 DISSECTED PIRATE PERCH NESTS. The adults were collected at or within several meters of the nest.

Nest No.	Stage A	Stage B	Stage C	Stage D	Stage E	Stage F	Total number embryos[b]	Number males at or near nest[b]	Number females at or near nest[b]
1	0	0	3	53	26	0	82	11	1
5	42	0	0	0	73	225	340	1	0
6	71	59	15	14	95	27	281	2	1
7	1047	212	38	1	3	0	1301	3	1
8	378	0	0	0	0	0	378	2	0
9	0	163	0	0	0	0	163	1	0
10	606	421	362	434	797	116	2736	14	2
11	1160	233	200	206	295	125	2219	10	1
12	10	12	18	0	2	3	78	3	0
13	5	38	0	0	0	0	43	1	1
14	231	36	26	0	61	90	444	13	4
15	4	7	20	7	20	1	59	2	3
16	833	2	0	0	28	27	890	9	2
17	36	3	0	4	28	5	76	2	0
18	391	26	39	17	227	104	804	3	1
19	826	74	121	145	42	11	1219	2	0
20	277	18	10	0	0	41	346	2	0
21	343	11	4	9	1	3	371	6	0
22	0	0	0	0	0	8	8	3	0
23	14	56	0	0	0	0	70	1	0
24	74	0	97	27	12	0	210	2	0
25	102	148	13	1	38	20	322	2	1
26	2	1	0	0	3	90	186	8	0

[a] Stage A, egg from fertilization to blastula, no visible tissues; Stage B, early embryo with neural tube visible; Stage C, embryo with complete neural tube and prosencephalon visible; Stage D, embryo with optic vesicles visible; Stage E, recently hatched embryo with full yolk sac and no melanophores visible; Stage F, hatched embryo with partially absorbed yolk sac and visible melanophores. Developmental stages based on Martin and Hubbs (1973) and Hardy (1978).

[b] Undoubtedly in at least some cases, not all individuals were collected because of logistical constraints.

part on rootlet density. Surface entrances to the canals (Fig. 2B) were similar in diameter but sometimes situated in depressions about 2–5 cm wide and 3–5 cm deep, their edges often matted down. Some canals appeared to be shallow punctures extending inward only 3–4 cm, whereas others progressed deep into the root mass. A few eggs were sometimes visible in the canal mouth or its surrounding depression (Fig. 2B), but most were deep enough inside to be invisible from the surface (Fig. 2C). In flowing root masses, holes most often were on the underside.

Rootlet density influenced the distribution of eggs within a cluster. In coarse root masses with large interstitial spaces, eggs entangled among the roots were concentrated near the access canal and became more diffuse in fanlike patterns for distances of up to 5 cm. These patterns looked as though the eggs had been sprayed from the canal access point into the rootlets (Fig. 2C). In denser, less easily penetrated root masses with smaller interstitial spaces, more eggs were clumped in the main canals.

In loose root masses, we filmed Pirate Perch repeatedly thrusting their heads into the roots and wriggling back and forth, as though boring new holes or perhaps enlarging preexisting ones. However, some of the root masses used as nests seemed far too dense for the fish alone to have bored deeply. These canals must have been made by other organisms.

Larval and juvenile salamanders often resided deep inside the burrows that Pirate Perch used as spawning sites. On two occasions, we also found unhatched salamander embryos deep inside a root mass, together with an adult. We also observed dobsonfly larvae in the canal systems where Pirate Perch spawned.

In our raceway experiments, both juvenile *Desmognathus* salamanders and dobsonfly larvae produced small diameter burrows that ran deep into virgin root masses, including areas of fine compact rootlets. These canals appeared similar in structure to those that we observed routinely in the field.

Reproductive behavior.—Camera 1 provided our single best spawning observation. The filmed se-

quence of events (excerpts of which can be viewed at website www.genetics.uga.edu/Asayanus/) began about 20 min before the actual spawning act.

In this prelude period, at least six different fish (probably more, but the view was restricted) were swimming within a few centimeters of the root mass. One of these visited the focal canal periodically and faced it for short periods of time. Just before the spawn, this male stationed himself immediately below the opening, his ''chin'' resting on its entrance floor. A female moved in from above and positioned herself above the male, facing the hole. After backing away from the hole and returning several times, she entered headfirst and remained there for slightly less than four seconds, undulating briefly. Presumably after laying eggs, the female backed out of the hole and swam out of camera view.

Immediately, the male entered the hole headfirst, where he remained for five seconds. While inside, he released two separate, clearly visible clouds of milt, each time undulating his body violently. He then backed out of the hole but remained at its entrance. During the spawn, both male and female penetrated the root mass to approximately the depth of their anal fin (Fig. 3). We detected what appeared to be similar behavior on other occasions, but the fish generally swam too far into the root mass to permit further behavioral observations (or even to detect the presence of egg and milt clouds that would confirm spawning).

Following the spawning act described above, the male swam out of the hole briefly. As another fish approached, the original spawner positioned himself between the entrance and the intruder and then dove back headlong into the hole (to the depth of his anal fin), a maneuver that effectively plugged the entrance. Several minutes after the initial spawn, while maintaining this position, the focal male released a third burst of milt. He remained in the hole for several more minutes. Then, when another individual tried to bore into the spawning area from a lower portion of the root mass, the guarding male left the spawning hole and plunged back into the roots below, between the intruder and the nest (as if to head the intruder off). For at least another 20 min, the original spawner remained in or near the hole, defending it aggressively. Filming continued, but the spawning male went out of the field of view. Over the next two hours, at least one fish (presumably the same male) occasionally visited the hole and stuck its head inside but did not stay there, and no aggressive behaviors were seen.

We likewise filmed on five different nights (for 2–4 h each). Although certain nesting sites were hot spots of episodic activity, we never observed an individual fish to dominate a specific entrance persistently for an extended time. Also, no aggression was ever observed toward other fish species that approached the root masses. However, it should be emphasized that activities inside a root mass were not visible, and on occasion we did discover male Pirate Perch wedged in spawning canals of dissected root masses.

The lower surfaces of flowing root masses often were associated with considerable Pirate Perch activity, with at times more than a dozen individuals engaged in almost continual movements between the stream bottom and roots (Fig. 3). The most frequent behavior involved swimming in a vertical orientation from the substrate to the root mass, where a fish might come to rest hanging vertically by its extended pectoral fins among the roots, drift back to the stream bottom, or continue swimming up and over the roots and out of camera view. Sometimes, before swimming upward, the vertically aligned fish would sweep the substrate by violently thrashing its tail. During flurries of activity, at least six Pirate Perch (there were probably more, out of the cameras' view) were observed tail thrashing the substrate at once, producing clouds of sediment in the water column. After one such flurry, several fish dove into the root mass at one time and undulated forcefully. We suspect that tail-thrashing behavior may account for the saucer-like substrate depressions noticed in aquaria-housed Pirate Perch (Katula, 1987, 1992).

Genetic analyses.—The three microsatellite loci each displayed many alleles in our sample of Pirate Perch adults, yielding high heterozygosities and strong parental exclusion probabilities. Primers for locus AS13 (5′GTATAAAACCATC CTTCACTTGC3′ and 5′TGTGTTCGTGAGTG TGGCT3′) resulted in a total of seven alleles and $H_e = 0.629$. Primers for locus AS72 (5′CC TCCAATACTGTTCGGTGAG3′ and 5′AGTGG AAAGTGGGATGAGCAAA3′) showed a total of 11 alleles and $H_e = 0.815$. Locus 16.2 (primers 5′TGTTACTGTGGTGGGCTGCC3′ and 5′GTT GTTAGAATACACCGACT3′) was the most polymorphic marker, with 18 alleles and $H_e = 0.911$. Significant genotypic disequilibria between loci were not detected in the adult sample, nor were departures of genotypic frequencies from Hardy-Weinberg expectations (all $P > 0.05$).

A total of 351 embryos from three nests was genotyped (Table 2). Nest 10 was sampled most

TABLE 2. NUMBER OF OBSERVED ALLELES AT THE MOST POLYMORPHIC MICROSATELLITE LOCUS (AS 16.2) WITHIN EACH DEVELOPMENTAL STAGE (TABLE 1) OF PIRATE PERCH EMBRYOS, AND THE DEDUCED MINIMUM NUMBER OF THEIR PARENTS, AT NESTS 5, 7, AND 10.

Developmental phase (number genotyped)	Number of different alleles	Minimum number parents
Nest 5		
A (n = 24)	5	3
E (n = 2)	4	2
total (n = 26)	9	5
Nest 7		
A (n = 11)	7	4
B (n = 33)	8	4
C (n = 23)	5	3
total (n = 67)	11	6
Nest 10		
A (n = 54)	15	8
B (n = 54)	15	8
C (n = 47)	12	6
D (n = 52)	12	6
E (n = 51)	14	7
total (n = 258)	21	11

extensively, with a mean of 52 embryos from each of the five distinct developmental stages examined. In that nest, each stage proved to be the result of at least 6–8 spawning adults, and in each case there must have been multiple parents of both sexes (because no single set of alleles was shared across all embryos). When the data from all developmental stages in nest 10 were combined, it is evident that at least 11 different parents contributed to those progeny. The genotypic data cannot exclude the possibility that some parents of individual clutches may have spawned on multiple occasions within the nest.

Smaller numbers of embryos also were analyzed from nests 5 and 7 (Table 2). Again in each case, the genetic analyses indicate that multiple parents of both sexes had spawned successfully. Although more sophisticated methods are also available to provide less conservative estimates of numbers of parents, the qualitative results would remain the same as those found in our estimates.

DISCUSSION

Gilliams first described the Pirate Perch in 1824, but almost two centuries later this species' natural spawning behaviors and sites of field oviposition had continued to elude natural historians. Our discovery that Pirate Perch spawn in the canals of root masses represents the first documentation of this behavior in any species of North American fish. Our field observations in conjunction with genetic analyses not only provide the first documentation of natural reproductive behaviors in this species, but they also may have solved a longstanding enigma regarding the functional significance of this species' odd morphological attribute—a forward-shifted urogenital opening.

Spawning substrate.—Pirate Perch probably gain several benefits from depositing eggs in root masses. First, this behavior should enable developing embryos and fry to avoid or withstand fast-flowing water outside the protected nest. In other fish species inhabiting lotic environments, additional spawning behaviors known to circumvent water-velocity effects include: flow avoidance behind obstructions or in backwaters (e.g., North American sunfishes); egg burial in the substrate (salmonids and some darters); attachment of eggs to the underside of objects (sculpins and some darters); and deployment of adhesive eggs or even larvae (e.g., some minnows; Fletcher and Wilkins, 1999). Second, root masses probably make Pirate Perch eggs and embryos less exposed to damaging siltation. Third, root masses likely afford the offspring considerable protection against predation, especially by other fishes. An additional key benefit of root spawning (given the forward-shifted urogenital pore) is discussed below.

Symbiosis.—Many fish species are known to capitalize on the habitat modifications of other species during spawning. For example, many cyprinid species are known to spawn on nests created by other nest-building cyprinids and centrarchids (Fletcher and Burr, 1992; Johnston and Page, 1992). Such relationships may be obligative or facultative (Johnston and Page, 1992; Wallin, 1992) and could potentially be mutualistic or parasitic (Fletcher, 1993; Johnston, 1994). A number of costs and benefits are involved in these nest associations, but the one primarily applicable to Pirate Perch is the modification of habitat into a suitable spawning substrate by the nest-building species. All costs and benefits of the symbiotic interaction between Pirate Perch and the burrowing salamanders and dobsonflies were not assessed (i.e., whether the relationship is mutualistic, commensal, or parasitic was not determined). Nevertheless exploitation of the burrowing efforts of the salamanders and dobsonflies undoubtedly opens up spawning habitat to Pirate Perch that otherwise would not be available. The canals also fre-

quently allow deposition of eggs deeper into the root masses. The observation of Pirate Perch at times forming their own canals indicates a facultative association, and given the diverse habitat that the Pirate Perch resides in and its wide geographic range, use of canals created by other organisms (including a number of species of aquatic salamanders) is likely.

We suspect that the salamanders and dobsonflies may benefit from this association by occasionally preying on Pirate Perch eggs and larvae. Dobsonfly larvae are voracious predators on aquatic insects (Stewart et al., 1973; Pennak, 1978) and probably feed on small fish also. Adult salamanders feed opportunistically on insects and other aquatic invertebrates (Carr, 1940; Burton, 1976). Potential predation on Pirate Perch offspring by the canal builders could represent a cost to the Pirate Perch.

Male aggregations.—Although Pirate Perch are usually considered highly solitary, Abbott (1861) noted that adults school in the spring, and Katula (1992) reported male cohabitation of aquarium cavities during spawning periods. Our current data confirm and extend the notion that Pirate Perch congregate in small areas while spawning (Table 1). Sometimes, spawning was concentrated in a single root mass even when others nearby remained unexploited. For example, at nest site 11, two large root masses were adjacent, one of which contained multiple egg clusters, whereas the second was unused. Such findings suggest that adult Pirate Perch may be attracted by social interactions, as in colonially nesting fish species (Bietz, 1981; Gross and MacMillan, 1981). Although we have observed over 30 males at a root mass, females were never seen congregating in large numbers and appeared to enter the nesting locations only when ready to spawn. This spawning behavior of females is further elucidated by the discrepancy between the low number of females collected around each nest site and the genetic finding of multiple females contributing to each nest.

Benefits of a jugular vent.—Our discovery of large numbers of freshly spawned embryos and larvae inside root masses, coupled with our failure to detect any progeny in the branchial chambers of adults, indicates that Pirate Perch at our study site do not routinely gill-brood their offspring. Instead, the peculiar jugular placement of the urogenital pore would make the adults well suited for depositing eggs and sperm deep into tight chambers within the root masses. Each spawner swims head-first into a canal to deposit gametes. Gametic release from an anterior position also allows a Pirate Perch to back out of the canal with less dislodgment of its eggs or sperm. Furthermore, during sequential spawning in the headfirst position, an extensive array of sensory pores on the Pirate Perch's head (Moore and Burris, 1956) may help adults locate eggs previously deposited in a dark canal.

Although Pirate Perch are not branchial brooders, several observations suggest that they may spit eggs into the root masses. First, considerable force would seem to be required to spray eggs as far into the roots' interstitial spaces as we observed. Second, many canals appeared to be too narrow for the female to raise her head sufficiently to spray eggs effectively from the urogenital pore. Third, egg spitting would be consistent with the following observations by others: that released ova slide forward along a groove from the vent to the gill chamber (Martin and Hubbs, 1973); that the egg stream is split by the protuberance of thoracic muscle (Jenkins and Burkhead, 1994); and that ova were present in the branchial cavity of a preserved female (Boltz and Stauffer, 1986).

Parental care versus defense of paternity.—Aggregations of adults, the presence of several different age-class embryos within a nest, and the overall large number of eggs present at one time indicate that a given root mass is used repeatedly and by multiple spawners. The genetic data (Table 2) confirm that many parents contribute successfully to offspring within a nest, and that this is true even for nestmate embryos of identical developmental stage.

Immediately after a spawning event, a Pirate Perch male clearly defends the site of oviposition, driving away other males and using his body to plug an entrance to the egg-laden canal. Within two hours, however, we observed no further aggression near the hole entrance, nor did any fish persistently remain in the viewing area. Thus, we found no evidence for extended parental care in this species. Instead, we strongly suspect that aggressive behaviors of males at the nest, following the spawn, reflect paternity defense under the intense competition for fertilization success that must attend these group-spawning situations.

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Literature Cited

Abbott, C. C. 1861. Notes on the habits of *Aphredoderus sayanus*. Proc. Acad. Nat. Sci. Phila. 1861:95–96.

———. 1868. List of vertebrate animals of New Jersey, p. 807–808. *In:* The geology of New Jersey. G. C. Cook (ed.). Board of Managers, Newark, NJ.

———. 1870. Notes on fresh-water fishes of New Jersey. Am. Nat. 4:107.

Becker, H. R. 1923. The habitat of *Aphredoderus sayanus* in Kalamazoo County, Michigan. Occ. Pap. Mus. Zool. 138:1–4.

Bietz, B. F. 1981. Habitat availability, social attraction and nest distribution patterns in Longear Sunfish (*Lepomis megalotis peltastes*). Environ. Biol. Fish. 6: 193–200.

Boltz, J. M., and J. R. Stauffer Jr. 1986. Branchial brooding in the Pirate Perch, *Aphredoderus sayanus* (Gilliams). Copeia 1986:1030–1031.

———, and ———. 1993. Systematics of *Aphredoderus sayanus* (Teleostei: Aphredoderidae). *Ibid.* 1993:81–98.

Breder Jr., C. M., and D. E. Rosen. 1966. Modes of reproduction in fishes. Natural History Press, Garden City, NY.

Brill, J. S. 1977. Notes on abortive spawning of the Pirate Perch, *Aphredoderus sayanus*, with comments on sexual distinctions. Am. Curr. 5:10–16.

Burton, T. M. 1976. An analysis of the feeding ecology of the salamanders (Amphibia, Urodela) of the Hubbard Brook Experimental Forest, New Hampshire. J. Herpetol. 10:187–204.

Carr, A. F. 1940. A contribution to the herpetology of Florida. Biol. Sci. Ser., Univ. Fla. Publ. 3:1–118.

DeWoody, J. A., D. E. Fletcher, S. D. Wilkins, W. S. Nelson, and J. C. Avise. 2000. Microsatellite analyses of parentage and nest guarding in the Tessellated Darter (Pisces: Percidae). Copeia 2000:740–747.

Fletcher, D. E. 1993. Nest association of dusky shiner (*Notropis cummingsae*) and Redbreast Sunfish (*Lepomis auritus*), a potentially parasitic relationship. *Ibid.* 1993:159–167.

———, and B. M. Burr. 1992. Reproductive biology, larval description, and diet of the North American Bluehead Shiner, *Pteronotropis hubbsi* (Cypriniformes: Cyprindae), with comments on conservation status. Ichthyol. Explor. Freshwaters 3:193–218.

———, and S. D. Wilkins. 1999. Glue secretion and adhesion by larvae of Sailfin Shiner (*Pteronotropis hypselopterus*). Copeia 1999:274–280.

———, ———, V. McArthur, and G. K. Meffe. 2000. Influence of riparian alteration on canopy coverage and macrophyte abundance in Southeastern USA blackwater streams. Ecol. Eng. 15:S67–S78.

Fontenot, Q. C., and D. A. Rutherford. 1999. Observations on the reproductive ecology of Pirate Perch *Aphredoderus sayanus*. J. Freshwater Ecol. 14: 545–550.

Gilliams, J. 1824. Description of a new species of fish of the Linnean genus *Perca*. J. Acad. Nat. Sci. Phila. 4:80–82.

Gross, M. R., and A. M. MacMillan. 1981. Predation and the evolution of colonial nesting in Bluegill Sunfish (*Lepomis macrochirus*). Behav. Ecol. Sociobiol. 8:163–174.

Hardy Jr., J. D. 1978. Family Aphredoderidae, p. 15–23. *In:* Development of fishes of the mid-Atlantic Bight: an atlas of egg, larval, and juvenile stages. Vol. 3. U.S. Fish and Wildlife Service, Publ. FWS-OBS-78/12, Washington, DC.

Jenkins, R. E., and N. M. Burkhead. 1994. The freshwater fishes of Virginia. American Fisheries Society, Bethesda, MD.

Johnston, C. E. 1994. Nest association in fishes: evidence for mutualism. Behav. Ecol. Sociobiol. 35: 379–383.

———, and L. M. Page. 1992. The evolution of complex reproductive behaviors in North American minnows (Cyprinidae), p. 600–621. *In:* Systematics, historical ecology, and North American freshwater fishes. R. L. Mayden (ed.). Stanford Univ. Press, Palo Alto, CA.

Jordan, D. S. 1878. A catalogue of the fishes of Illinois. Ill. State Lab. Nat. Hist. Bull. 1:37–70.

Katula, R. S. 1987. Spawning of the Pirate Perch recollected. Am. Curr. 20:9.

———. 1992. The spawning mode of the Pirate Perch. Trop. Fish Hobby. 40:156–159.

Kellogg, K. A., J. A. Markert, J. R. Stauffer Jr., and T. D. Kocher. 1998. Intraspecific brood mixing and reduced polyandry in a maternal mouth-brooding cichlid. Behav. Ecol. 9:309–312.

Lee, D. S. 1980. *Aphredoderus sayanus* (Gilliams), p. 484. *In:* Atlas of North American freshwater fishes. D. S. Lee, C. R. Gilbert, C. H. Hocutt, R. E. Jenkins, D. E. McAllister, and J. R. Stauffer Jr. (eds.). North Carolina State Museum of Natural History, Raleigh.

LeSueur, C. A. 1833. *Aphredoderus*, p. 445–455. *In:* Histoire Naturale des Poissons. G. Cuvier and A. Valenciennes (eds.). Levrault, Paris.

Maniatis, T., E. F. Fristch, and J. Sambrook. 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Mansueti, A. J. 1963. Some changes in morphology during ontogeny in the Pirate Perch, *Aphredoderus s. sayanus*. Copeia 1963:546–547.

Martin, F. D., and C. Hubbs. 1973. Observations on the development of Pirate Perch, *Aphredoderus sayanus* (Pisces: Aphredoderidae) with comments on yolk circulation patterns as a possible taxonomic tool. *Ibid.* 1973:377–379.

Monzyk, F. R., W. E. Kelso, and D. A. Rutherford. 1997. Characteristics of woody cover used by brown

madtoms and Pirate Perch in coastal plain streams. Trans. Am. Fish. Soc. 126:665–675.

MOORE, G. A., AND W. E. BURRIS. 1956. Description of the lateral line system of the Pirate Perch, *Aphredoderus sayanus*. Copeia 1956:18–22.

MURDY, E. O., AND J. W. E. WORTHAM JR. 1980. Contributions to the reproductive biology of the Eastern Pirateperch, *Aphredoderus sayanus*. Va. J. Sci. 31: 20–27.

NELSON, J. S. 1994. Fishes of the world. 2d ed. Wiley-Interscience, New York.

PARKER, N. C., AND B. A. SIMCO. 1975. Activity patterns, feeding and behavior of the Pirate Perch, *Aphredoderus sayanus*. Copeia 1975:572–574.

PENNAK, R. W. 1978. Fresh-water invertebrates of the United States. 2d ed. John Wiley and Sons, New York.

PFLIEGER, W. L. 1975. The fishes of Missouri. Missouri Department of Conservation, Western Publishing Co., Jefferson City.

RAYMOND, M., AND F. ROUSSET. 1995. GENEPOP. Vers. 1.2: population genetics software for exact tests and ecumenicism. J. Hered. 86:248–249.

ROBISON, H. W., AND T. M. BUCHANAN. 1988. Fishes of Arkansas. Univ. of Arkansas Press, Fayetteville.

STEWART, K. W., G. P. FRIDAY, AND R. E. RHAME. 1973. Food habits of hellgrammite larvae, *Corydalus cornutus* (Megaloptera: Corydalidae), in the Brazos River, Texas. Ann. Entomol. Soc. Am. 66:959–964.

WALLIN, J. E. 1992. The symbiotic nest association of Yellowfin Shiners, *Notropis lutipinnis*, and Bluehead Chubs, *Nocomis leptocephalus*. Environ. Biol. Fish. 33: 287–292.

WHITEHURST, D. K. 1981. Seasonal movements of fishes in an eastern North Carolina swamp stream, p. 182–190. *In:* Proceedings of the Warmwater Streams Symposium. American Fisheries Society, Bethesda, MD.

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