

## ABSTRACT

FAVROT, SCOTT DOUGLAS. Sicklefin Redhorse Reproductive and Habitat Ecology in the Upper Hiwassee River Basin of the Southern Appalachian Mountains. (Under the direction of Dr. Thomas J. Kwak).

The sicklefin redhorse *Moxostoma* sp. (Catostomidae) is a potamodromous, undescribed and imperiled species endemic to a restricted geographic range in the Blue Ridge physiographic province of North Carolina and Georgia. Research designed to study the reproductive and habitat ecology of this species revealed that Hiwassee River tributaries were frequently occupied during the spawning season; lower reaches of tributaries and Hiwassee River were primarily occupied during the postspawning season; and lower lotic reaches of Hiwassee River were occupied most during winter. Sicklefin redhorse selected annual, seasonal, and spawning microhabitat nonrandomly, and were generally associated with swift thalweg currents, shallow depths, and coarse substrates (e.g., boulder and bedrock) supporting river weed *Podostemum ceratophyllum*; however, different microhabitats were selected seasonally and during spawning. During 2007, mean daily water temperature for observed sicklefin redhorse spawning was 17.5 °C (11.0–21.1 °C). Male and female sicklefin redhorse conducted spawning migrations simultaneously; however, males occupied spawning tributaries longer than females. All sicklefin redhorse that conducted spawning migrations during both 2006 and 2007 demonstrated spawning tributary fidelity. Foraging was the most common behavior observed for adult sicklefin redhorse, and bedrock was the dominant foraging substrate. Sicklefin redhorse reproductive behavior was generally similar to congenics, but courting fish exhibited pod-courting behavior and females displayed an

undescribed repetitious postspawning digging behavior. Spawning substrate samples revealed very coarse gravel and small cobble as dominant substrates. My results on sicklefin redhorse increase knowledge of their reproductive and habitat ecology, seasonal behavior, and suggest critical management issues.

I also quantified the spawning migration of black redhorse *Moxostoma duquesnei*, golden redhorse *Moxostoma erythrurum*, river redhorse *Moxostoma carinatum*, sicklefin redhorse, and silver redhorse *Moxostoma anisurum* with resistance board weirs and prepositioned areal electrofishers (PAEs) during the springs of 2006 and 2007 in Valley River, North Carolina. Spawning was observed for all five redhorse species in 2007 at water temperatures generally ranging 8–21 °C and mean daily Valley River flows ranging 1.7–4.5 m<sup>3</sup>/s. Silver redhorse migrated and spawned first, followed by black redhorse, sicklefin redhorse, golden redhorse, and river redhorse with considerable temporal overlap. Male golden redhorse and river redhorse displayed agonistic behavior and spawning site fidelity, while female sicklefin redhorse displayed spawning site fidelity. Silver redhorse and river redhorse were not significantly different in size (total length and weight;  $P > 0.05$ ), while the other redhorse species differed significantly ( $P < 0.05$ ). The spawning migration chronology between males and females was significantly different ( $P < 0.05$ ) for black redhorse and sicklefin redhorse, but not for the other three species. All five species selected spawning microhabitat nonrandomly. Interspecific redhorse spawning site microhabitats were generally significantly different ( $P < 0.05$ ) due to deviation of one or two species. My findings among redhorse species suggest that seasonal, habitat, and ethological reproductive

isolating mechanisms are functioning within Hiwassee Basin; however, anthropogenic activities and alterations could diminish these reproductive barriers.

I compared resistance board weirs and prepositioned areal electrofishers to determine sampling efficiency for migrating potamodromous fishes. Fish species richness and diversity were higher for PAE total catch, while weir catch had higher species dominance. Total PAE catch by number was much higher than total weir catch, but PAE total biomass was lower than that of weirs. PAE sampling was biased with water velocity, but weirs did not demonstrate that attribute. On average, PAE fish mortality was five times higher than weir mortality. My results comparing gear efficiency will assist fisheries managers in selecting the most appropriate gear to address research and management objectives when sampling medium-sized rivers.

Sicklefin Redhorse Reproductive and Habitat Ecology in the Upper Hiwassee River Basin of  
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by  
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## **DEDICATION**

This thesis is dedicated to my father John Favrot for taking the time to introduce me to the outdoors and fishing and, my mother Kathy Favrot for always being there. I also dedicate this thesis to my wife Lindsay Favrot for her understanding and constant support.

## **BIOGRAPHY**

I am a native of the southern Appalachian Mountains and grew up in Black Mountain, North Carolina, situated immediately south of Mount Mitchell and the Blue Ridge Parkway. As a child, I was encouraged to explore nature by my parents and quickly became a nuisance to them by constantly wandering off to the nearest mountain seep, spring, creek, or stream to flip rocks for spring lizards. It did not take long to learn that these salamanders were the best fish bait one could use. I started fishing for anything that would bite in a local lake and caught fish ranging from bluegill to striped bass, likely stocked by locals. My father introduced me to the opening day of the North Carolina trout season when I was 5 years old on a small mountain stream named Curtis Creek, flowing through Pisgah National Forest. This day, I believe, is when the seed was planted and my future was set in motion. I did not miss an opening day of trout season with my father for the next 20 years and was typically wetting a line even when he could not join. The stream that taught me to trout fish flows through the heart of Black Mountain and is named the Swannanoa River. I quickly became bored with capturing weak-tailed, pectoral fin missing, and uneducated stocked trout and only fished backcountry streams for wild trout and occasionally indigenous speckled trout. These experiences fostered a desire within me to strive toward a career working within clear cold mountain streams, and eventually a yearning to understand montane stream ecology and protect these pristine natural resources that had provided me with endless opportunity for adventure and enjoyment.

I was accepted to North Carolina State University in 1997 and promptly began enrolling in courses required for a B.S. degree in Fisheries and Wildlife Sciences with a concentration in fisheries science. I graduated in 2001, and accepted a volunteer position in Yellowstone National Park (YNP) conducting backcountry radio-tracking of westslope cutthroat trout and always yelling “Hey, bear!” as grizzly bears were always near. I volunteered my time toward this position for the next six months until I was forced to return to western North Carolina by malnourishment and the end of the 2002 sampling season. I returned to Yellowstone National Park in 2003 as water quality crew leader and was highly influenced by the professionalism and guidance of Supervisory Fisheries Biologist Dr. Todd Koel and Aquatic Ecologist Jeff Arnold. Although I regarded YNP as heaven on earth, I resigned my position in 2004 and returned to Black Mountain again, this time in hopes of pursuing a serious relationship with a long time girlfriend.

While working as an environmental consultant in Cary, North Carolina, I contacted Dr. Tom Kwak inquiring about the availability of possible research assistantships and was informed of a project studying the reproductive ecology of an undescribed nongame sucker in the mountains of western North Carolina. I was keenly interested and immediately excited when I was offered the position to conduct ground-breaking fisheries research in a southern Appalachian stream similar to the streams I fished as a child. While serving as research assistant and pursuing my Master’s Degree in Fisheries Science, I married that girlfriend (Lindsay Favrot) I left YNP for. I have recently accepted an Assistant Project Leader position in La Grande, Oregon, and plan to move there in January of 2009. Future plans

include committing my passion for pristine nature and sedulous work ethic to the natural resources of northeastern Oregon, exploring the northern Rockies further, releasing my first Chinook salmon and steelhead, processing my first pronghorn and bull elk, enjoying the sight of cupped and coming, and summiting young jagged, snow-covered peaks of the northern Rocky Mountains as apposed to the old smooth, hardwood-covered peaks of the southern Appalachians which will always be home.

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**Chapter I – Sicklefin Redhorse Reproductive and Habitat Ecology in the Upper  
Hiwassee River Basin of the Southern Appalachian Mountains**

## **Abstract**

The sicklefin redhorse *Moxostoma* sp. (Catostomidae) is a potamodromous, undescribed and imperiled fish species endemic to a small geographic range in the Blue Ridge physiographic province of North Carolina and Georgia and currently a candidate to be protected under the Endangered Species Act (1973). I assessed spatial and temporal bounds of the spawning migration, quantified seasonal weekly movement patterns, identified seasonal microhabitat suitability, and characterized behavior of the Hiwassee Basin sicklefin redhorse population. I implanted 34 adult fish from Valley and Hiwassee rivers with radio transmitters during March and April of 2006 and 2007 between Mission Dam and Hiwassee Lake, North Carolina. Fish were relocated weekly from late-March to early-July in 2006 and 2007 and once during mid-January in 2007 and 2008 to determine seasonal migration patterns.

Sicklefin redhorse migrated into tributaries during the spawning season; most ascended Valley River, but Brasstown Creek, Hanging Dog Creek, and Nottely River were also occupied. Tributaries were most frequently occupied during the spawning season, lower reaches of tributaries and Hiwassee River were primarily occupied during the postspawning season, and lower reaches of Hiwassee River were occupied most during winter. Radio-tagged fish were never observed to consistently occupy Hiwassee Lake and appear to only move through Hiwassee Lake during spawning migrations to and from Valley River, Hanging Dog Creek, and Nottely River.

Sicklefin redhorse selected annual, seasonal, and spawning microhabitat nonrandomly. Sicklefin redhorse were associated with swift thalweg currents, shallow depths, over coarse substrates (e.g., boulder and bedrock) supporting river weed *Podostemum ceratophyllum*. However, different microhabitats were selected seasonally and during spawning, with marginal habitat, shallower depths, slower current velocities, smaller substrates (e.g., cobble), cover (e.g., boulder), near cover distances, and substrate lacking river weed being preferentially selected while spawning. Thalweg habitat, deeper depths, swifter velocities, abundant river weed, and coarser substrates with no cover were selected during the spawning and postspawning seasons, while deeper depths and slower velocities were optimal during winter. During 2007, mean daily water temperature for observed sicklefin redhorse spawning was 17.5 °C (11.0–21.1 °C).

Male and female sicklefin redhorse conducted spawning migrations simultaneously, but males occupied spawning tributaries longer than females. All sicklefin redhorse that conducted spawning migrations in 2006 and 2007 did so to the same spawning tributary and generally to the same spawning reach between years. A cold front that crossed the study area during 2007 temporarily terminated the spawning migration and caused some fish to return to Hiwassee River. Similarly, sicklefin redhorse were not observed within the upper reaches of Nottely River downstream of Nottely Dam where hypolimnetic releases remain unaltered by meteorological conditions. Foraging was the most common behavior observed for adult sicklefin redhorse, and bedrock was the dominant foraging substrate annually.

Sicklefin redhorse reproductive behavior was typical for the genus *Moxostoma*; however, fish exhibited atypical roaming and pod-forming courting behavior, and females displayed spawning site fidelity, while males displayed no agonistic behavior. In addition, an undescribed repetitious post-spawning digging behavior by females was observed. Spawning substrate samples revealed that very coarse gravel and small cobble were dominant, and were on average 3.0% fines (i.e.,  $\leq 2.0$  mm). A Fredle index value of 28.2 suggests that spawning substrate permeability is moderately diminished or at half potential. Eggs were recovered from 1 of 31 spawning sites excavated and were adhered to gravel covered by small cobble. Sicklefin redhorse generally occupied Hiwassee River from late-spring through late-winter while foraging from extensive outcroppings of rugose bedrock supporting river weed, and Hiwassee River tributaries during the spring for spawning. My results increase knowledge of sicklefin redhorse reproductive ecology and seasonal behavior, suggest critical issues, and guide management important to the life history of the species.

## **Introduction**

Despite mounting recognition by authorities in the United States (Endangered Species Act, ESA) and Canada (Committee on the Status of Wildlife in Canada, and the Species at Risk Act, SARA), nongame fishes typically become imperiled prior to receiving management consideration or conservation efforts (Cooke et al. 2005). The fish assemblage of the southeastern United States is among the most diverse in North America (Burr and Mayden 1992), being comprised of 44 recognized catostomids, of which 17 species are

redhorses of the genus *Moxostoma* (Warren et al. 1997; Cooke et al. 2005). Partially, as a result of suckers being commonly classified as economically and recreationally inconsequential by the general public and regulatory entities, several redhorse species have recently been either rediscovered following formal conception as extinct (e.g., robust redhorse *Moxostoma robustum*) or remain undescribed (e.g., Carolina redhorse and sicklefin redhorse *Moxostoma* spp.). Knowledge, substantiated by quantitative data, on the reproductive and habitat ecology of the sicklefin redhorse is crucial to effectively implement management plans.

Identifying, conserving, and protecting critical habitat associated with imperiled and endangered species is vital to ensure species recovery and persistence. In some cases, where life history information is known, operational conditions of hydropower facilities have been renegotiated to provide flows necessary during critical periods (e.g., spawning and overwintering; Cooke et al. 2005). While modifications in flow may partially alleviate certain negative consequences of hydroelectric dams, principal disturbances remain (e.g., barrier effect, genetic isolation, critical habitat loss). Identifying a critical habitat subset is essential knowledge for regulatory entities responsible for managing and reducing detrimental effects of environmental manipulations such as those affecting the sicklefin redhorse (Rosenfeld 2003).

The sicklefin redhorse is a colorful medium-sized fish with a deeply falcate (sickle-shaped) dorsal fin, adapted to swift waters of moderate gradient. This fish was recognized as a distinct species in 1992 by Dr. Robert E. Jenkins, who is currently in the process of

describing the species (Robert E. Jenkins, personal communication). Sicklefin redhorse are dissimilar from other *Moxostoma* species and are endemic to a relatively small geographic range, comprised of the Hiwassee and Little Tennessee river systems of the upper Tennessee River drainage (Ohio River Basin), in the Blue Ridge physiographic province of North Carolina and northern Georgia (Jenkins 1999). Historically, sicklefin redhorse likely inhabited the majority of large streams and rivers in the Hiwassee and Little Tennessee systems, but currently inhabit less than 20% of each the Hiwassee and Little Tennessee systems (Jenkins 1999).

Many riverine potamodromous fishes (i.e., fish that reside and migrate entirely within freshwater) require clean substrate and well-oxygenated lotic habitats to complete their life cycle (Balon 1975). A reduction in sicklefin redhorse abundance and distribution, similar to western United States potamodromous species, may be attributed to habitat degradation and fragmentation among other causal factors (e.g., Warren et al. 2000). Steep slopes combined with prevalent mining, livestock grazing, and agriculture during the late 1800s and early 1900s resulted in extensive sedimentation throughout western North Carolina (Jenkins 1999). Consequential loss of spawning and foraging habitat was exacerbated by inadequately treated municipal sewage and industrial wastes that were routinely deposited into the Hiwassee and Little Tennessee systems until the early 1960s. More recently, Mission Reservoir was drained in 1997 to reduce alluvium deposition, resulting in extensive sediment deposition in the Hiwassee River extending from Mission Dam into Hiwassee Lake (Jenkins 1999).

Extensive dam construction occurred within the Hiwassee and Little Tennessee systems between 1911 and 1957 (Etnier and Starnes 1993). Hydroelectric dams have been associated with blocking fish migration corridors (Holden 1979), decreased growth rates as a function of decreased temperatures (Clarkson and Childs 2000), decreased dissolved oxygen concentrations (Hill 1980), obstructing sediment transport (Kondolf 1997; Camargo and Voelz 1998), and an unstable flow regime (Minckley et al. 2003). Resulting reservoirs have drastically altered habitat previously inhabited by sicklefin redhorse and fragmented populations and subsequently, gene pools (Jenkins 1999). A large portion of shallow and moderate gradient riverine habitat, conducive to sicklefin redhorse spawning and foraging, was converted into deep, stratified, lacustrine habitat (e.g., Hiwassee Reservoir and Fontana Reservoir) not suitable for spawning (Jenkins 1999). In addition to downstream effects of hydroelectric dams, less abrupt but evident upstream disturbances have profound effects on the perpetuity of potamodromous species (Winston et al. 1991; Pringle 1997; Morita and Yamamoto 2002). In addition to large obvious migration corridor barriers, smaller barriers (e.g., beaver dams, culverts, low-head dams) exist within the upper reaches of many sicklefin redhorse spawning tributaries.

Habitat fragmentation is detrimental to population persistence (Wilcox and Murphy 1985). Genetic, demographic, and environmental stochasticity have profound effects on species constrained to small and fragmented geographic ranges, resulting in an elevated probability of extinction (Shaffer 1981; Lande 1988). Small populations tend to have fewer alleles within their gene pools, rendering the populations less adaptive to environmental

change and more susceptible to local extinction (Primack 1993). Angermeier (1995) observed that Virginia fish extirpation patterns show that aquatic degradation is extensive and multifaceted, and he suggested that extirpations in Virginia and other regions of North America are the result of cumulative effects of multiple anthropogenic impacts. Similar to the sicklefin redhorse, Angermeier (1995) concluded that extirpation patterns of Virginia fishes indicate that species with a limited physiographic range or a limited range of water sizes that display migratory behavior or that are ecologically specialized are at an elevated risk of extirpation or extinction as a result of an exacerbated vulnerability to reduced numbers, fragmentation, and isolation. Sicklefin redhorse currently display all of these attributes, and are highly susceptible to extinction. The sicklefin redhorse is currently a candidate to be listed under the Endangered Species Act of 1973 (USFWS 2005; 2008).

Adult sicklefin redhorse are most commonly found in riffles, runs, and flowing portions of pools of warm, moderate-gradient streams and rivers. Foraging substrate includes silt-free gravel, cobble, boulders, bedrock, sticks, coarse woody debris, and submerged aquatic vegetation (i.e., river weed *Podostemum ceratophyllum*; Jenkins 1999). Sicklefin redhorse, similar to other *Moxostoma* species, forage on benthic macroinvertebrates, small bivalves, and gastropod mollusks (Jenkins 1999). Sicklefin redhorse, an obligate riverine species, originated in lotic environments yet is tolerant of lentic habitat at certain stages of the life cycle (i.e., juveniles). The maximum known longevity of a sicklefin redhorse is 22 years; however, they reach maturity at ages 5–7 for males and 7–8 for females (Jenkins 1999, 2005). Juvenile sicklefin redhorse have been observed and collected in reservoirs at higher

frequencies near the dam, river mouths, and within tributary arms (Jenkins 1999). Juvenile sicklefin redhorse are scarce in streams and are believed to migrate to lower river reaches and reservoirs after emergence (Jenkins 1999).

During late-April to mid-May, adult sicklefin redhorse migrate from lower stream reaches to upper stream reaches for the purpose of spawning (Jenkins 1999). Several integrated environmental cues (temperature, photoperiod, and flow) are believed to trigger this mass spawning migration (Kwak and Skelly 1992). Temperatures during the spawning migration range from 10 °C to 16 °C (Jenkins 1999).

Sicklefin redhorse spawning typically occurs in the middle and upstream reaches of rivers and near the shoreline of moderate to swift runs and riffles. Similar to other *Moxostoma* (Curry and Spacie 1984; Page and Johnston 1990; Kwak and Skelly 1992), sicklefin redhorse spawn over coarse gravel and small cobble (Jenkins 2005). Distinct from other *Moxostoma*, the sicklefin redhorse is strictly a roaming, troupe and pod-forming, gang-spawner (Jenkins 1999). Site preparation is absent and eggs are thought to be covered by substrate during the act of quivering; males display no overt agonistic behavior during courting or spawning, and females exhibit high fidelity for oviposition sites (Jenkins 1999).

In addition to sicklefin redhorse, the black redhorse *Moxostoma duquesnei*, golden redhorse *Moxostoma erythrurum*, silver redhorse *Moxostoma anisurum*, smallmouth redhorse *Moxostoma breviceps*, and river redhorse *Moxostoma carinatum* also inhabit the Hiwassee and Little Tennessee river systems. Sicklefin redhorse spawning coincides with the late spawning of black redhorse and early spawning of golden redhorse. The black redhorse and

golden redhorse initiate spawning following the peak spawning of the silver redhorse and smallmouth redhorse. Commencement of river redhorse spawning coincides with golden redhorse peak spawning (Jenkins 1999).

Reduction in recruitment of western United States potamodromous catostomid fishes has been partially ascribed to competition and predation by nonnative fishes. Predation by flathead catfish *Pylodictis olivaris* and channel catfish *Ictalurus punctatus* has been correlated with reductions in razorback sucker *Xyrauchen texanus* recruitment (Marsh and Langhorst 1988; Marsh and Brooks 1989). Minckley (1983) ascertained that predation by introduced ovivorous and piscivorous fishes is the most single important factor in the extirpation of the razorback sucker.

The Hiwassee and Little Tennessee river systems contain 16–19 nonnative fish species, of which 15 are omnivores, ovivores, or piscivores (NCDWQ 2006). Wheeler et al. (2003) found that 32.9% of nonnative blueback herring *Alosa aestivalis* diets containing prey items, collected during April and May in the Hiwassee River, contained fish eggs, of which 98.8% were white bass *Morone chrysops* eggs, while 14.3% of diets containing prey items contained larval fish, of which 50% were catostomid fishes.

Increasing our understanding of sicklefin redhorse reproductive and habitat ecology will provide essential information to regulatory agencies that may guide management, protection, and preservation of this rare, endemic, and imperiled species. Implementing sound management designed to increase survival and recruitment pivots on understanding sicklefin redhorse reproductive ecology and possible recruitment bottlenecks. Successful

recovery efforts are encumbered by a lack of detailed information on natural history and ecology of early life stages of catostomids (Cooperman and Markle 2003). Spawning habitat needs to be identified and categorized relative to the geographic range of the sicklefin redhorse. Identifying spawning triggering mechanisms (e.g., photoperiod, temperature, and discharge) and resulting *Moxostoma* spawning chronology may direct the establishment of guidelines toward restoration of suitable flows downstream of hydroelectric dams. Establishing flow regimes that are conducive to sicklefin redhorse spawning, based on data collected from unregulated spawning tributaries, could mitigate habitat degradation effects and restore function to critical habitat.

### **Objectives**

This research was designed to identify and describe the reproductive behavior and critical habitat of the sicklefin redhorse considering the restricted geographic range, degraded and fragmented habitat, and recent introduction of predacious species to guide future management decisions. Specifically, my objectives were to research sicklefin redhorse reproductive ecology by (1) identifying the temporal and spatial bounds of the spawning migration using radio-telemetry; (2) quantifying weekly directional movement patterns during the spawning period; (3) identifying and characterizing annual, seasonal, and spawning microhabitat suitability by comparing microhabitat use to available microhabitat.

## **Methods**

### *Site Description*

This study was conducted within the upper Hiwassee Basin of the southern Blue Ridge Province in the southern Appalachian Mountains of western North Carolina and northern Georgia (Figure 1). The majority of the upper Hiwassee Basin is positioned within the northeast-trending Murphy lithotectonic belt, extending northeast from Cartersville, Georgia, to Bryson City, North Carolina and between the Unaka Range to the west and the Blue Ridge Range to the east (Power and Forrest 1971). The Murphy Belt is composed of crystalline rocks such as relatively inert dark shale, sandstone, metasilstone, marble, calcareous siltstone, and clean quartzite (Hatcher and Goldberg 1991).

Valley River, a tributary to the Hiwassee River and a known spawning tributary of sicklefin redhorse, was chosen as the primary stream in which to conduct this research. Valley River is an unregulated, seventh-order, moderate gradient tributary of the upper Hiwassee River system in the upper Tennessee River drainage and drains approximately 303 km<sup>2</sup>. Valley River is approximately 47.1 km long and has a maximum headwater elevation of 1,339 m. Similar to other watersheds within the Hiwassee Basin, the Valley River watershed typifies a trellis drainage pattern as a result of parallel stratigraphic ridges associated with anticlines and synclines created from folded sedimentary sequences (Twidale 2004). The high-gradient watershed that encompasses the tributaries and head waters of Valley River is primarily composed of deciduous forest, while lower Valley River is largely surrounded by land devoted to agriculture. Valley River converges with Hiwassee River 47

km downstream from where Valley River originates in the Snowbird Mountains. The Valley River and Hiwassee River confluence is periodically inundated by Hiwassee Lake when reservoir levels are at full pool.

Hiwassee River, residing within the upper Tennessee River drainage, is a highly regulated system that originates on the northwestern slopes of the Blue Ridge Mountains in northern Georgia. Since the Hiwassee Basin sicklefin redhorse population appears restricted to upstream of Hiwassee Dam and downstream of Mission Dam (Jenkins 1999), this is the only section of Hiwassee River that was researched during this study. At the Valley River and Hiwassee River confluence, the Hiwassee River is a seventh-order stream that drains 1,092 km<sup>2</sup>. This upper portion of the Hiwassee River watershed is approximately 81% forested, 8% agriculture, 6% developed, and 5% other. Hiwassee River, from the headwaters to the Valley River and Hiwassee River confluence, is approximately 79.4 km long and has a maximum headwater elevation of 1,422.3 m. The majority of Hiwassee River's substratum is composed of arcuate bedrock outcroppings best described as "rugose" in nature. These bedrock outcroppings, or bedrock "flats", constitute the entire stream bed for many extensive and considerable reaches of river (e.g., 1–2 km). River weed, a freshwater aquatic dicotyledon lacking true roots and the only North American member of a predominately tropical family (i.e., Podostemaceae), is abundant within Hiwassee River and the lower reaches of most spawning tributaries.

Hiwassee Lake is impounded by Hiwassee Dam, which was completed in 1940 with recreation, hydroelectric power generation, and flood control as functions (NCDENR 2002).

Hiwassee Dam is 93.6 m high and 419.4 m across. Hiwassee Lake is a 2,464-ha oligotrophic impoundment with 262 km of shoreline and a drainage area of 2,507 km<sup>2</sup>. The mean depth of Hiwassee Lake is 47 m and the hydraulic retention time is 116 days (NCDENR 2002).

Hiwassee Lake has a diverse fish assemblage composed of ovivores (e.g., blueback herring), planktivores (e.g., threadfin shad *Dorosoma petenense* and gizzard shad *Dorosoma cepedianum*), and piscivores (e.g., black crappie *Pomoxis nigromaculatus*, white bass, largemouth bass *Micropterus salmoides*, smallmouth bass *Micropterus dolomieu*, and walleye *Stizostedion vitreum*) (available online at [http://www. Esb.enr.state.nc.us/BAU.html](http://www.Esb.enr.state.nc.us/BAU.html)). Hiwassee Lake's walleye recruitment has experienced a significant decline since 1997, after the first capture of blueback herring (Wheeler et al. 2004). Blueback herring occupy tributaries (e.g., Hanging Dog Creek and Valley River) utilized by spawning walleye, white bass, and redhorse species during the spring. Blueback herring demonstrated significant predation on white bass eggs in Hanging Dog Creek and Hiwassee Lake (Wheeler et al. 2004), possibly indicating a limiting mechanism for walleye recruitment.

### *Radio Telemetry*

Twenty-five adult sicklefin redhorse were implanted with Advanced Telemetry Systems radio transmitters (Model F1820) with a 12 h/d duty cycle on March 30, March 31, and April 5 in 2006. An additional nine adult sicklefin redhorse were implanted with a radio transmitter on March 9 in 2007 (Table 1). In addition, a 125 kHz 12 mm passive integrated transponder (PIT) tag (Biomark, Inc.; Model TX1400L) was implanted into the muscle near

the base of the dorsal fin of all radio-tagged fish (Jenkins and Smith 1990). Twenty-six additional sicklefin redhorse were PIT-tagged, but not radio-tagged, during this period. Tagged fish were captured by boat electrofishing, using a Smith-Root 2.5 GPP electrofishing unit with pulsed DC current (60 Hz) at 3.0–5.0 A. Considerable effort was exerted to obtain adult sicklefin redhorse from both Hiwassee River and Valley River to reduce capture location bias. Fish maturity determination was based on criteria found in Jenkins (1999).

Specifically, in 2006, six radio transmitters were implanted in adult sicklefin redhorse obtained approximately 3 km downstream from the confluence of Valley River and Hiwassee River and just upstream from Hiwassee Lake, and 19 transmitters were implanted in fish captured in Valley River approximately 0.5–2.0 km upstream of the confluence of Valley River and Hiwassee River. In 2007, an additional nine radio transmitters were implanted in adult sicklefin redhorse captured in Hiwassee River approximately 4.5 km upstream from the confluence of Valley River and Hiwassee River.

Following capture, total length (mm, TL), weight (g), water temperature (°C), and capture location geographic coordinates (UTM) were obtained for all adult sicklefin redhorse. Determination of sex was accomplished by gamete expression and percent tubercle development. Gamete expression was classified based on gamete maturation stage (i.e., not ripe, hard squeeze ripe, slight squeeze ripe, running ripe), and percent tubercle development was visually and tactilely estimated.

Adult sicklefin redhorse weighing greater than 425 g were utilized to ensure that radio transmitters weighed less than 2% of the fish's total body weight (Table 1; Winter

1996); mean percent of body mass relative to transmitters utilized was 0.7% (SE 0.0003).

All radio transmitters operated between 48.0 and 49.0 MHz and emitted a signal at a rate of 34 pulses per minute, guaranteeing a battery life of 287 days and an expected battery life of 575 days. Radio transmitters utilized had a mean weight of 8.19 g (SE 0.05) in air.

Sicklefin redhorse transmitter implantation surgeries were conducted on the stream bank promptly following capture. Immediately following capture, adult sicklefin redhorse chosen for radio-tag implantation were individually placed in an aerated 40 L container containing a 35–40 mg/L Benzocaine solution until a loss of equilibrium and reduced opercular rate was achieved. Following the anesthetized sicklefin redhorse displaying the symptoms of stage 4 anesthesia (Summerfelt and Smith 1990) (mean 4.5 minutes, SE 0.17), a sterilized transmitter was inserted intraperitoneally through a 1.5 cm incision anterior to the pelvic girdle and offset 1.5 cm left of the ventral midline. Transmitters with a trailing antenna were employed to maximize field range. The trailing antenna was coiled up and inserted into the body cavity to minimize mortality as external wire antennas may be associated with mortality and infection of tissues around the exit of the antenna (Matheney and Rabeni 1995). The tip of each trailing antenna included a 3 mm Scotchcast resin bead to prevent peritoneal irritation. Following transmitter implantation, sterile, non-absorbable, monofilament suture material (Monosof 3–0) with a 24 mm 3/8 circle, reverse cutting needle was used to close the incision (Wagner and Cooke 2005). Mean total surgery time for all radio-tagged sicklefin redhorse was 7.9 minutes (SE 0.35). Once the incision was closed, fish were placed into an aerated, covered tank and monitored until normal equilibrium and

operculum rate were regained (mean 2.6 minutes, SE 0.27). Following initial recovery, implanted fish were transferred to a 2.12-m<sup>3</sup> coated wire mesh instream cage (mean 3.2 h, SE 0.16) to ensure immediate post-surgery survival prior to release.

In general, radio-tagged fish were tracked weekly during the reproduction period (April–June), following a 14-day post-surgical period to ensure normal behavior (Stasko and Pincock 1977). Weekly radio-tracking was terminated on 8 July 2006 and 9 June 2007. To document seasonal patterns, additional tracking occurred 12–13 October in 2006, 11–14 January in 2007, and 14–17 January in 2008 to obtain postspawning and winter relocations. Tracking was not conducted during October 2007, because relocations obtained during October 2006 revealed that all radio-tagged fish had not moved substantially since July 2006.

Four river reaches [Brasstown Creek (23.8 km), Hanging Dog Creek (14.4 km), Hiwassee River (17.1 km), Valley River (32.2 km)] were tracked by canoe weekly in 2006. Nottely River (24.0 km) was tracked on 22 June 2006 from Nottely Dam to Hiwassee Lake in an attempt to relocate one missing radio-tagged sicklefin redbreast. In 2007, Valley River and Hiwassee River were tracked on a weekly basis, while Brasstown Creek, Hanging Dog Creek, and Nottely River were tracked on a bi-weekly basis. Upper Hiwassee Lake, upstream from Lake Marker Number 7, was tracked by motor boat on a weekly basis from 13 May to 7 July in 2006 and 17 April to 9 June in 2007. On 24 June 2006 and 9 June 2007, the entirety of Hiwassee Lake, from Hiwassee Dam to the Valley River and Hiwassee River confluence, was tracked in an attempt to relocate missing radio-tagged fish. Several smaller

tributaries within the Hiwassee River Basin were tracked by foot or vehicle in 2006 and 2007 in an attempt to relocate missing fish (Table 2).

Typically, I relocated undisturbed sicklefin redhorse using an ATS Model R2100 receiver and a handheld loop antenna from a two-person canoe. Upon receiving a signal from a radio transmitter, the canoe was promptly maneuvered to the stream bank and the exact fish location was determined using triangulation techniques. Once the exact location was determined, visual observation was attempted to establish activity (i.e., courting, spawning, foraging, resting, and migrating). Geographic coordinates were obtained using a hand-held global positioning system unit (Garmin GPS 5) for all relocation sites. Microhabitat use variables (depth (m), bottom velocity (m/s), mean column velocity (m/s), dissolved oxygen (mg/L), temperature (°C), dominant substrate, subdominant substrate, cover type, distance to cover (m), distance to bank (m), and occurrence of river weed (i.e., present or absent) were measured and collected at the exact relocation site for all relocated fish. While tracking by canoe, numerous undisturbed but untagged adult sicklefin redhorse were observed. Behavior and microhabitat use data were also collected for these undisturbed fish.

Considerable effort (4,350 person hours, 15.1 hours/day, 2006; 3,570 person hours, 15.0 hours/day, 2007) was expended between 2006 and 2007 to conduct the necessary field work required to accomplish my research objectives. A total of 106 tracking sessions were completed resulting in 261 and 215 relocations and 149 and 67 visual observations in 2006 and 2007, respectively, yielding 692 total fish locations. A total of 1,091.6 river km (503

hours) and 902.7 river km (450 hours) were tracked in 2006 and 2007, respectively. An average of 4.2 km was canoed to obtain a single radio-tagged fish relocation and 2.9 river km to obtain a single fish location (radio-tagged or visual observation).

Tracking within multiple water bodies and high mobility of tagged fish during the spawning season prevented the relocation of every fish on a weekly basis. I assumed that all tagged fish in streams tracked were detected, due to shallow depths and narrow stream widths. Non-detection of tagged fish was not assumed when tracking Hiwassee Lake due to signal attenuation from low conductivity and depths exceeding 35 m (Freund and Hartman 2002). On 9 June 2007, a radio transmitter was attached to a buoy and deployed at a depth of 35 m at a location 100 m upstream from Hiwassee Dam. A strong signal was detected while conducting test tracking transects up to a distance of 100 m from the buoy. This result suggests that had tagged fish routinely utilized Hiwassee Lake during the extent of this study, my tracking methods would have been successful at detecting their presence at even the deepest sections of the reservoir.

### *Spawning Observations*

In 2006, effort devoted toward observing and obtaining spawning microhabitat data was restricted to visually scanning downstream riffles and runs while conducting radio-tracking sessions (canoeing). Despite this technique proving successful at identifying and observing numerous spawning black redhorse, golden redhorse, river redhorse, and silver redhorse, I only identified two active sicklefin redhorse courting troupes. In 2007, increased

effort to observe sicklefin redhorse spawning was conducted by foot rather than canoe. Observers used binoculars with polarized lenses to identify sicklefin redhorse from the bank. Following positive identification of a sicklefin redhorse, observers attempted to ascertain specific fish behavior (i.e., courting or spawning). When an active sicklefin redhorse troupe was observed, effort was made to identify an oviposition site for that troupe by observing spawning or courting over a silt-free oval depression. Identified depressions were categorized as either a courting or spawning oviposition site, and courting oviposition sites were treated as locations where spawning had recently occurred.

Once a depression was positively identified as an oviposition site, reproductive behavior was documented and quantified. Reproductive behavior quantified included number of fish engaged, spawning act duration, prespawning and postspawning behavior, and species engaged in the act. In addition to all oviposition sites being photographed, when possible, spawning and courting acts were video recorded for later analysis. Geographic coordinates were obtained using a hand-held global positioning system unit (Garmin GPS 5) for all spawning and courting oviposition sites. Following the collection of microhabitat data, substrate samples were collected from the center of the oviposition site (i.e., pit).

#### *Microhabitat Use and Availability*

Microhabitat data were collected at each exact location occupied by a relocated or visually observed sicklefin redhorse. Oviposition site microhabitat data were collected from the center and immediately upstream of each spawning or courting oviposition site.

Microhabitat availability data were collected in a line-transect survey of Brasstown Creek, Hanging Dog Creek, Hiwassee River, Nottely River, and Valley River when flow conditions were similar to those associated with microhabitat use data (Table 3–10). Microhabitat availability data collected at each transect point included depth (m), bottom velocity (m/s), mean column velocity (m/s), dominant substrate, subdominant substrate, cover type, distance to cover (m), distance to bank (m), and occurrence of river weed. The line-transect method was used because it has minimal measurement error and is more repeatable than visual techniques (McMahon et al. 1996; Stanfield and Jones 1998). Additional morphological stream characteristics obtained during habitat availability surveys included bank angle (°), undercut bank distance (m), and 30-m riparian land use (%). Microhabitat availability data and morphological stream characteristics for Hiwassee River were collected during early-July 2006; for Nottely River in late-May 2007; and for Valley River, Hanging Dog Creek, and Brasstown Creek in early-July 2006 and mid-January 2008. Transects were evenly spaced two mean stream widths (2MSWs) apart and microhabitat variables were measured at evenly-spaced points across each transect (Simonson et al. 1994). A total of 142 transects were surveyed and 1,815 survey points were measured, resulting in approximately 13 points per transect. A total of 7.56 km of the 111.5 km (~ 7.0%) regularly radio-tracked were included in these microhabitat availability surveys (Table 11; Figure 2).

For both microhabitat use and availability, a top-set wading rod was used to measure depth to the nearest centimeter. A Marsh-McBirney flow meter (Model 2000) was used to measure bottom and mean current velocity (m/s). Mean current velocity was measured in the

water column at a depth 60% from the surface in water depths of 0.75 m or less, and in the water column at depths 20% and 80% from the surface, which were averaged to produce a mean column velocity for depths greater than 0.75 m (McMahon et al. 1996). Dominant and subdominant substrates were visually determined by visually estimating percent composition of surface substrates and were classified based on a modified Wentworth particle size classification (Table 12; Bovee 1986).

Visual estimates of surface substrates are subject to observer bias (Platts et al. 1983); therefore, substrate samples were obtained from oviposition locations to accurately and precisely describe spawning substrate. Subsurface substrate samples were only collected from oviposition sites for which spawning was visually confirmed ( $N = 31$ ). Depth of samples taken was only as deep as the average depth of egg deposition (Lotspeich and Everest 1981). Sample depths were 10 cm, as depth of egg deposition by the river redhorse (sister species to the sicklefin redhorse; Harris 2002) is <10 cm in Valley River, North Carolina (Byron J. Freeman, University of Georgia, unpublished data).

Initially, core substrate samples were attempted with a McNeil hollow-core sampler. A McNeil sampler has been shown to be the most accurate device for assessing substrate composition (Young et al. 1991). All attempts to utilize a McNeil sampler to obtain a spawning substrate sample were ineffective due to the coarseness of the utilized spawning substrate and resulting inability to insert the hollow-core sampler into the substrate to the desired depth (i.e. 10 cm). Thus, a round point shovel was used to collect substrate samples. Shovel samples have been shown to be similar to McNeil core samples and sample larger

particle size classes more effectively than core samplers (Young et al. 1991). Following all other variable measurements, a shovel was used to excavate the spawning oviposition site to an approximate depth of 10 cm. All substrate samples were decanted and placed into a resealable polyurethane container following visual examination for eggs.

In the laboratory, each substrate sample was oven dried at 60°C for 24 h or to a constant weight. Boulder, cobble, and very coarse gravel constituents of each sample were individually identified and categorized by hand-measuring the intermediate axis (b-axis; nearest 1.0 mm) and weighed separately (nearest 0.01 g). The remaining portion of each sample was sorted through five Tyler USA standard testing sieves (mesh sizes 31.5, 16.0, 9.5, 2.0, and 0.074 mm) placed in geometric progression on a mechanical shaker (Tyler Ro-Tap Sieve Shaker) for 10 minutes. Sorted sieve contents were then weighed separately (nearest 0.01 g). Fines (<2.0 mm) were then transferred to a muffle furnace and heated to 420°C for 2 h. Ashed samples were removed and promptly weighed (nearest 0.01 g) to obtain percent organic fines.

Nearest dominant cover type was visually determined by establishing the presence or absence of cover and then determining the distance to the fish location. Cover types included were no cover, coarse woody debris, fine woody debris, root wad, emersed aquatic vegetation, submersed aquatic vegetation, terrestrial vegetation, undercut bank, and boulder. Vertically protruding bedrock, relative to surrounding substratum, was classified as cover type boulder. Cover types were considered associated with fish occurrence when the cover

was 2 m or less from the fish location. Presence or absence of river weed was determined for each fish location and was considered present if it occurred within 2 m of the fish location.

In addition to collecting an instantaneous temperature measurement at each fish location, continuous hourly water temperature data were collected using HOBO Water Temp Pro v2 Loggers (Onset Computer Corporation) from 11 January 2007 to 11 January 2008 for middle Hanging Dog Creek and lower Valley River. Additional loggers were deployed in Hiwassee River, upper Valley River, and middle Valley River, but malfunctioned and failed to collect water temperature data. In addition, daily water temperature data were obtained for Hiwassee River (1 January 2006 to 31 December 2007) from the Murphy Water Treatment Plant, owing to fulfillment of responsibilities to the Public Water Supply Section under the North Carolina Department of Environment and Natural Resources. As a result of Hiwassee River daily water temperature data being collected at 08:00 daily, recorded water temperatures from hourly temperature logger data obtained from Valley River and Hanging Dog Creek corresponding to 08:00 were utilized to maintain analogous temporal water temperature data and permit spatial comparisons. Mean daily water temperatures were utilized relative to Valley River spawning and courting observations.

Spawning migration initiation, during periods of migratory restlessness (i.e., zugunruhe), has been reported to be correlated with flow (Hayes 1953; Junk 1989; Kwak and Skelly 1992, Smith et al. 1994). Flow in cubic feet per second (cfs), for Valley River, was acquired from U.S. Geological Survey gauging station 03550000 (available online at <http://waterdata.usgs.gov/nwis/uv?03550000>) and converted to m<sup>3</sup>/s.

### *Two-way Resistance Board Weirs and PIT-Tagged Fish*

Two modified two-way resistance board weirs were installed in Valley River 15–18 April 2006 and 20–23 March 2007 to describe directional movement of sicklefin redhorse. Weir construction and installation was based on methods described by Torbin (1994) and Stewart (2002, 2003), while the resistance boards were constructed following the design of Mogen (1996). Weir sampling concluded on 30 June in 2006 and 9 June in 2007, following the conclusion of the *Moxostoma* spawning migration. Weirs were installed 11.8 km and 22.4 km upstream from the Valley River and Hiwassee River confluence. During the 2006 and 2007 spawning season, concurrent 24-h samples were collected for both weirs twice per week in 2006, and four times per week in 2007. Sites appropriate for weir installation were rare due to scarce suitable stream reaches and readily accessible private property. Large cobble and small cobble dominated the substrate composition with moderate to high embeddedness at the downstream location and low to moderate embeddedness at the upstream location. Stream depth (0.25–0.50 m) and mean column velocity (0.10–0.60 m/s) at both installation sites were consistently moderate along the base (i.e., anchor cable) of the weir.

In 2006, modified fyke nets functioned as the upstream and downstream traps for each weir (Figure 3). This technique, though successful, proved problematic due to excessive debris collection and large hole formations. Holes were likely created by non-target species (e.g., muskrats *Ondatra zibethicus*) or predatory animals (e.g., common snapping turtle *Chelydra serpentina* and northern river otter *Lontra canadensis*) attempting

to acquire trapped migratory fish. Live traps, constructed out of angle iron and metal conduit, were installed on 15 June 2006, despite a nearly concluded spawning season, to replace the problematic modified fyke nets (Figure 3).

As a result of difficulties encountered in 2006 (i.e., debris collection and hole formations), vertically fixed black polyurethane mesh netting was replaced with vertical 3.05-m (10.0-ft) long fixed weir segments constructed out of angle iron and metal conduit in 2007 (Figure 3). All fixed weir segments had a gap width of 38.1 mm (1.5 in) and had an approximate height of 1.52 m (5.0 ft). This alteration successfully reduced debris collection, gap and hole evolvment, and head differential.

The downstream weir was checked every morning followed by the upstream weir. Captured fish were removed from each trap and relocated to a coated wire mesh live cage positioned in a slack section of the stream. Following removal from the mesh live cage, total length (mm), weight (g), sex, maturation stage, tubercle development, and migration direction (i.e., upstream or downstream) were recorded for each fish. In addition, all sicklefin redhorse were scanned with a Mini Portable Tag Reader (Biomark, Inc.) to determine recapture status. Unmarked fish received a 125-kHz 12-mm passive integrated transponder (PIT) tag (Biomark, Inc., Model TX1400L) which was implanted into the muscle tissue near the base of the dorsal fin. Annual absolute growth was calculated for fish recaptured in 2007 that were tagged in 2006. Spawning season absolute growth was calculated for fish that were captured twice during the same spawning season.

Several deceased sicklefin redhorse were collected from upstream margins of weir panels after floating downstream. These fish were not included in any statistical analysis, because occurrence at the weir appeared to be attributed to postspawning mortality, rather than migration. Captured fish were released in the direction they were migrating, and all appeared in good condition upon release.

Of the 341 sicklefin redhorse captured during this study, either by electrofishing techniques or two-way resistance board weir, all fish were PIT-tagged except 86 fish due to equipment failure from 31 March to 5 April during 2007. From data collected on these recaptured adult sicklefin redhorse, spawning season and annual absolute growth were estimated (Table 13).

### *Statistical Analysis*

Spatial Analyses.—Median weekly linear range was calculated seasonally and annually for radio-tagged sicklefin redhorse. Linear ranges were categorized as either upstream or downstream movement (i.e., directional). Linear ranges were estimated using methods similar to those described by (Vokoun 2003). Fish location coordinates were imported into ArcView 9.2. The thalweg was then delineated using a National Hydrology Dataset flow line data layer obtained from the United States Geological Survey (available online at <http://nhdgeo.usgs.gov/viewer.htm>). Four data layers composed of one of the spawning tributaries and Hiwassee River were created for subsequent manipulation using shareware arcscripts Add Points Evenly Along a Line (Lead 2002) and Nearest Neighbor 3.1

(Weigel 2002). Points, spaced 10 m apart, were added to each thalweg data layer beginning at the headwaters of each spawning tributary. For all fish locations, the nearest neighboring 10-m point was identified using Nearest Neighbor 3.1. The accumulation of 10-m thalweg points were used to calculate weekly total linear stream distance between relocation points, which were classified as either upstream or downstream movement, relative to the prior week. In addition to weekly directional linear range and annual and seasonal linear range, I calculated distance from the Valley River and Hiwassee River confluence as this enabled the use of every relocation point which subsequently increased sample size. The Valley River and Hiwassee River confluence was selected as a reference point due to a centralized location within the portion of Hiwassee Basin that sicklefin redhorse currently occupy.

Similarly, linear range was calculated on an annual and seasonal basis. Linear range was calculated for the winter season using the last relocation obtained during the postspawning season (i.e., mid-October) and single relocation events obtained during winter. Relocations for all radio-tagged sicklefin redhorse were segregated by sex and number of days residing within a spawning tributary for 2006 and 2007. Spawning tributary residence times were subsequently enumerated and separated into 20-day intervals by sex, and differences were tested between the sexes by a Kolmogorov-Smirnov two-sample test (K-S Test, Sokal and Rohlf 1981).

Seasonal Behavior.—Relocated undisturbed sicklefin redhorse, when a visual observation was obtained, were monitored from the stream bank to determine dominant behavior or activity. Observed activities were classified and separated into those that

occurred during either the spawning season or nonspawning period (i.e., postspawning season and winter). Undisturbed behaviors for all relocated radio-tagged and visually observed sicklefin redhorse were quantified and categorized into five behaviors (foraging, resting, courting, spawning, migrating) based on the particular period in which they occurred (spawning or nonspawning). A likelihood-ratio chi-square test was performed comparing spawning season activity and nonspawning period activity.

Microhabitat.—Sicklefin redhorse microhabitat use and availability data were stratified by stream (Beaverdam Creek, Brasstown Creek, Hanging Dog Creek, Hiwassee River, Nottely River, and Valley River). In addition, microhabitat data were stratified by reproductive behavior (courting and spawning) and season (spawning, postspawning, and winter) (Tables 3–9). Annual, seasonal, and observed spawning microhabitat use data were compared to corresponding microhabitat availability data.

Pit and flat oviposition microhabitat variables depth, bottom velocity, mean column velocity, and dominant substrate were compared using the Student's *t* test to ascertain if utilized microhabitat (i.e., pit) varied from theoretically undisturbed pre-spawning microhabitat (i.e., flat). The Student's *t* test was employed to avoid resolution loss associated with nonparametric tests and justified due to the majority of the pit and flat microhabitat samples being normally distributed. Microhabitat variables distance to bank, cover type, and distance to cover were not compared between the pit and flat as these variables were primarily synonymous between the two different locations.

Non-random microhabitat use was tested using a K-S test comparing utilized microhabitat to available microhabitat for all continuous variables (i.e., depth, bottom velocity, mean column velocity, dominant substrate, distance to cover, and distance to bank). Dominant substrate was considered a continuous variable due to the continuity of substrate particle sizes. An analogous likelihood-ratio chi-square test was performed on categorical variables (i.e., cover and river weed occurrence) to test for nonrandom microhabitat use. A temporal (i.e., seasonal) shift in microhabitat use was examined by comparing (K-S test) utilized seasonal microhabitat. In addition, the K-S test was performed on utilized spawning microhabitat and nonspawning microhabitat occupied during the spawning season to ascertain if a functional difference occurred between microhabitat used within the spawning season. Additionally, relocation and visual observation microhabitat data collected during an unseasonable cold front in 2007, following the onset of spawning migration, were compared to spawning season microhabitat data collected pre and post-cold front seasonable conditions.

Empirical microhabitat use and availability data were used to estimate microhabitat suitability. Suitability was calculated by dividing percent microhabitat use by percent microhabitat available for each interval of the corresponding frequency distribution for each variable and then dividing the resulting quotients by the maximum quotient of all intervals. Waters (1976) and Bovee (1986) describe microhabitat suitability as a weighting factor derived by comparing the frequency distribution of utilized microhabitat to that of available microhabitat. Microhabitat suitability ranges from 0 to 1, with 0 indicating the lower limits of suitable microhabitat and 1 representing preferred or optimal microhabitat (Waters 1976;

Bovee 1986). The transferability of optimal habitat is likely limited to stream reaches similar to the specific stream reaches from which the utilized and available microhabitat were obtained (i.e., Category II) (Bovee 1986). For this study, in an attempt to negate bias associated with Category II criteria, influence of limited available microhabitat data were eliminated from suitability analyses by omitting rare available microhabitat yielding Category III criteria (Bovee 1986). The purpose of this modification is to enhance reasonable transferability of the suitability criteria to lotic water bodies (i.e., Little Tennessee Basin) that may diverge from those where suitability criteria originated (i.e., Hiwassee Basin). This modification will also enable application of suitability criteria to streams exhibiting periods of aberrant flow (i.e., hydroelectric release and severe drought) and guide the establishment of minimum flows for hydroelectric dams.

Principal component analysis (PCA) enables the cumulative interaction among several microhabitat variables to be examined and prioritized by importance. PCA techniques analyze multiple continuous variables and create uncorrelated linear combinations (i.e., principle components) that explain the greatest amount of variation. PCA was conducted on all continuous microhabitat variables (depth, bottom velocity, mean column velocity, dominant substrate, distance to cover, and distance to bank) to ascertain selected annual, seasonal, and spawning macrohabitat. I retained components with eigenvalues greater than 1.0 as recommended by Kaiser (1960), Stevens (1996), and Kwak and Peterson (2007). Habitat availability scoring coefficients were then used to calculate utilized habitat principle component scores. A K-S two-sample test was performed on scores of each

retained principal component to test for significantly different frequency distributions for microhabitat use and availability, and between seasonal and oviposition site microhabitat use.

Spawning and nonspawning seasonal activities that were determined to be foraging behavior (i.e., gulping or “sucking” behavior that resulted in subterminal lips coming into direct contact with substratum) were further analyzed by dividing each foraging act into bins according to substrate particle size (e.g., clay, silt, sand, gravel, cobble, boulder, bedrock ) in an attempt to determine seasonal dominant foraging substrate. A K-S test was performed on utilized spawning season foraging substrate and nonspawning season (i.e., postspawning season and winter) foraging substrate to ascertain if a temporal difference or seasonal shift occurred between dominant foraging substrates. In addition, a likelihood-ratio chi-square test was performed comparing utilized dominant substrate to available dominant substrate relative to the presence or absence of river weed to ascertain if sicklefin redhorse utilization of dominant substrate is influenced by the presence of river weed on an annual basis excluding relatively brief periods during the actual act of spawning.

Spawning substrate particle size distribution was characterized by calculating the geometric mean diameter ( $D_g$ ) (Shirazi and Seim 1981) and median diameter ( $D_{50}$ ) of sorted substrate samples. Spawning substrate relative quality was determined by calculating the Fredle index ( $f_i$ ). Bounds of central tendency (i.e., spread) were determined by plotting particle size diameter for each particle size class against cumulative percent mass on a log-probability graph. From this cumulative plot,  $D_{25}$  and  $D_{75}$  were calculated and are defined as the substrate diameter below which 25% and 75% of a sample fall. Statistical software

package SAS/STAT 9.1 (SAS Institute Inc., 2003) was used to conduct all statistical analyses. A significance level ( $\alpha$ ) of 0.05 was applied to all statistical tests.

## **Results**

On 30 March (water temperature, 14°C), 31 March (15°C), and 5 April (15°C) in 2006, 25 adult sicklefin redhorse were collected from lower reaches of Valley and Hiwassee River and surgically implanted with radio transmitters (Table 1). Of the 25 tagged fish, 15 were male and 10 were female. Males had a mean total length (TL) of 479.0 mm (SE 6.6) and a mean weight of 973.7 g (SE 36.4); females had a mean total length (TL) of 507.1 mm (SE 7.2) and a mean weight of 1,218.6 g (SE 47.8). Males had a mean tubercle development of 61.0% (SE 4.6); females displayed no tubercle development. One male fish expressed milt when slightly squeezed, while no females were ripe.

On 9 March 2007 (water temperature, 10°C), an additional nine sicklefin redhorse were collected from middle Hiwassee River and surgically implanted with radio transmitters. Three of the nine tagged fish were male and six were female. Males had a mean total length (TL) of 527.3 mm (SE 24.2) and a mean weight of 1,325.7 g (SE 175.3); females had a mean total length (TL) of 523.3 mm (SE 12.3) and a mean weight of 1,285.7 g (SE 62.5). Males had a mean tubercle development 50.0% (SE 5.8); females had a mean tubercle development of 1.7% (SE 1.7). Gamete expression was absent for all implanted fish during 2007 at the time of surgery.

Of 25 fish implanted and released in 2006, seven mortalities occurred; six within one month of surgical implantation and one during mid-June 2006. No mortalities were observed for fish implanted in 2007. Data collected for all deceased fish that displayed abnormal behavior or no movement prior to confirmed mortality were excluded from subsequent analyses. Microhabitat and movement data for two deceased fish that made upstream spawning migrations prior to mortality were included in subsequent analyses because mortality could not be attributed to complications associated with surgical implantation, and behavior prior to death was not discerned as abnormal.

Out of 25, 17 sicklefin redhorse made upstream spawning migrations in 2006. Of these 17 fish, 14 subsequently made spawning migrations up Valley River, two made a spawning migration up Hanging Dog Creek, and one made a spawning migration up Brasstown Creek. Post-implantation, three female radio-tagged fish promptly returned to Hiwassee River from Valley River. One fish that made a spawning migration up Valley River, and was subsequently relocated on 30 April, was not relocated again until 12 October 2006 in Hiwassee River and not relocated in Valley River again during this study.

In 2007, of the remaining 18 radio-tagged sicklefin redhorse from 2006, 12 made an upstream spawning migration. Of these 12 fish, 10 made upstream migrations in Valley River, one made an upstream migration in Hanging Dog Creek, and one made an upstream migration in Nottely River. In 2007, the fish that made an upstream migration in Hanging Dog Creek was the same fish that made an upstream migration in Hanging Dog Creek in 2006. The fish that made an upstream migration in Nottely River is the same fish that was

relocated in Valley River once and then not relocated again until relocated in Hiwassee River in October 2006. All 10 fish that migrated up Valley River in 2007, either conducted spawning migrations up Valley River in 2006 or were not documented to conduct a spawning migration.

Of the nine sicklefin redhorse radio-tagged in 2007, six made an upstream spawning migration and three remained in Hiwassee River the remainder of the study. Of the six fish that made an upstream spawning migration, four migrated up Valley River and two migrated up Brasstown Creek. Of the three fish that did not migrate, all were female. During this study, female radio-tagged fish that did not conduct an upstream spawning migration outnumbered male fish seven to one (87.5%). The majority of radio-tagged adult sicklefin redhorse conducted spawning migrations up Valley River, while Brasstown Creek, Hanging Dog Creek, and Nottely River were less frequently ascended. Radio-tagged fish that migrated upstream for spawning in 2006 and 2007 did so to the same tributary both years.

In 2006, 19 sicklefin redhorse were captured moving upstream past each two-way resistance board weir on Valley River. Fifteen females and four males composed the catch for the downstream weir; six females and thirteen males composed the catch for the upstream weir. Total sicklefin redhorse biomass for the downstream weir upstream trap was 23,999 g; total biomass for the upstream weir upstream trap was 19,904 g. In 2007, 201 sicklefin redhorse were captured in the downstream weir and 28 were captured in the upstream weir. The downstream weir upstream trap catch was composed of 133 females and 51 males; the downstream trap catch was composed of 13 females and 4 males. The upstream weir

upstream trap catch was composed of 16 females and 9 males; the downstream trap catch was composed of 3 females. Total sicklefin redhorse biomass for the downstream weir upstream trap was 236,739 g; total biomass for the upstream weir upstream trap was 29,784 g. Total sicklefin redhorse biomass for the downstream weir downstream trap was 21,620 g; total biomass for the upstream weir downstream trap was 3,113 g.

Captured adult sicklefin redhorse during 2006 and 2007 exhibited similar mean weekly percent tubercle development and maturation stages; however, females exhibited later tubercle development in 2007 compared to 2006 (Figure 4). Male sicklefin redhorse exhibited 60–80% tubercle development by late-March and early-April and maturation stages ranging from slight squeeze ripe (2) and running ripe (3) during mid-April. Male sicklefin redhorse mean weekly tubercle development peaked in mid- to late-April and then sharply declined during May, while maturation stage peaked during late-April to mid-May. Female adult sicklefin redhorse mean weekly percent tubercle development did exhibit some divergence between years, while female maturation stages were temporally consistent between 2006 and 2007. Female sicklefin redhorse mean weekly tubercle development was considerably less (0–15%) compared to males and appeared to peak during early-April in 2006, while peaking during early-May in 2007. Female mean weekly maturation stage was also considerably less [not ripe (0) to hard squeeze ripe (1)] compared to males; however, similar to males, peaked in mid to late-April. During 2006, the last ripe female (i.e., HSR) and the last ripe male (i.e., SSR) were captured on 2 May and 31 May, respectively. During 2007, the last ripe female (i.e., RR) and the last ripe male (i.e., HSR) were captured on 3 May

and 16 May, respectively. Water temperature was considerably colder during early and mid-April in 2007 compared to 2006. This water temperature variation coincides with the tubercle development disparity exhibited by female sicklefin redhorse (Figure 4).

All fish were initially captured prior to or during an upstream spawning migration and recaptured while attempting to pass a weir in an upstream direction. Of the 255 PIT-tagged fish, 17 were recaptured (Table 13). Of these 17 recaptured fish, 15 fish were initially captured within a two-way resistance board weir in Valley River during the upstream spawning migration and two fish were initially captured on 9 March 2007 in Hiwassee River during boat electrofishing efforts to capture fish for radio-tag implantation. Of 17 PIT-tagged fish recaptured at a resistance board weir, two were recaptured in 2006 at the upstream weir following an initial capture at the downstream weir and 15 fish were recaptured in 2007. Of the 15 recaptured fish in 2007, six were from the 79 PIT-tagged fish implanted in 2006. During the spawning season, fish experienced negative mean absolute growth. Annually, fish experienced mean absolute growth of 0.13 g/d (Table 13). Annually, recaptured fish, on average, weighed more and were longer in 2007 than when initially captured in 2006 (Table 13). Annually, males exhibited a lower absolute growth than did females (Table 13). During the spawning season, recaptured fish weighed less and were shorter than when initially captured (Table 13). During the spawning season, males exhibited a negative mean absolute growth; females exhibited a positive mean absolute growth (Table 13). Annually, female fish, on average, accrued greater than three times more

mass than male fish; male fish, on average, accrued approximately six times more length than females.

Radio-telemetry and observational data revealed that sicklefin redhorse, within the Hiwassee Basin, utilize five distinct spawning tributaries (Valley River, Brasstown Creek, Hanging Dog Creek, Nottely River, and Beaverdam Creek). Spawning migration data obtained from weir samples, in conjunction with radio-telemetry data and microhabitat data, revealed spawning migration patterns and possible proximate environmental stimuli. Distinctive temporal suitable microhabitat, timing of observed spawning acts, percent tubercle development, and maturation stage, in addition to movement patterns discerned from weir sampling and radio-telemetry relocation data were used to identify three discrete functional seasons. Spawning season began on March 1 and ended on May 31; postspawning season began on June 1 and ended on November 15; and winter season began on November 16 and ended on February 29. Dates selected to bound the spawning season were determined based on earliest evidence of spawning migration initiation and occurrence of latest observed spawning act. Generally, median weekly linear movement and distance from the Hiwassee River and Valley River confluence was greatest during the spawning season and minimal during postspawning and winter seasons; however, linear range was greater during the winter when compared to the postspawning season. During 2006 and 2007, basin-wide dispersion was greatest during the spawning season as a result of upstream spawning migrations; however, restricted to lotic stream reaches of lower Valley River and all of Hiwassee River located between Hiwassee Lake and Mission Dam during the postspawning season (Figure

5). Basinwide dispersion was most restricted during the winter and primarily confined to downstream portions of Hiwassee River (Figure 5).

Valley River (76.9%) was most intensively used by radio-tagged migratory sicklefin redhorse during the spawning season, followed by Brasstown Creek (11.5%), Hanging Dog Creek (7.7%), and Nottely River (3.8%). However, it should be noted that of six fish tagged in 2007 from middle Hiwassee River that conducted a spawning migration, 33.3% utilized Brasstown Creek as a spawning tributary and 66.7% used Valley River, indicating that Brasstown Creek is a significant spawning tributary in Hiwassee Basin. Observational data indicate that Beaverdam Creek is minimally used as a spawning tributary (i.e., two observed courting sicklefin redhorse). Fish typically occupied spawning tributaries from mid-March through mid-May and Hiwassee River the remainder of the year; however, several fish continued to occupy lower reaches of spawning tributaries through October before returning to Hiwassee River during the winter. Following return to Hiwassee River, postspawning fish dispersed from Mission Dam to Hiwassee Lake. Radio-tagged fish that were relocated in middle to upper Hiwassee River during the postspawning season were relocated in extreme lower Hiwassee River during winter. All fish that made spawning migrations during 2007 that had previously migrated during 2006 ascended the same spawning tributary both years, indicating that sicklefin redhorse return to the same spawning tributary from year to year and possibly display a high degree of natal stream spawning fidelity similar to some salmonid species.

### *Linear Range*

Sicklefin redhorse linear range estimates revealed considerable between-season differences. Linear ranges were greatest during the spawning season and minimal during the postspawning and winter seasons following return to Hiwassee River from spawning tributaries. Despite linear ranges being larger during the spawning season, relocations of radio-tagged fish during the spawning season indicate that sicklefin redhorse are highly mobile prior to arriving at utilized spawning grounds during the beginning of the spawning season (i.e., early-March), rather sedentary following occupancy of spawning grounds (i.e., mid-March–May), and highly mobile after spawning grounds are abandoned corresponding with the end of the spawning season (i.e., late-April–late-May).

Specifically, median annual and seasonal linear ranges were similar to corresponding mean linear ranges. Median annual linear range was 19.4 km, slightly larger than the median spawning linear range of 17.8 km (Table 14). Wide ranges in linear range among individuals indicate substantial spawning migration and movement variability within a given spawning season and indicate that not all adult sicklefin redhorse make a spawning migration each year. However, 34 (85%) spawning season linear range estimates were greater than 5.0 km, 14 (35%) were greater than 20 km, and four (10%) were greater than 29 km suggesting that the vast majority of adult fish undertake a substantial spawning migration.

Sicklefin redhorse had a median postspawning linear range of 0.1 km with a wide range among individuals (Table 14), indicating a reduction in movement after the spawning season following downstream movements from spawning tributaries and reestablishment of

previously occupied lower reaches of Hiwassee and Valley rivers. Four fish exhibited a postspawning season linear range greater than 11.0 km that was the result of delayed downstream movement following an upstream migration during the 2006 spawning season. Three additional fish exhibited similar prolonged spawning tributary occupancy in 2007. After excluding these five individuals, the maximum postspawning linear range estimate was 4.5 km.

Sicklefin redhorse in winter showed a median linear range of 3.4 km with a minimum of zero (no movement) and a maximum of 13.6 km (Table 14), suggesting that sicklefin redhorse utilize stream reaches during the winter that are different than those utilized during the postspawning season. Twelve fish had a winter linear range greater than 1.0 km, and five fish had a winter linear range greater than 5.0 km. During the winter of 2006, 17 of 18 radio-tagged fish were relocated during mid-January; 16 fish were in Hiwassee River, and one fish was in the lower reaches of Valley River where available microhabitat (i.e., depth, mean column velocity, substrate) strongly resembled that available in Hiwassee River.

In 2006, annual median linear distance from the confluence of Valley and Hiwassee rivers was 4.8 km (Table 15). Spawning season linear distance from confluence had a median of 7.7 km (Table 15). The majority of sicklefin redhorse utilized middle and lower reaches of spawning tributaries during the spawning season and occasionally upper reaches; 50 (47%) spawning season relocations were greater than 10 km from the confluence and 24 (22%) were greater than 20 km from the confluence of Valley and Hiwassee River. Median postspawning season linear distance from the confluence was 4.7 km (Table 15),

demonstrating that the majority of fish that made and upstream spawning migration had moved toward the confluence but a number of fish still occupied the upper reaches of spawning tributaries during the beginning of the postspawning season as well as the upper reaches of Hiwassee River below Mission Dam. Seventy-five (63%) of the postspawning season relocations were less than 5.0 km from the confluence. It should be noted that in 2006, the lower 1.6 km of Valley River and lower 2.3 km of Hiwassee River were inundated by Hiwassee Lake, and at no time was a radio-tagged fish observed consistently utilizing these reaches during those lentic hydrologic conditions. Six radio-tagged fish remained in lower Valley River and occupied habitat just upstream (50–100 m) from the lower reaches of Valley River that were inundated. As the postspawning season progressed and Hiwassee Lake continued to impound more of Valley River, each fish was subsequently found further upstream keeping pace with the impounded water line. Due to a severe drought in the southeastern United States and subsequent low Hiwassee Lake levels, the Valley and Hiwassee River confluence was not inundated in 2007. No radio-tagged fish were relocated within the extreme lower reaches of Valley River during the postspawning season in 2007 that were intensively utilized in 2006 just upstream of impounded Hiwassee Lake. Other than variation associated with the impoundment of the confluence of Valley and Hiwassee rivers relative to the postspawning season, distance from confluence trends were highly similar between 2006 and 2007.

Winter median linear distance from the confluence was 2.6 km (Table 15), suggesting that sicklefin redhorse migrate toward Hiwassee Lake or the extreme lower reaches of

Hiwassee River during winter. Twelve (71%) of the 17 mid-January relocations were less than 4 km from the confluence of Valley and Hiwassee River; four (24%) relocations were occupying microhabitat just upstream of Hiwassee Lake located 3 km downstream of the confluence and one (6%) relocation was obtained at the confluence. The five relocations that occurred on 12 January and 14 January 2007 within a lotic environment, were the only relocations recorded at or below the confluence of Valley River and Hiwassee River during this study that were not otherwise occupying a downstream spawning tributary.

In 2007, annual median linear distance from the confluence of Valley and Hiwassee rivers was very similar to estimates calculated for 2006 (Table 15). Conversely, spawning season median linear distance was 4.8 km in 2007 as apposed to 7.7 km during 2006 (Table 15), suggesting that sicklefin redhorse conducted shorter spawning migrations during drought conditions in 2007. Dissimilar from 2006, only 68 (39%) spawning season relocations were greater than 10 km from the confluence and 26 (15%) relocations were greater than 20 km from the confluence during 2007. Postspawning linear distance from confluence estimates for 2007 were similar to those calculated for 2006.

Sicklefin redhorse median annual weekly linear upstream and downstream movement was 0.09 km (Table 16). Annual weekly maximum upstream linear movement was 21.1 km; the maximum downstream weekly linear movement was 30.5 km (Table 16). Annual weekly directional movement estimates suggest that sicklefin redhorse are a rather sedentary fish throughout the year except during migrations to and from spawning grounds and winter habitat. Specifically, sicklefin redhorse conduct a rather expedient upstream spawning

migration and are relatively sedentary during spawning tributary occupancy. Generally, sicklefin redhorse return to Hiwassee River following a brief occupancy of a spawning tributary and are rather sedentary during the postspawning season. However, radio-telemetry data and undisturbed visual observations reveal that sicklefin redhorse continuously move and reposition within a specific macrohabitat for the purpose of foraging, resting, or spawning.

Spawning season median weekly directional movement estimates were greater than both annual and postspawning estimates (Table 16). Specifically, 34% to 22% of sicklefin redhorse moved more than 1 km during a given week of the spawning season; 13% to 15% moved more than 5.0 km in a given week, during the spawning season, respectively. Postspawning season median weekly directional movement estimates were less than both spawning and annual estimates, indicating sedentary behavior during occupancy of Hiwassee River and lower reaches of utilized spawning tributaries.

Several radio-tagged fish appeared to transition between two spawning grounds during this study. One female fish, during the 2006 spawning season, consistently occupied a specific riffle 29.4 km upstream from the Valley River mouth during a time period ranging from 23 April to 18 May; however, this fish was relocated at a riffle 900 m further upstream on 11 May and again on 26 May before relocated in Hiwassee River on 2 June, indicating a degree of “wandering” among multiple spawning sites within a relatively small stream reach.

Fish ( $N = 7$ ) that conducted two (both 2006 and 2007) annual spawning migrations during this study utilized spawning grounds associated with the same stream reach each year.

On average, spawning grounds utilized in 2007 were 2.27 km (SE, min–max; 1.01, 0.20–7.59) from those utilized in 2006. Once a radio-tagged fish occupied a spawning area, reduced movement was documented until emigration back to Hiwassee River or lower spawning tributary reaches. Mean linear movement, while occupying spawning grounds, was 1.04 km (SE, min–max; 0.20, 0.02–3.90). Similarly, following occupancy of postspawning stream reaches, site fidelity was resolute; mean linear movement while occupying postspawning habitat was 0.14 km (SE, min–max; 0.04, 0.01–1.58). On average, postspawning habitat utilized in 2007 was 0.23 km (SE, min–max; 0.17, 0.00–1.60) from that utilized in 2006. Movement and stream reaches occupied by radio-tagged fish indicate that sicklefin redhorse utilize the same stream reaches within the same spawning tributary for spawning purposes annually; and utilize the same stream reaches within Hiwassee River during the postspawning season from year to year. Specifically, several fish were habitually relocated to the same cobble riffles in 2007 that were utilized during the 2006 spawning season and the same bedrock outcroppings in 2007 that were utilized in 2006 during the postspawning season.

#### *Migration, Flow, and Water Temperature*

Sicklefin redhorse movement, during the spawning season, appeared associated with increasing discharge volume (Figures 6–7). During the week of 16 April 2007, when upstream spawning migration rate increased, flow increased from 4.08 m<sup>3</sup>/s to 4.84 m<sup>3</sup>/s between 14 and 15 April. In addition, flow increased from 3.94 m<sup>3</sup>/s to 5.13 m<sup>3</sup>/s between 31

March and 2 April when 52 fish were captured migrating upstream in the downstream weir. Similarly, during the week of 28 May 2006, migratory fish occupying upper Valley River began downstream migrations during three separate hydrograph pulses measuring 6.23 m<sup>3</sup>/s on 21 May, 5.69 m<sup>3</sup>/s on 27 May, and 8.07 m<sup>3</sup>/s on 1 June (Figure 6). Overall, the hydrograph for Valley River during 2007 was relatively flat (i.e., relatively homogenous daily flow volumes), but consistently declined during the spawning season, associated with drought conditions (Figures 6–7).

Low discharge volumes appeared to be associated with reduced spawning tributary occupancy time between years. Stream discharge in Hiwassee Basin and specifically Valley River was significantly lower during 2007 compared to 2006 ( $P < 0.0001$ ,  $t = 4.681$ , Student's  $t$ -test). Radio-tagged fish generally returned downstream a month earlier during the week of 30 April in 2007 compared to the week of 28 May in 2006 (Figure 6). In addition, downstream migration, during severe drought conditions in 2007, was not associated with elevated flows (Figure 6).

Low spawning tributary discharge was also associated with a reduced probability to migrate for spawning and a tendency to restrict spawning reaches to lower portions of tributaries. During 2007, the mean daily flow for Valley River from 1 February to 30 June was 3.89 m<sup>3</sup>/s (SE, min–max; 0.189, 1.25–19.37); during 2006, the mean daily flow for that same period in Valley River was 5.11 m<sup>3</sup>/s (SE, min–max; 0.161, 1.84–12.12). During the higher stream flows in 2006, 1 of 20 (5.0%) radio-tagged fish failed to migrate for spawning; 5 of 27 (18.5%) fish failed to migrate during the spawning season in 2007 during lower

stream flows. Radio-telemetry data revealed that radio-tagged sicklefin redhorse migrated farther upstream in Valley River during the spawning season in 2006 than in 2007 as well. Additionally, downstream weir sicklefin redhorse catches during 2007 were significantly higher than corresponding upstream weir catches; both weirs collected identical numbers of upstream migratory sicklefin redhorse in 2006. Specifically, the upstream weir caught 86.1% fewer upstream migratory fish compared to the downstream weir during 2007.

While stream flow was anecdotally related to migration, water temperature appeared to have the most significant and consistently detectable effect on sicklefin redhorse migration during the spawning season. During radio-tag implantations in late-March and early-April 2006, sicklefin redhorse were staging for or beginning upstream spawning migrations. Water temperatures ranged from 14.0 °C to 15.0 °C during this period. Mean weekly water temperature during the spawning season and calculated from relocation temperature data, was 16.84 °C (min–max; 15.74 °C to 18.53 °C) during 2006, and 16.39 °C (min–max; 12.08 °C–21.77 °C) during 2007. Similarly, water temperatures measured with each sicklefin redhorse weir catch during the spawning season, had a mean of 16.01 °C (min–max; 14.5 °C–17.4 °C) during 2006, and 14.73 °C (min–max; 9.0 °C–20.2 °C) during 2007. During 2006, the last upstream migratory sicklefin redhorse was captured on 10 May when the water temperature was 16.1 °C, and on 4 May in 2007 when the water temperature was 17.5 °C. In 2007, the earliest an upstream migrating sicklefin redhorse was captured was 21 March when the water temperature was 12.5 °C. The majority of radio-tagged fish returned to lower spawning tributary reaches and Hiwassee River in 2006 during late-May and early-June

when mean weekly water temperature at sicklefin redhorse relocations was 18.39 °C, and late-April and early-May in 2007 when mean weekly water temperature was 18.67 °C.

Downstream migration from spawning tributaries was associated with warming water temperatures coinciding with the end of the spawning season; however, downstream migration to lower Hiwassee River was associated with decreasing water temperatures during winter. During the postspawning season, when radio-tagged fish displayed sedentary behavior in lower Valley River and Hiwassee River, mean weekly water temperature associated with relocation events was 19.75 °C (min–max; 15.47–23.04 °C) in 2006 and 21.50 °C (min–max; 21.10–21.89 °C) in 2007. Mean weekly water temperatures at radio-tagged fish locations during mid-January (i.e., winter), when radio-tagged fish primarily occupied lower Hiwassee River, were 8.63 °C in 2006 and 5.5 °C in 2007.

During the spawning season, unseasonably cold water coincided with spawning migration termination and spawning tributary descent. In 2007, a cold front passed through the study area approximately 6–12 April suggesting a threshold effect pertaining to water temperature (< 10 °C) and sicklefin redhorse migration. Prior to the cold front (25 March–5 April), mean hourly water temperature was 16.12 °C (min–max; 11.86 °C–18.96 °C) and sicklefin redhorse were actively migrating upstream; during the cold front, mean water temperature decreased to 9.25 °C (min–max; 6.05 °C–14.74 °C), and no sicklefin redhorse were migrating. Daily catches from the downstream weir also demonstrated the effect, where prior to the cold front (25 March–5 April), 85 (42.3%) sicklefin redhorse were captured; during the cold front, 3 (1.5%) sicklefin redhorse were captured; and post-cold

front catch (13 April to 4 May) when mean water temperature was 15.03 °C (min–max; 8.99 °C–21.25 °C), was 113 (56.2%) sicklefin redhorse.

Spatially disproportional occupancy of spawning tributary downstream reaches was observed in Nottely River where hypolimnetic discharge occurs downstream of Nottely Dam. On 22 June 2006 and 29 April 2007, Nottely River was radio-tracked from Nottely Dam to Hiwassee Lake. On 22 June 2006, numerous (> 100) undisturbed sicklefin redhorse were observed in a river reach located 22.1 rkm downstream from Nottely Dam and upstream of Hiwassee Lake, occupying swift shallow water, flowing over rugose bedrock, with a water temperature of 15.3 °C. On 29 April 2007, several (< 25) undisturbed sicklefin redhorse (including one radio-tagged fish) were observed in a river reach located 7.9 rkm downstream of Nottely Dam occupying deep slow water, flowing over gravel and cobble, with a water temperature of 14.4 °C. Macrohabitat occupied and behavior observed during 2007 were indistinguishable from those observed during the cold front that occurred in early-April. Water temperatures directly below Nottely Dam were 13.0 °C on 22 June 2006 and 11.1 °C on 29 April 2007; this cold water likely restricted sicklefin redhorse to downstream reaches of Nottely River.

For all ( $N = 43$ ) observed sicklefin redhorse spawning and courting acts that occurred during this study, mean water temperature was 17.45 °C (min–max; 11.0 °C–21.1 °C), indicating a wide temperature range for sicklefin redhorse spawning. For observed spawning and courting acts ( $N = 34$ ) during 2007 in Valley River, mean water temperature at the precise time of the observed act was 17.60 °C (min–max; 14.0 °C–20.8 °C); mean time of

day these acts occurred was 15:54 (min–max; 11:27–21:30). Mean daily water temperature, obtained from hourly Valley River temperature data, for days on which spawning and courting acts occurred was 17.14 °C (min–max; 13.09 °C–19.00 °C).

### *Residence Time and Behavior*

Residence Time.—The number of days that radio-tagged fish occupied spawning tributaries varied between sexes with male sicklefin redhorse staying in spawning tributaries longer than females (Figure 8). During 2006 and 2007, males ( $N = 14$ ) spent a mean of 108 days (min–max; 0–289) within their representative spawning tributaries; females spent a mean of 35 days (min–max; 0–290) in spawning tributaries. Of 18 females, 17 (94.4%) remained within their utilized spawning tributary between 0–80 days; of 14 males, 9 (64.3%) remained within their utilized spawning tributaries between 0–80 days. Of the 14 male and 18 female radio-tagged fish that were determined to survive until the end of this study, one male fish and nine female fish failed to conduct a spawning migration in either 2006 or 2007. However, all males conducted a spawning migration at some point (i.e., 2006 or 2007) during this study; 3 of 9 female fish were not observed to migrate up a spawning tributary during a spawning season. Nevertheless, male and female spawning tributary residence times were significantly different ( $P = 0.0035$ ), indicating that females typically only briefly occupy spawning tributaries (i.e., 1 month); males typically occupied spawning tributaries considerably longer (i.e., 1–10 months) during this study before returning to lower Hiwassee River in winter.

Seasonal Behavior.—Instream visual observations suggest that spawning and courting behaviors during the reproductive season displace foraging that occurs during the remainder of the year. During the spawning season (1 March–31 May) 47.1% of visual observations were of fish foraging, while 22.1% and 17.2% were of fish in the act of courting and spawning, respectively (Figure 9). Resting behavior was ascribed to 12.3% of total spawning season visual observations, and migration behavior was rarely encountered (Figure 9). During the nonspawning period (i.e., 1 June–29 February), 87.0% of visual observations were fish exhibiting foraging behavior. Similar to the spawning season, 12.2% of total nonspawning period visual observations were recognized as resting behavior (Figure 9). Visually observed behaviors during the spawning and nonspawning periods were significantly different ( $P = <0.0001$ ); however, foraging behavior accounted for the vast majority of visually observed behaviors during both periods (Figure 9). During the spawning season, foraging behavior was frequently observed within the oviposition site immediately following a quivering act, and anecdotal observations indicate that these fish were foraging on eggs that were not buried during the act of quivering. During the nonspawning season, foraging and resting behaviors were commonly observed in sequence such that fish would hold in low velocity or protected locations prior to relocating to a foraging area only to return to the protected location.

When not engaged in courting or spawning behaviors, sicklefin redhorse utilized coarser substrates (i.e., those larger than very coarse gravel) (87.7%) much more frequently than finer substrates (12.3%). Among these coarse substrates, sicklefin redhorse occupied

bedrock and boulders more frequently (77.3%) than small and large cobble (22.7%). Of these most coarse dominant substrates, bedrock was used 80.0% of the time compared to small, medium, and large boulders. River weed was generally absent more frequently than present among utilized dominant substrates finer than large boulder, and considerably more frequently absent than present for available substrates smaller than large boulder. River weed was present ( $N = 229$ ) more frequently than absent ( $N = 192$ ) only for bedrock (Table 17) compared to available dominant substrates. For utilized dominant substrates larger than gravel, presence of river weed (%) was generally significantly greater than that for available dominant substrate ( $P < 0.05$ ; Table 17). Occupied large boulder and bedrock had river weed presence estimates of 87.5% and 92.9%, respectively. Conversely, available dominant substrate classes boulder and bedrock had river weed presence estimates of 40.3% and 54.4%, respectively (Table 17). Such a divergence indicates that adult sicklefin redhorse occupy substrates larger than gravel that support river weed beds significantly more frequently than such combinations are available. This is specifically the case for large boulders and bedrock ( $P < 0.0001$ ; Table 17). Small boulder was the only particle size class larger than gravel that did not indicate a significant difference ( $P = 0.5097$ ) from availability relative to river weed occurrence (%).

Coarse substrates were predominately observed as foraging substrates annually. During the spawning season, bedrock (43.8%), cobble (37.5%), and boulder (12.5%) were the most frequently utilized foraging substrates (Figure 10). Similarly, during the nonspawning period, bedrock (85.1%) and boulder (12.2%) were the most frequently utilized

foraging substrates. Silt, sand, and gravel were observed to be minimally utilized as foraging substrates annually. During the early and middle portions of the spawning season, the majority of foraging observations occurred at or near utilized spawning grounds where available substratum was primarily composed of particle sizes conducive to spawning (i.e., very coarse gravel–large cobble) and relatively free of river weed. During the end of the spawning season, after several radio-tagged fish had relocated to Hiwassee River and lower Valley River, the majority of foraging observations were observed in stream reaches in which the stream bed was primarily comprised of rugose bedrock supporting dense river weed beds. Foraging on boulders and bedrock not supporting river weed was not observed during this study. Protrusion of lips, indicating foraging from cobble during the early and middle sections of the spawning season was sporadic and rare over a large stream reach; lip protrusions, while foraging from bedrock supporting river weed, during the end of the spawning season and the nonspawning period occurred in rapid succession within a relatively small area (e.g., 10 m<sup>2</sup>). During the postspawning season, adult sicklefin redhorse were observed maneuvering through a stream reach comprised of clean cobble with intermittently spaced boulders supporting river weed. Foraging was not observed to occur over the clean cobble in these reaches; fish only foraged from the sporadically spaced boulders covered in river weed. Within lower Valley River and Hiwassee River, adult sicklefin redhorse were observed foraging from inverted bedrock, supporting dense river weed mats, between resting periods within bedrock troughs immediately downstream of the section of raised bedrock being utilized for foraging. In addition, adult sicklefin redhorse were encountered foraging

within deep (i.e., ~ 2 m), high gradient, entrenched swiftly flowing stream reaches located within middle Hiwassee River when river weed was abundantly present. Following periods of precipitation that resulted in elevated stream levels, several radio-tagged and visually located sicklefin redhorse were observed holding position within exceptionally shallow (i.e., 0.10–0.20 m) water flowing at extremely high velocities (i.e., 1.25–1.50 m/s) exhibiting foraging behavior. Typically, the dorsal fin and occasionally the entire surface of the back of the fish continuously broke the surface while foraging. These foraging observations occurred over boulders and bedrock supporting dense mats of river weed that, prior to the precipitation event, were only barely inundated by flowing water (i.e., <0.10 m).

Spawning Behavior.—Valley River yielded the majority of the spawning observations for which spawning behavior could be quantified. Of the 31 sicklefin redhorse spawning acts observed in which behavior could be quantified, 29 were in Valley River, 1 occurred in Brasstown Creek, and 1 occurred in Hanging Dog Creek. Of the 8 courting acts in which behavior could be quantified, 6 occurred in Valley River, 1 occurred in Hanging Dog Creek, and 1 occurred in Beaverdam Creek. The mean number of individuals engaged in courting behavior was 11.8 (SE, min–max; 2.43, 2–20); the mean number of individuals engaged in spawning behavior (i.e., quivering) was 3.8 (SE, min–max; 0.43, 2–15). Mean duration of quivering was 6.4 seconds (SE, min–max; 0.66, 3–15). No agonistic behavior was observed by males; however, on numerous occasions a presumed male was seen quickly swimming into and past a troupe of quivering fish and consequently terminating the quivering session. During the one (5.9%) courting act observed in Beaverdam Creek, two

sicklefin redhorse were observed displaying courting behavior with a black redhorse troupe ( $N = 10$ ). On three (9.7%) occasions, two in Valley River and one in Brasstown Creek, a single black redhorse was observed actively engaged in quivering behavior with a spawning sicklefin redhorse troupe.

Many aspects of sicklefin redhorse reproductive behavior were similar to that reported for other *Moxostoma* species (Hackney et al. 1967; Curry and Spacie 1984; Page and Johnston 1990; Kwak and Skelly 1992; Cooke and Bunt 1999). Similar to observations by Jenkins (1999), I observed no site preparation by male sicklefin redhorse. Upon locating suitable spawning habitat, adult sicklefin redhorse utilized several oviposition sites within a relatively small area (e.g.,  $\sim 9\text{--}12\text{ m}^2$ ). Females, prior to quivering sessions, typically would hold motionless resting on substratum directly downstream of the selected oviposition location. Males did not display any territorial (i.e., agonistic) tendencies related to an oviposition site. Males were commonly observed nudging females displaying oviposition site fidelity. No quivering acts materialized from these observed nudging acts and males, following a female appearing unreceptive, would typically swim upstream while occasionally displaying foraging behavior. On five occasions, an adult sicklefin redhorse was observed breaking the surface of the stream displaying behavior comparable to surface foraging. This behavior was only observed during the spawning season within spawning tributaries and is possibly similar to other *Moxostoma* species that have been observed to break the surface (e.g., jumping) during spawning (Burr and Morris 1977). Typically, a spawning trio (i.e., one centralized female and two straddling males) was observed when quivering occurred.

However, on three (9.7%) occasions, only two fish (i.e., one male and one female) were observed during a quivering act; and on two (6.5%) occasions supernumerary males (i.e., >10) were observed to join a spawning trio following quivering initiation.

Behavior described by Jenkins (1999) (i.e., pod- or troupe-forming) was commonly observed at spawning sites in between quivering sessions. Pods were usually roaming, as a collective unit, and typically occurred within slack and deeper water downstream of raised large boulders. Occasionally, a pod would hold position within slack protected water as strong swift currents were deflected around the courting fish. Fish comprising a pod were tightly formed, exhibiting complex and organized movement behavior, and appeared to be composed of numerous (i.e., 10–15) smaller males which were slowly and orbital in nature swarming around and gently nudging a single larger female. Typically, male fish were observed foraging immediately downstream of and deeply within the oviposition site, at times approaching vertical orientation (i.e., perpendicular to substratum) as the head was maneuvered into the bottom of the depression. I observed no female sicklefin redhorse foraging on previously deposited and unburied eggs.

Selected spawning sites were frequently associated with cover; small to large boulder or an embedded log typically and directly bordered the oviposition location. Occasionally, raised embedded large cobble or a small boulder constituted the downstream boundary of the oviposition site. Only one (3.2%) spawning act occurred in the absence of cover. Substratum depressions were generally associated with occupied cover, and spawning typically occurred within these preexisting depressions. During all sicklefin redhorse

quivering acts, participating fish displayed minimal tendency to rise anteriorly and disturb the substrate with their caudal and anal fins. Fish primarily remained almost parallel to flow and substrate although, minimal anterior rise and posterior sag was present, and despite being minimal, produced a substantial silt plume downstream. For 8 of 31 (25.8%) spawning sessions, an observed secondary body undulation behavior was distinctly different from quivering. Approximately 1–2 minutes following conclusion of quivering, the female returned to the oviposition site and violently thrashed her caudle and anal fins against the substrate immediately upstream of the oviposition site while progressing upstream. Males closely followed the female upon return to the oviposition site and prior to the secondary behavior (i.e., powerful rapid tail flexures). This behavior started at the oviposition site and progressed upstream approximately 0.5 m to 2.0 m. At each oviposition site for which this behavior was observed, the behavior was consistently repeated. Females exhibiting this behavior displayed significant anterior rise during these violent upstream movements, resulting in substantial sediment plumes. The mean time interval between these unique events, for which video recordings were obtained ( $N = 18$ ), was 101.1 seconds (SE, min–max; 20.81, 17–245). These separate and distinct violent upstream movements occurred for 1–2 hours following quivering. Females appeared to remain completely upright during these tail flexures (i.e., postspawning-digging). One excavated oviposition location contained buried eggs, and tail thrashings in rapid succession were observed at this location. Despite there being no prevalent sand, fine gravel, or medium gravel in close proximity to the oviposition site, eggs had adhered to such substrates beneath a layer of small cobble.

### *Microhabitat Use and Suitability*

Use and Availability Comparisons.—Microhabitat use and availability univariate frequency distributions were significantly different for all variables (depth, bottom velocity, mean column velocity, dominant substrate, cover, distance to cover, distance to bank, and occurrence of river weed) during all seasons as well as annually ( $P < 0.05$ ), with the exception of distance to cover ( $P = 0.1498$ , Annual;  $P = 0.7300$ , Postspawning;  $P = 0.9896$ , Winter) and occurrence of river weed ( $P = 0.4260$ , Winter) (Table 18; Figures 11–17). Such significant divergence between utilized and available habitat indicates that sicklefin redhorse nonrandomly select specific microhabitats for life history requirements (e.g., spawning, foraging, resting). During winter 18 of 20 (90%) observations were not associated with cover (Table 18). For depth, bottom velocity, mean column velocity, and dominant substrate, oviposition microhabitat from the center of the oviposition site and that immediately upstream were not significantly different ( $P > 0.05$ ; Table 19). Thus, microhabitat data obtained from the center of the oviposition site (i.e., pit) were employed for all analyses.

Occupied microhabitats were different than those available, and typically different among seasons. Annual and seasonal mean distance to bank values of occupied microhabitats varied greatly from those available (Table 18). Distance to bank use means were greater than available means, indicating that sicklefin redhorse select habitat near the thalweg. However, observed spawning use and available distance to bank means demonstrated the reverse trend (Table 18, Figure 11), indicating that sicklefin redhorse select microhabitat near the bank for oviposition sites. Use mean depth values were consistently

greater than available mean depths annually and seasonally (Table 18), but the trend was reversed during spawning (Table 18, Figure 12), indicating that sicklefin redhorse select shallower habitat than that available, on average, for spawning and deeper habitat than that available the remainder of the year. Annual, seasonal, and observed spawning use bottom velocity means were consistently greater than available bottom velocity means (Table 18, Figure 13), indicating that sicklefin redhorse select habitats with bottom velocities greater than those available, and this same trend occurred for mean column velocity (Table 18, Figure 14). Annual and seasonal use and available dominant substrate mode values were consistently bedrock; however, observed spawning and courting acts were most frequently associated with small cobble (Table 18, Figure 15). Occupied river weed means were greater than available means annually and during the spawning and postspawning seasons (Table 18). For observed spawning and courting acts, river weed occupancy was zero despite river weed occurring within stream reaches utilized for spawning (Table 18), indicating that spawning sicklefin redhorse avoid conditions or stream reaches conducive to river weed when spawning. Sicklefin redhorse utilized habitat annually that was not associated with cover; however, sicklefin redhorse utilized spawning habitat associated with cover (most frequently boulder) despite cover not being readily available (Table 18, Figure 16). Of the 42 oviposition sites, 33 (78.6%) were associated with boulders, six (14.3%) were associated with coarse woody debris, and three (7.1%) had no association with cover (Figure 16). Annually and seasonally, use and available distance to cover mean values demonstrate little variation (Table 18). Conversely, observed spawning and courting acts had a use distance to

cover mean that was considerably less than the available distance to cover mean (Table 18, Figure 17), indicating that sicklefin redhorse typically select oviposition locations that are in close proximity to cover. Of the 42 spawning and courting acts observed where available microhabitat was surveyed, 28 (66.7%) observations occurred less than 0.25 m from cover (e.g., boulder or coarse woody debris) (Table 18).

Suitable and Optimal Microhabitat.—Suitable microhabitat ranges for sicklefin redhorse revealed optimal microhabitats. Distances  $\geq 20$  m were optimal distances from bank for all seasons as well as annually; however, distances 4.0–12.0 m were similarly suitable during the spawning season when fish were occupying spawning tributaries (Figure 18). Short distances from bank were optimal for oviposition sites (Figure 18). Moderately deep water was optimal during the spawning and postspawning seasons as well as annually; however, shallow water was most suitable for spawning and courting acts, and deep water was optimal during winter (Figure 19). Relatively swift bottom velocities were most suitable during the spawning season, for oviposition sites, and annually; slower bottom velocities were optimal during the postspawning season and during winter (Figure 20). A broad range of mean column velocities were optimal during the postspawning season (Figure 21). Annual and spawning and postspawning season mean column velocities indicate that sicklefin redhorse select extremely swift currents during these periods; however, winter and oviposition suitabilities indicate that slower mean column velocities are optimal (Figure 21). Bedrock and cobble were the most suitable dominant substrates during the spawning and winter seasons as well as annually (Figure 22). Cobble was the most suitable dominant

substrate for oviposition sites; bedrock was the most suitable dominant substrate during the postspawning season (Figure 22). No cover was most suitable during all seasons and annually (Figure 23); coarse woody debris and boulder were optimal cover categories at oviposition sites (Figure 23), indicating that spawning sicklefin redhorse prefer some type of embedded large object in close proximity to their oviposition site (i.e., spawning pit). No obvious trend was present relative to distance to cover annually and for all functional seasons; however, close proximity to cover was optimal relative to oviposition sites (Figure 24).

Annual, Seasonal, and Oviposition Comparisons.—Characteristics of microhabitats occupied by sicklefin redhorse varied among seasons. Similarly, microhabitat use varied within the spawning season at oviposition sites and during a cold front that moved through the study area during the spawning migration in 2007 (Table 20). Spawning season distance to bank distributions were significantly different from those of the postspawning and winter seasons (Table 20, Figure 25), likely a factor of stream width as the wider Hiwassee River was occupied predominately during the postspawning and winter seasons and more narrow spawning tributaries were occupied during the spawning season. These seasonal differences indicate that, other than during the act of spawning, sicklefin redhorse utilize thalweg habitat as apposed to marginal habitat. This notion is bolstered by the fact that postspawning and winter distance to bank distributions were not significantly different (Table 20, Figure 25). Spawning season depth distributions were significantly different from postspawning and winter depth distributions; postspawning season depth distributions were significantly

different from winter season depth distributions (Table 20, Figure 26). Sicklefin redhorse occupied deeper water during winter compared to during the spawning and postspawning season (Table 20, Figure 26). For bottom velocity, the only statistically significant difference occurred between the spawning season and the 2007 cold front ( $P = 0.0019$ ; Figure 27). Mean column velocities were significantly different during the winter season when compared to the postspawning season (Table 20, Figure 28). Swifter mean velocities were occupied more frequently during the winter season than during the spawning and postspawning seasons (Table 20), despite the winter bottom velocity distribution not being statistically different from either the spawning or postspawning season distributions (Table 20, Figure 28). Utilized dominant substrates during the spawning season were significantly different from dominant substrates used during the postspawning season and winter (Table 20, Figure 29). Utilized postspawning dominant substrates were not significantly different from winter dominant substrates (Table 20, Figure 29). Bedrock was the most frequently utilized dominant substrate during all seasons (Table 18); however, cobble was occupied almost as often as bedrock during the spawning season. Bedrock was utilized considerably more often during the postspawning and winter season compared to the spawning season (Figure 29). Comparisons of seasonal cover use reveal that winter use is not significantly different from spawning and postspawning pertaining to cover use (Table 20; Figure 30). Cover use during the spawning season was highly significantly different from that during the postspawning season (Table 20, Figure 30). For all seasons, no cover was most frequently utilized followed by boulder (Table 18). For all seasonal comparisons, distance to cover was not

significantly different (Table 20, Figure 31). Seasonal comparisons of river weed occurrence reveal that postspawning habitat use was highly significantly different from spawning and winter season habitat use for presence of river weed (Table 20); spawning season use was not significantly different from winter use (see below; Table 20).

To ascertain the differences and similarities between utilized oviposition microhabitats and the general microhabitat use during the spawning season, oviposition microhabitat was compared to all microhabitats quantified during the spawning season. Distinct differences were detected between spawning and courting act microhabitat compared to spawning season relocation and visual observation microhabitat. Oviposition microhabitat distance to bank, depth, mean column velocity, dominant substrate, cover, distance to cover, and river weed occurrence were all highly significantly different from general spawning season microhabitat ( $P < 0.005$ , Table 20); only bottom velocity was not significantly different ( $P = 0.053$ ; Table 20, Figure 27). Oviposition distance to bank was shorter than spawning season values; depth was shallower; bottom and mean column velocities were swifter; dominant substrates were regularly very coarse gravel and small cobble rather than bedrock; boulders and coarse woody debris (i.e., embedded logs) cover was usually associated with oviposition sites as apposed to a lack of cover during the spawning season; and oviposition locations were routinely less than 0.50 m from associated cover as opposed to greater than 1 m for spawning season locations (Table 18, Figures 25–31). River weed did not occur at any oviposition locations, while 56% of measured locations during the spawning season supported river weed growth (Table 18).

Significant differences between utilized microhabitat during a cold front that interrupted the upstream spawning migration and utilized microhabitat during the spawning season under seasonal temperature conditions were detected. Cold front distances to bank, cover type, distance to cover, and mean column velocity were not significantly different from those of the seasonal spawning season microhabitat variables (Table 20). Cold front microhabitat depth was highly significantly different from that of the seasonal spawning season ( $P = <0.0001$ ; Table 20, Figure 26). Cold front microhabitat bottom velocity was significantly different from that of the seasonal spawning season ( $P = 0.0019$ ; Table 20, Figure 27). Cold front microhabitat dominant substrate was significantly different from that of the seasonal spawning season ( $P = 0.0001$ ; Table 20, Figure 29). Cold front microhabitat variable river weed occurrence was significantly different from that of the seasonal spawning season ( $P = 0.0070$ ; Table 20). These statistically significant differences that occurred following considerable decreases in spawning tributary water temperature indicate a reactive habitat shift during an upstream spawning migration. Specifically, as a result of colder water temperatures, migratory sicklefin redhorse shifted from shallow swift currents and coarse substrate that encourage river weed growth to deeper habitat with slower currents and fine substrates that are not conducive to river weed growth. Observations during tracking sessions in Valley River, Brasstown Creek, and Hanging Dog Creek that coincided with this early-April cold front in 2007, corroborate these macrohabitat shifts. During these tracking sessions, numerous (i.e.,  $> 50$ ) sicklefin redhorse were observed motionless in the tail-end of calm deep pools with large numbers (i.e.,  $> 200$ ) of additional *Moxostoma* species (black

redhorse, golden redhorse, river redhorse, silver redhorse, and smallmouth redhorse). Moreover, two (14.3%) radio-tagged fish that had previously commenced an upstream spawning migration in Valley River from Hiwassee River returned to reoccupy their previous locations in Hiwassee River following declining water temperatures. This finding indicates that water temperatures considerably colder from those that are associated with upstream spawning migrations may not only cause some migratory fish to postpone upstream migration, but can result in a percentage of fish prematurely descending spawning tributaries and terminating their spawning migration until water temperatures increase.

Multivariate Analyses.—Annually, for all seasons, and for oviposition sites, sicklefin redhorse occupied macrohabitat nonrandomly for components 1 and 2, except for component 2 during the spawning season and winter ( $P < 0.05$ ; Table 21). Significant difference ( $P < 0.05$ ) was found between all seasonal and oviposition site comparisons for both components 1 and 2, except for the comparison between the spawning season and winter (PC 2,  $P = 0.1383$ ; Table 22) and the postspawning season and winter (PC 1,  $P = 0.1335$ ; PC 2,  $P = 0.5448$ ). Principal Component Analysis (PCA) during the spawning season as well as annually indicated that bottom velocity, mean column velocity, dominant substrate, distance to bank, and depth were important microhabitat variable combinations in determining what habitats adult sicklefin redhorse occupied (Tables 23–24, Figure 32). Components 1 and 2 explained a cumulative variance of 59.8% annually and during the spawning season (Tables 23–24), and 59.7% during the postspawning and winter seasons (Tables 25–26). For bottom velocity and mean column velocity, annually and for all seasons, loadings were significantly

large enough to indicate a considerable influence on both PC1 and PC2 (Tables 23–26, Figure 32); depth loadings were never large enough to indicate influence on PC1 however, always large enough to indicate considerable influence on PC2 (Tables 23–26, Figure 32).

Annually and during the spawning season, adult sicklefin redhorse were most commonly relocated or visually observed occupying the thalweg of swift runs and riffles where coarse substrates were prevalent (high PC1 scores) and were rarely relocated or visually observed in marginal stream habitat where minimal flows encouraged deposition and retention of fine substrates (i.e., silt and sand) (low PC1 scores; Figure 32). When fish were observed near shore, occupied macrohabitats were typically shallow and swift (low PC2 scores); fish were less commonly associated with the slow flowing currents of deep, low gradient pools (high PC2 scores; Figure 32). During the postspawning season, adult sicklefin redhorse were most commonly relocated or visually observed occupying the thalweg of swift runs and riffles where coarse substrates (i.e., boulder and bedrock) were prevalent (high PC1 scores) and were less likely to utilize marginal stream habitat (low PC1 scores) than during the spawning season (Figure 32). In addition, during the postspawning season, relocations and visual observations typically occurred within runs, characterized by bedrock flats, at intermediate depths and moderate flows associated with the mid-channel of Hiwassee River and lower Valley River (intermediate PC2 scores; Figure 32). During the winter, adult sicklefin redhorse were relocated occupying the thalweg of extreme lower Hiwassee River and utilizing bedrock flats where water velocities were intermediate to swift and cover was rarely associated with use (high PC1 scores; Figure 32). Additionally, relocations generally

occurred within water currents that tended to be swifter and depths that were significantly deeper than those during the spawning and postspawning seasons (high PC2 scores; Figure 32). These spatial and temporal shifts in microhabitat use indicate that adult sicklefin redhorse use varying lotic macrohabitats nonrandomly.

From the oviposition microhabitat PCA, components 1 and 2 had a cumulative explained variance of 61.1% for available microhabitat (Table 27). Combined microhabitat variables distance to bank, bottom velocity, mean column velocity, and dominant substrate constituted component 1 (Table 27, Figure 33). Component 2 combined microhabitat variables were bottom velocity, mean column velocity, distance to cover, and depth (Table 27, Figure 33).

Oviposition locations of adult sicklefin redhorse were most commonly in marginal stream habitat close to riffles and swift runs where intermediate to coarse substrates (very coarse gravel and small cobble) were prevalent (intermediate PC1 scores; Figure 33). Oviposition sites were usually close to cover and typically located in shallow water with swift currents (low PC2 scores); oviposition locations were never associated with slow currents or deep water (high PC2 scores; Figure 33). Tightly grouped sicklefin redhorse spawning scores, more so than annual and seasonal scores, indicate that oviposition sites represent specific microhabitat that is selected nonrandomly (Figure 32 and 33).

Oviposition Substrate Composition.—On average, small cobble (40.9%) and very coarse gravel (21.3%) were most commonly represented in oviposition substrate samples; however, large cobble (13.2%) and coarse gravel (11.0%) were also abundant. Assessment

of clay, silt, and sand revealed that oviposition substrates samples contained on average 3.0% fines that was 2.4% organic (Table 28). Measures of central tendency revealed a mean geometric mean diameter ( $D_g$ ) of 54.0 mm (i.e., very coarse gravel) and a mean median diameter ( $D_{50}$ ) of 78.7 mm (i.e., small cobble) (Table 28). To assess particle size unity or permeability, a sorting coefficient ( $S_o$ ) was calculated for each substrate sample; the mean sorting coefficient for sicklefin redhorse oviposition sites was 1.93 (Table 28). Since a sorting coefficient ( $S_o$ ) of 1.0 equals unity or maximum permeability, substrates utilized for spawning in the Hiwassee Basin are not perfectly sorted or at unity, indicating some level of degradation (natural or unnatural). Since  $D_g$  equals 54.0 mm, the Fredle index would need to be exactly equal to 54.0 to indicate that utilized spawning substrates exhibit maximum possible pore size and permeability. With a calculated Fredle index value of 28.2, collected spawning substrates appear to be at approximately half potential relative to pore size and permeability.

Microhabitat Availability.—Stream morphology characteristics, obtained from habitat availability surveys, reveal that streams and rivers utilized by sicklefin redhorse within the upper Hiwassee Basin are relatively similar (Table 10). Spawning tributaries had similar mean stream widths ranging from 12.1 m (Hanging Dog Creek) to 24.6 m (Nottely River); Hiwassee River was considerably wider with a mean stream width of 50.2 m (Table 10). In addition, all streams appeared to be moderately to highly entrenched with bank angles ranging from 104° (Nottely River) to 133° (Hanging Dog Creek). For all streams, available depths averaged 0.39 m and ranged from 0.28 m (Brasstown Creek) to 0.53 m (Nottely

River). Mean bottom velocity among all streams was 0.09 m/s and ranged from 0.06 m/s (Nottely River) to 0.12 m/s (Valley River and Brasstown Creek). Average mean column velocity was 0.21 m/s and ranged from 0.15 m/s (Hanging Dog Creek) to 0.27 m/s (Hiwassee River). Bedrock was the modal dominant substrate for Valley River, Brasstown Creek, and Hiwassee River, while Hanging Dog Creek and Nottely River were dominated by fine substrates (e.g., silt and sand). The subdominant substrate for all spawning tributaries and Hiwassee River was sand. Nearly half of all habitat availability points were within 2 m of some sort of cover, ranging from 45% (Brasstown Creek) to 69% (Hanging Dog Creek). River weed was relatively scarce in spawning tributaries, ranging from 18% (Valley River) to 27% (Brasstown Creek) occurrence, but was abundant in Hiwassee River (56%). Mean undercut bank distance (m) revealed relatively minor lateral bank erosion at base flow levels with mean undercut distances ranging from 0.08 m (Valley River) to 0.20 (Brasstown Creek). Analysis of riparian zone land use, within a 30 m buffer at survey reaches, revealed that approximately 60% of Valley River's riparian zone was in agriculture or developed while the other 40% was forested. The riparian zones of Brasstown Creek and Hiwassee River were 30–40% developed, while the riparian zone of Hanging Dog Creek and Nottely River were 65.0–95% forested (Table 10).

## **Discussion**

Movement patterns and linear range results of this study should be interpreted as descriptive for adult sicklefin redhorse and applicable to current conditions in the Hiwassee

Basin that are influenced by anthropogenic activities, including dam operations and changes in watershed land use; however, the sicklefin redhorse population that I studied in the Hiwassee Basin, is one of only two known to occur, and these results presumably represent the species' behavior and ecology under current conditions. Examination of natural populations with biological rather than physical boundaries are preferred (Gerking 1950) if movement and home range estimates are desired on which to base management decisions. Both sicklefin redhorse populations occupy basins (i.e., Hiwassee and Little Tennessee) characterized as highly fragmented and impounded, and this inevitably has altered stream reach continuity, availability, and suitability. Had the status of Hiwassee Basin hydrologic and watershed continuity characteristics resembled pre-anthropogenic disturbance conditions, very different movement patterns and seasonal linear ranges might have resulted.

Water temperature appeared to be an important proximate physical factor influencing spawning migration timing. Water temperature has been associated with triggering of upstream spawning migration for robust redhorse (Grabowski and Isely 2006), northern hog sucker (*Hypentelium nigricans*) (Matheney and Rabeni 1995), and razorback sucker (Modde and Irving 1998). Curry and Spacie (1984) reported that spawning occurred one month earlier during 1977 relative to 1978 and 1979, due to an early warming trend, for several sucker species in Deer Creek, Indiana. During 2006 and 2007, radio-telemetry and two-way resistance board weir data indicated that sicklefin redhorse commence upstream spawning migrations when water temperatures are 10.0–12.0 °C and peak when temperatures are 15.0–16.0 °C. Radio-tagged fish relocations, weir samples, and observational data, corresponding

with a late-March to early-April cold front in 2007, that indicated a spawning migration temporary termination, bolsters the notion that water temperature may be an important factor influencing timing and intensity of the spawning migration. Similarities between percent tubercle development and maturation stages between 2006 and 2007, despite considerably different water temperature, may suggest that a uniform stimulus (e.g., photoperiod) is responsible for physiological changes that prepare sicklefin redhorse for spawning migration (Huber and Bengston 1999).

Suppressed or aborted spawning migration runs have been documented for numerous potomodromous and anadromous fishes related to unseasonably low flows (Purkett 1961; Paukert and Fisher 2001; Firehammer and Scarnecchia 2006, 2007; Paragamian and Kruse 2001; Mitchell and Cunjak 2007; Erkinaro et al. 1999). Premature abandonment of spawning grounds has also been contributed to rapidly decreasing spring flows (Paragamian and Kruse 2001). High spring flows or spates have been proposed to improve the chance of successful spawning and ultimately recruitment of upstream migratory fishes by preventing the deposition of fine particulates (i.e., silt), ensuring inundation of oviposition sites, and facilitating the downstream emigration of emergent larvae to suitable downstream rearing habitat. Barton (1980) found that spring discharge volumes, in an Alberta tributary, accounted for greater variation in upstream migrating white sucker and longnose sucker *Catostomus catostomus* numbers than did temperature; however, the greatest amount of upstream migration variation was explained by the product of discharge and temperature, suggesting interactive effects (Lucas and Baras 2001). For sicklefin redhorse, stream flow

appeared to be a proximate factor influencing spawning migration magnitude, distance, and duration, while water temperature appeared to influence migration timing considerably.

Spatiotemporal identification and characterization of potomodromous fish home range is critical for management. Gerking (1953) defined home range for fishes as “the area over which the animal normally travels”, leaving ample room for interpretation and discussion, due to the ambiguousness related to the term “normal”, and there has been a copious amount of both (Mohr 1947; Funk 1957; Gowan et al. 1994; Gowan and Fausch 1996; Smithson and Johnston 1999; Rodriguez 2002). Hansteen et al. (1997) and Vokoun (2003) recognized that consideration of a temporal aspect is crucial when estimating and reporting home range. Unfortunately, my relocation sample sizes were not sufficiently large enough to estimate stream reaches having a defined probability of occurrence during a specified time period (e.g., kernel density estimates) (Van Winkle 1975; Kernohan et al. 2001). I calculated linear range as the difference between the two most distant relocation points for an individual fish in relation to a given time period (i.e., annual and seasonal). Thus, I defined sicklefin redhorse linear range as a stream reach occupied or utilized during a specific time period (e.g., spawning season) to fulfill specific life history requirements (e.g., foraging and spawning).

Sicklefin redhorse weekly directional linear range and distance from the Valley River and Hiwassee River confluence estimates revealed behavior that was highly mobile during the spawning season and extremely sedentary during the postspawning and winter seasons. Quantitative data describing the movement of other *Moxostoma* species is lacking; however,

sicklefin redhorse display similar movement patterns to those of the robust redhorse in that movement is highest during the spring spawning migration and minimal during the winter (Grabowski and Isely 2006). Conversely, sicklefin redhorse migrate shorter distances compared to the robust redhorse and utilize spawning tributaries rather than spawning within the mainstem larger river. Use of spawning tributaries by other *Moxostoma* species has been documented for the shorthead redhorse (Sule and Skelly 1985), black redhorse, and golden redhorse (Curry and Spacie 1984; Kwak and Skelly 1992). No radio-tagged sicklefin redhorse conducted upstream migrations completely within Hiwassee River during the spawning season, and no courting or spawning behavior was observed within Hiwassee River, possibly owing to the lack of suitable spawning substrate (i.e., very coarse gravel–large cobble) between Mission Dam and Hiwassee Lake.

The use of multiple spawning sites or “wandering” has been documented for several potomodromous fishes; specifically, the robust redhorse (Grabowski and Isely 2006), razorback sucker (Tyus and Karp 1990; Modde and Irving 1998; Mueller et al. 2000), and paddlefish (Paukert and Fisher 2001; Stancill et al. 2002). Grabowski and Isely (2006) speculated that this “wandering” mechanism possibly indicates a degree of habitat suitability evaluation prior to spawning site commitment. Both seasonal linear range and distance to confluence estimates indicated that adult sicklefin redhorse were less mobile in 2007 than in 2006 during the spawning season. This is likely related to both low flows brought on by a severe drought and a prolonged cold front resulting in low temperatures during the 2007 spawning season.

High site fidelity has been documented for numerous riverine catostomids including the white sucker (Olson and Scidmore 1963; Werner and Lannoo 1994), salish sucker *Catostomus* sp. (Pearson and Healey 2003), robust redhorse *Moxostoma robustum* (Grabowski and Isely 2006), and razorback sucker (Modde and Irving 1998); in addition to, several large riverine fishes including the Colorado pikeminnow *Ptychocheilus lucius* (Tyus 1990; Irving and Modde 2000), paddlefish *Polyodon spathula* (Lein and DeVries 1998; Stancill et al. 2002; Zigler et al. 2003; Firehammer and Scarnecchia 2006, 2007), Gulf sturgeon *Acipenser oxyrinchus desotoi* (Wooley and Crateau 1985; Foster and Clugston 1997; Heise et al. 2004) and numerous site fidelity traditionalists (i.e., salmonids) (Harcup et al. 1984; Brown and MacKay 1995; Dittman and Quinn 1996; Northcote 1997; Swanberg 1997; Burrell et al. 2000; Hilderbrand and Kershner 2000). Grabowski and Isely (2006) emphasized that their radio-tagged robust redhorse exhibited a high degree of site fidelity (i.e., 0.1 rkm) for specific staging and overwintering macrohabitats typically after concluding an extensive migration (i.e., 100 rkm) and attribute this level of fidelity to the restricted movement paradigm (RMP; Gowan et al. 1994). Sicklefin redhorse similarly displayed a very high degree of spawning ground and postspawning site fidelity, albeit not as rigid as the robust redhorse, in addition to spawning tributary fidelity. It is not clear why the sicklefin redhorse displayed relatively less spawning ground site fidelity compared to the robust redhorse; however, possible explanations may be that robust redhorse spawning macrohabitats in the Savannah River are extremely rare (only two known gravel bars), the severe drought that affected the Hiwassee Basin in 2007, and the cold front that interrupted

the 2007 sicklefin redhorse spawning migration in 2007. Grabowski and Isely (2006) conceded that their findings are novel among catostomid literature and concluded that no one clear pattern can be drawn. The level of similarity between the sicklefin redhorse and the robust redhorse for site fidelity is in accord with findings for other potamodromous fishes and bolsters the notion that a pattern among catostomids may exist.

Site fidelity and minimal movement during all seasons of both 2006 and 2007, except during the actual act of migration to and from spawning tributaries and overwintering areas, is in strict accordance with the RMP coined and then challenged by Gowan et al. (1994), but first described by Gerking (1959). The RMP is a widely supported phenomenon associated with many riverine fishes that states that adult riverine fishes, not associated with spawning migrations (Gerking 1959), are predominately sedentary and typically reside within a single riffle or pool (e.g., Gerking 1953; Bachman 1984) or inhabit a 20–50 m stream reach (e.g., Miller 1957; Rodriguez 2002) throughout their life. Gerking (1959) qualified the “restricted movement” notion by stating the following:

“That stray (i.e., mobile) fish occur has never been doubted, and their importance in repopulation of decimated areas and the distribution of the species is not questioned. Stray fish should be found simply as a consequence of biological variation. It was never intended to deny the existence of strays, or to imply that all races of the same species have the same behavior...For example, a fish may return to familiar surroundings if caused to move by unfavourable conditions or by spawning requirements.”

Gowan et al. (1994) challenged the validity of the RMP for salmonids based on a literature review and their own research that found extensive immigration into a stream reach that had been artificially enhanced. Gowan et al. (1994) suggested that these immigrations would not have occurred if the RMP was a valid and true concept and concluded that many salmonids are mobile during periods other than the spawning season and many previous studies validating the RMP unintentionally contain biases by not accounting for detection of marked fish outside of the “home section”. Rodriguez (2002) offered an alternative explanation that high turnover rates, obtained during mark-recapture studies, are indicative of low displacement (i.e., <50 m) rather than high mobility and suggested that low displacement is covered by the RMP. Furthermore, Gerking (1959) acknowledged 35-years prior to Gowan et al. (1994) that “stray fish” (i.e., mobile fish) exist and should be considered a function of “biological variation”. In accord with the RMP, radio-tagged sicklefin redhorse displayed spawning stream and site homing or fidelity, extreme site fidelity during the postspawning season, downstream displacement during the winter season when upstream conditions within upper Hiwassee River are less favorable, straying movements, and high mobility associated with spawning migrations. Radio-telemetry data in conjunction with weir data clearly indicate that sicklefin redhorse are sedentary fish that briefly exhibit extensive seasonal movements to fulfill life history requirements (i.e., reproduction and overwintering) conducive to survival.

Funk (1957) suggested that fish populations are composed of two distinct subpopulations, one functioning as rather mobile and the other characterized as sedentary.

Several researchers have concluded that their findings support Funk's (1957) two subpopulation conclusion (Heggenes et al. 1991, Freeman 1995; Smithson and Johnston 1999; Schmetterling, and Adams 2004). Lucas and Baras (2001) noted that, in a substantial number of studies that conclude, as Funk (1957) did, that a movement dichotomy exists within a single species occupying identical stream reaches based on mark-recapture data, the possibility exists that subsequent conclusions or suggestions are the result of poor temporal resolution, and that radio-telemetry techniques capture a higher spatial resolution related to fish movements or lack thereof. This is not to say that fish populations are not composed of both mobile and sedentary fish subpopulations; however, low to moderate displacements by a small proportion of the population, especially when the abandoned habitat is reoccupied or displacement occurs during unfavorable conditions, is not evidence of such a population dichotomy. The majority of radio-tagged sicklefin redhorse that conducted significant migrations did so in conjunction with spawning requirements and subsequently returned precisely to previously occupied stream reaches or conducted downstream migrations during winter in conjunction with cold water temperatures. Parsimoniously, variation in individual sicklefin redhorse movement is likely associated with individual requirements and limitations rather than as complicated as the existence of two subpopulations.

Skipped spawning migration (i.e., spawning omission) is a widely occurring phenomenon for migratory fishes (Rideout et al. 2005; Secor 2008). Four adult females and one adult male radio-tagged sicklefin redhorse failed to migrate during the spawning season in 2006 or 2007; however, those fish conducted a spawning migration at least once during

my two year study. Skipped spawning likely occurs, although it is not certain, as a result of a compromise between present and potential reproduction (Bull and Shine 1979; Rideout et al. 2005). By postponing upstream spawning migration, adult sicklefin redhorse likely acquire growth advantages, ultimately increasing survival, during the skipped year and subsequently maximize lifetime reproductive success. Secor (2008) suggested that migration, for the purpose of spawning, is the principle “accessory” cost experienced by migratory fish associated with reproduction.

Similarly, despite many of the adult sicklefin redhorse returning to Hiwassee River immediately following the spawning season, a significant proportion and mostly males utilized lower sections of a spawning tributary for some portion of the postspawning season during 2006 and 2007. Koed et al. (2002) documented similar findings for pikeperch (*Stizostedion lucioperca* (L.)) in Gudena River, Denmark, and concluded that downstream migration for that species was not highly concerted and indicated considerable individual variability. Sicklefin redhorse are sedentary fish appearing to remain within a very restricted home range when not conducting extensive migrations, and this range can vary among functional seasons (e.g., spawning); however, if currently occupied habitat becomes unfavorable as a result of biological variation or more suitable habitat is encountered during spawning, winter, or exploratory (i.e., straying fish) migrations, it is beneficial to relocate to more favorable habitat. Sicklefin redhorse appeared to utilize three home ranges during this study. These three home ranges were typically located within the middle to upper reaches of a spawning tributary during the spawning season, the middle to lower reaches of Hiwassee

River and the lower reaches of utilized spawning tributaries during the postspawning season, and the extreme lower reaches of Hiwassee River during the winter season.

Longer spawning tributary residence time for male fish, such as that found for Hiwassee Basin sicklefin redhorse, has been documented for blueback herring (Loesch and Lund 1977), Atlantic sturgeon *Acipenser oxyrinchus* (Van Eenennaam et al. 1996), Arctic charr *Salvelinus alpinus* L. (McCubbing et al. 1998), and striped bass *Morone saxatilis* (Carmichael et al. 1998). Van Eenennaam et al. (1996) reported that male Atlantic sturgeon occupy spawning tributaries considerably longer than females, and this may be indicative of spawning tributary resident males; however, male abundance increases when females enter the spawning tributary during the spawning season. Despite several species exhibiting longer male resident times postspawning and immediate descention by females, explanations for this sexual dichotomy are lacking. Males and females are likely responding to varying physiological and environmental migration cues. Baker (1978) and Carmichael et al. (1998) suggested that initiation of migration is based on a “migration threshold” and natural selection encourages divergent stimuli relative to the migration threshold and sex. Additionally, Baker (1978) proposed that the threshold for males is related to the maturation stage or receptiveness of females and the threshold for females is possibly physiological. Adult male sicklefin redhorse not only hold to spawning grounds longer than females, many relocate to lower reaches of the utilized spawning tributary, rather than to Hiwassee River like females do, and remain there until the onset of winter when males relocate to lower Hiwassee River. Female fish typically partition disproportionately more energy toward

production of relatively few large gametes, while males devote far less energy toward many small gametes (Quinn 2005). As a function of this anisogamy, male fish generally can allocate more energy toward longevity of spawning ground occupancy. I hypothesize that female sicklefin redhorse, due to high energetic expenditures during oogenesis (i.e., the female process of gametogenesis or formation of ovum) and upstream migration, immediately return to Hiwassee River for optimal foraging and growth conducive microhabitat to recover from the spawning season and prepare for winter and subsequent spawning seasons; males remain on spawning grounds as long as receptive females are present and upon the chance that a late arriving female may arrive on the spawning grounds, and utilize less optimal lower reaches of spawning tributaries during the postspawning season as these reaches are suitable for recovery and growth following less demanding energetic expenditures during spermatogenesis and upstream migration from lower Hiwassee River. Unexpectedly, and perhaps contrary to this hypothesis, mark-recapture data suggest that males experience a greater negative absolute growth relative to females during the spawning season; however, females display an annual growth rate nearly three times that of males suggesting that returning to Hiwassee River following spawning is beneficial relative to growth and ultimately survival.

A sexual dichotomy related to spawning migration chronology has been reported for numerous catostomids such that male fish precede female fish up spawning tributaries and arrive at spawning grounds prior to females (Hackney et al. 1967; Page and Johnston 1990; Vokoun et al. 2003). I did not observe this phenomenon for the sicklefin redhorse. Radio-

telemetry and weir catches revealed that males and females arrive within spawning tributaries and onto spawning grounds simultaneously. Since sicklefin redhorse exhibit no spawning site preparation and are non-territorial, it is possible that there is no need for males to arrive at and occupy spawning grounds prior to females and that intraspecific sexual dichotomy of migration chronology is likely selected against without a reproductive or selective advantage.

Spawning female sicklefin redhorse appear to select natural depressions bordered by raised cover (i.e., boulder or log) either positioned on the lateral-right, lateral-left, or downstream of the selected oviposition site. Natural depressions, possibly a function of cover and downstream currents, may be preferred due to eddy currents within the depression that will retain deposited gametes. I suggest that if quiver duration is sufficiently long enough for eggs to be deposited, then that female will promptly begin routinely and violently thrashing her caudle and anal fin against the substrate (i.e., postspawning digging) immediately upstream of the utilized oviposition site in an attempt to relocate coarse substrate downstream and subsequently cover any fertilized eggs deposited and remaining within the naturally occurring depression. Healey et al. (2003) found that frequency of digging by female sockeye salmon *Oncorhynchus nerka* significantly increased postspawning for 20 to 30 minutes to cover the oviposition site. Tiffan et al. (2005), using dual-frequency identification sonar (DIDSON), documented that chum salmon *Oncorhynchus keta* following quivering, initiate a rapid succession of postspawning covering digs immediately upstream of the utilized oviposition site and that out of 10 spawning events, only three (30%) were conducted by repeat spawners that utilized covering digs to excavate a

second nest. This low percentage of postspawning digs that were complemented by subsequent immediate upstream oviposition sites suggests that the sole consequence of the majority of the postspawning digs was buried gametes. Similarly, no sicklefin redhorse were observed to utilize disturbed substratum for the purpose of quivering following postspawning digs and sicklefin redhorse postspawning digs, similar to Healey et al. (2003), were rapid in succession. It is important to note that several quivering events, typically short in duration (i.e., 3–5 seconds), were not associated with postspawning digs and occurred more than once at the same oviposition location similar to other *Moxostoma* species in Hiwassee Basin (e.g., golden redhorse and black redhorse). However, postspawning digs were associated with a single quivering event and were typically associated with prolonged (approximately 11 seconds) and undisturbed quivering sessions.

Chapman (1988) stated that understanding of salmonid redd construction advanced slowly, and this will likely be the case for sicklefin redhorse pertaining to postspawning digs and the specific function of nearby cover as well. The exact function of these unique and separate postspawning digs is unclear. A few additional possible explanations may include an undescribed courting behavior, uncovering of eggs for consumption, site preparation, substratum inquisition, or other undescribed behavior for the genus *Moxostoma*. Regardless of the evolutionary function of these observed postspawning digs, those that I observed likely facilitated the burial of any gametes that resulted from prior occurring quivering acts.

It is unclear if foraging on eggs by male sicklefin redhorse is filial cannibalism or heterocannibalism (Rohwer 1978). Cooke and Bunt (1999) reported that greater redhorse

were observed to commit both filial and heterocannibalism and that analysis of stomach contents confirmed their observations. However, Cooke and Bunt (1999) reported that females foraged on eggs; no sicklefin redhorse females were observed foraging on previously deposited and unburied eggs during my study. Jenkins (1999) also reported foraging behavior within oviposition sites by sicklefin redhorse. Possibly, consumption of unburied eggs, that are likely to perish as a result of interspecific predation, by male sicklefin redhorse evolved to improve male fitness and abate negative physiological effects associated with upstream spawning migration. However, I did not observe postspawning egg consumption by any of the other *Moxostoma* species observed spawning within Hiwassee Basin. This may be partially attributed to differences in reproductive strategies and reproductive isolating mechanisms. Black redhorse and golden redhorse oviposition sites typically occurred in finer substratum (i.e., gravel) increasing the likelihood of egg burial occurring during quivering. Male golden redhorse and river redhorse displayed territorial behavior, excluding heterocannibalism. If sicklefin redhorse eggs are not completely buried during the act of quivering due to coarse spawning substrate (e.g., cobble) or protected against heterocannibalism as a function of male agonistic behavior, then immediate recurring female postspawning digs may function to bury and protect potential zygotes from intraspecific predation in addition to interspecific predation, as well as to facilitate incubation.

Sicklefin redhorse appear to utilize shallow depths during high flows, which were previously inaccessible prior to a precipitation event, for foraging purposes due to the presence of previously unexploited benthic macroinvertebrate communities. A similar

scenario was observed by Grabowski and Isely (2006); they relocated radio-tagged robust redhorse to the floodplain of the Savannah River, Georgia and South Carolina, during spring floods. Grabowski and Isely (2006) concluded that these fish were utilizing the floodplain for foraging in preparation for the spawning season. However, sicklefin redhorse are likely exploiting previously inaccessible benthic macroinvertebrates as a function of precipitation events (i.e., increased discharge) and opportunistic foraging.

Grubaugh et al. (1997) documented within the Little Tennessee Basin, North Carolina, that river weed was rare in the upper reaches of the Little Tennessee River and its tributaries regardless of substratum size and abundant in the middle and lower reaches. River weed is likely less abundant within upper reaches of Hiwassee Basin spawning tributaries as a result of stable upstream reaches being conducive to depositional habitats that accumulate benthic organic matter (Grubaugh et al. 1997), frequent more intense spates as a function of impervious surface and riparian zone development that promotes bed form mobilization, siltation from development and agriculture, and sand abrasion during high flow (Connelly et al. 1999). Lack of river weed within upper reaches of Valley River may partially explain why the majority of adult sicklefin redhorse relocate to lower Valley River and Hiwassee River postspawning, where dominant substrates (i.e., bedrock) are not mobile and depositional habitats are routinely excavated by high flows. In addition, the substratum of lower Valley River and Hiwassee River are dominated by rugose bedrock and exceptionally conducive to river weed growth. Similarly, Tyus and Karp (1990) suggested that mass downstream movements of the razorback sucker were associated with foraging. During the

postspawning season and as a function of foraging on benthic macroinvertebrates occupying river weed beds, the relationship between river weed and sicklefin redhorse appears to be significant because foraging by relocated radio-tagged fish and visually observed fish was constantly associated with river weed during this period.

River weed differentiates Hiwassee Basin streams and rivers (as well as others) from other lotic environments dominated by macrophytes that require slow-flowing usually marginal habitats; Podostemaceae prefer open-canopy rapids (e.g., Everitt and Burkholder 1991). River weed attaches itself to stationary coarse substrates (i.e., small cobble–bedrock) using holdfasts, an adaptation which allows rapids and riffles to be occupied (Hill and Webster 1984). River weed increases stream bed stability (e.g., Fritz and Feminella 2003) and manipulates immediate hydrology by decreasing subsurface current velocities (e.g., Sand-Jensen 1998; Madsen et al. 2001; Dodds and Briggs 2002). Hill and Webster (1984) found that, within the New River, Virginia, river weed standing crop begins to increase in mid-May and decreases in late-August. In addition, river weed appears to significantly enhance benthic macroinvertebrate community abundance (Grubaugh et al. 1997; Hutchens et al. 2004) by increasing the surface area, due to extremely branched growth morphology. Hutchens et al. (2004) estimated that river weed increased the surface area of bedrock outcroppings, in the Little Tennessee River, North Carolina, by 3.2–4.2 m<sup>2</sup> (i.e., 3 to 4 times). Voshell (1985) estimated that river weed-covered substrates within the New River, West Virginia, yielded the highest benthic macroinvertebrate production reported to date (612 g/m<sup>2</sup>/year).

River weed flourishes on Hiwassee River rugous bedrock and other substantially raised coarse substrates free of fine sediments that occur within moderate to swift flowing currents. River weed is intolerant of desiccation (Everitt and Burkholder 1991) and excessive fine sediments (i.e., siltation) (Meijer 1976; Philbrick and Crow 1983). Fine sediments are abundant in the section of Hiwassee River between Mission Dam and Hiwassee Lake as a result of intensive riparian zone development, agriculture, and cattle infringement. Brasstown Creek appears to significantly contribute to Hiwassee River sediment loading. There is approximately 16.2 km of regulated unimpounded Hiwassee River between Mission Dam and Hiwassee Lake depending on the season and precipitation.

Benthic macroinvertebrate abundance and distribution is correlated with substrate quality, substrate composition, temperature, dissolved oxygen, and nutrients (Thorp and Covich 1991). Environmental alteration (e.g., dams and channelization) resulting in extensive degradation of microhabitat (e.g., river weed) will result in benthic macroinvertebrate abundance and distribution levels different from those with which sicklefin redhorse evolved. Management for river weed will likely benefit numerous species (benthic macroinvertebrates, mussels, fishes) including the sicklefin redhorse. Argentina (2006) found that habitats that experience river weed increases via artificial manipulation, supported significant increases in benthic fish abundances. In addition, benthic macroinvertebrate biomass and abundance were significantly higher in areas where river weed was added than in areas with reduced river weed coverage. Understanding foraging

behavior will facilitate basin-wide management decisions designed to improve foraging substrate.

Previous investigations studying *Moxostoma* species microhabitats focused on those associated with spawning (Curry and Spacie 1984; Kwak and Skelly 1992; Cooke and Bunt 1999; Grabowski and Isely 2007), and microhabitat requirements for functional seasons and life history requirements are largely unknown. However, a general pattern in *Moxostoma* of utilizing shallower depths during spawning, compared to overwintering depths, has been documented. Robust redhorse, while utilizing spawning gravel bars or shoals, select depths with a mean of 0.74 m during the spawning season (Grabowski and Isely 2007) and depths in the 3.0–5.0 m range (Grabowski and Isely 2006) during the winter. Although depths associated with winter were not measured, Kwak and Skelly (1992) documented within Stony Creek, Illinois, that golden redhorse and black redhorse oviposition sites have mean depths of 0.18 m and 0.22 m, respectively. Similarly, Cooke and Bunt (1999) documented within Grand River, Ontario, that greater redhorse *Moxostoma valenciennesi* utilize midstream riffles with a mean depth of 0.34 m during the act of spawning. Mean depths reported by Kwak and Skelly (1992) and Cooke and Bunt (1999) reveal that relatively shallow depths associated with oviposition sites are common among *Moxostoma* species; those reported mean depths by Grabowski and Isely (2006, 2007), in conjunction with the findings of my study, reveal that utilization of a wide range of depths between the spawning and winter season is common. With a wide range of depths being utilized by adult sicklefin redhorse, anthropogenic alterations (e.g., siltation, channelization, reservoirs) that contribute

to the loss of habitat complexity and the homogenization of available depths are likely to adversely affect the species.

Data provided by an unseasonable cold front and the affect that subsequent decreasing water temperatures had on the 2007 sicklefin redhorse upstream spawning migration provide insight into depths utilized and behavior exhibited relative to cold water regardless of functional season. In addition, nearly identical results from the sicklefin redhorse spawning migration and the hypolimnetic discharge from Nottely Dam suggest that unseasonably cold water temperatures interrupt or terminate the spawning migration. These findings bolster the idea that although hypolimnetic discharges from hydroelectric dams may be suitable to establish a nonnative trout fishery in a geographic region (e.g., southern Appalachian Mountains) that may or may not support salmonids during the summer months, unseasonably cold water temperatures ultimately are unsuitable for native potamodromous species. Unseasonable cold water, whether natural or human induced, such as those associated with cold fronts or hydroelectric dams that release hypolimnetic discharges appear to cause sicklefin redhorse to abandon upstream migration and move to relatively deeper water and downstream reaches similar to those occupied during the winter.

Deep water was not considered a form of cover during this study; however, adult sicklefin redhorse utilized greater depths during winter when compared to the spawning and postspawning seasons; suggesting that deep water is utilized as a form of energetic refuge during winter, similar to that during the 2007 spawning season cold front. Due to the heterogeneity of utilized rugose bedrock outcropping, it is possible that cover utilization and

therefore, microhabitat variable distance to cover were underestimated despite elevated or inverted bedrock being grouped with cover type boulder during this study. Nevertheless, sicklefin redhorse utilized bedrock troughs and crevices during this study and efforts aimed at reducing sediment loading, that will likely increase heterogeneity of bedrock, could be beneficial toward conservation and restoration efforts.

Similar to other *Moxostoma* species, oviposition sites utilized by adult sicklefin redhorse are generally located within shallow depths, swift velocities, water temperatures ranging from 14.0 °C–21.0 °C, and associated with substrates of medium coarseness (Curry and Spacie 1984; Page and Johnston 1990; Kwak and Skelly 1992; Cooke and Bunt 1999). Microhabitat use variables that displayed some level of divergence from or have not been reported for other *Moxostoma*, were dominant cover, distance to cover, distance to bank, and the absence of submerged aquatic vegetation. Sicklefin redhorse oviposition sites were typically positioned within a natural depression located proximately to a boulder or an embedded log (Figure 34). Spawning typically occurred between 2.0 and 4.0 m from the bank, and suitability indicates that this distance range is optimal for sicklefin redhorse spawning. Cooke and Bunt (1999) reported that greater redhorse utilize both marginal and midstream habitats during spawning. Sicklefin redhorse oviposition sites were located <4.0 m from the bank 76% of the time. Oviposition sites never supported river weed beds despite the plant being moderately available within spawning reaches. It is unclear if river weed is being avoided; however, the lack of river weed at oviposition sites, relative to availability, was significant ( $P = 0.0008$ ). Submersed aquatic vegetation has been reported to reduce

subsurface current velocities; it is possible that adult sicklefin redhorse select oviposition sites free of river weed to avoid reduced subsurface currents and subsequent sedimentation and low dissolved oxygen concentrations.

Spawning substrate composition samples confirmed visual estimations that small cobble and very coarse gravel were the dominant substrates at spawning sites. However, sorting coefficients ( $S_o$ ), a measure of particle size unity or permeability, indicate that substrates utilized by sicklefin redhorse have relatively low permeability, when coarse dominant substrates theoretically should promote extremely high permeability. A sorting coefficient of 1.0 indicates perfectly sorted gravel (i.e., unity) or approximate maximum permeability for that particular particle size; thus, for spawning tributaries in Hiwassee Basin, sicklefin redhorse spawning substrates exhibit subsurface pores that are filled with finer particles that impede permeability (i.e.,  $S_o > 1.0$ ). Using geometric mean diameter and sorting coefficient, a relatively high mean Fredle index of 28.2 (SE, 1.59) was calculated to serve as a baseline value to which future comparisons could be made to evaluate the status of varying spawning substrate quality (i.e., post disturbance or post enhancement). This mean Fredle index value, resulting from high levels of sedimentation within sicklefin redhorse spawning tributaries, is likely not representative of optimal spawning substrate. However, if sicklefin redhorse deposit their gametes within naturally occurring depressions and subsequently bury zygotes with postspawning digs, then subsurface oviposition substrate analyses may not accurately represent or describe incubation substrates.

### *Management Implications*

Comprehensive management and conservation of nongame fishes is rarely undertaken until it is deemed necessary to establish a recovery plan for an imperiled species (Cooke et al. 2005). Such an effort requires an understanding of life history and critical habitat. When this information is lacking, managing habitat often defaults to educated guesswork, which usually fails and has negative unforeseen consequences, or a conservative strategy of protecting everything is implemented, which often yields societal consequences that are difficult to justify (Rosenfeld 2003).

Potential basinwide management initiatives that could benefit the Hiwassee Basin and Little Tennessee Basin sicklefin redhorse populations include reductions in sedimentation loading (e.g., best management practices), delayed infiltration of runoff into streams to promote bed stability, migration barrier removal (e.g., dams, log jams, culvert road crossings), elimination or mitigation of hypolimnetic discharges from hydroelectric dams (e.g., Nottely Dam), delaying inundation of the Hiwassee River and Valley River confluence until after the spawning season, flushing Hiwassee River below Mission Dam prior to the river weed growing season to transport deposited sediments downstream, maintaining flows below those that promote the mobilization of bed form during the river weed growing season, implementation of a riparian zone buffer, implementation of minimum flows below hydroelectric dams that prevent river weed from being desiccated during drought years, and reintroductions of juvenile sicklefin redhorse propagated and collected (Grabowski and Isely 2005) using the most current technology from a brood stock that has been observed

spawning. Past efforts have been successful at restoration on the watershed scale within the Hiwassee Basin. In 1992, as part of Tennessee Valley Authority's (TVA) clean water initiative, 12 River Action Teams (RAT) were created to coordinate resource protection and recovery efforts within the Hiwassee Basin. From this program, watershed restoration was deemed best served when holistic resource management was implemented (Bowling et al. 1997). Future restoration objectives will likely be most effective if efforts are made to restore pre-anthropomorphic form and function to utilized streams while providing ample consideration of the requirements of principal stakeholders to enhance the probability that voluntary resolutions will be broadly implement and established (Bowling et al. 1997).

Future management might be guided by additional research designed to ascertain optimal rearing habitat for young of year (YOY) and juvenile sicklefin redhorse, as the ecology of these life stages remain relatively unknown for *Moxostoma* species and most stream fishes. In addition, research aimed at understanding the behavior of YOY after substratum emergence may indicate optimal rearing habitat at a broad spatial scale (e.g., spawning tributaries or lower lotic reaches of Hiwassee River). Estimating movement or residency of recently hatched sicklefin redhorse relative to spawning tributaries may guide other aspects of management (e.g., nonnative trout stocking). Currently, the majority of juvenile sicklefin redhorse has been collected from lentic waters (e.g., Hiwassee Lake) (Jenkins 1999). The functional role of reservoirs as juvenile sicklefin redhorse rearing habitat is unclear, as well as the influence of blueback herring on *Moxostoma* species within streams and reservoirs. Efforts to increase knowledge of predators of sicklefin redhorse

during all life stages would benefit future management (e.g., reintroductions). Similar to discovering that impounded reaches of Hiwassee River (i.e., Hiwassee Lake) reduce foraging and overwintering habitat of adults due to lentic habitat intolerance, knowledge of population bottlenecks, habitat requirements of all life stages, and mitigating management are critical to the ecological success of the sicklefin redhorse as well as other *Moxostoma* species.

Resource managers are increasingly pressured by multiple user groups to base resource management decisions on sound evidence and conclusive quantitative data. The results of this research can support sensitive decisions to manipulate aquatic habitat in an effort to protect and enhance the sicklefin redhorse, while attempting to satisfy the objectives of affected constituents (i.e., hydroelectric utilities, agriculturists, recreationalists, and environmentalists). Any legislation, policy, or otherwise sensitive decision that directly reduces alterations to critical habitats or water quality degradation would likely directly benefit the sicklefin redhorse (Cooke et al. 2005). Manipulations that directly benefit the sicklefin redhorse will likely benefit other aquatic organisms, such as coexisting *Moxostoma* species and threatened and endangered freshwater mussels that employ migratory fishes as obligatory hosts. Implementation of freshwater protected areas (Crivelli 2002), elimination of migration barriers, implementation of fish passage mechanisms, reduction of sediment deposition, and establishment of flow variability that mimics a natural hydrograph are resource management actions that may be considered to benefit the sicklefin redhorse (Cooke et al. 2005).

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Table 1.—Radio-tagged sicklefin redhorse characteristics for reproductive ecology study in Hiwassee Basin, North Carolina and Georgia. Radio-tagged fish that died at anytime during the study are omitted.

| Transmitter frequency (MHz) | Capture year | Capture stream | Spawning tributary | Sex    | Total Length (mm) | Weight (g) | Number of relocations |
|-----------------------------|--------------|----------------|--------------------|--------|-------------------|------------|-----------------------|
| 48.041                      | 2006         | Hiwassee River | Hanging Dog Creek  | Male   | 501               | 1,048      | 18                    |
| 48.201                      | 2006         | Hiwassee River | Valley River       | Female | 496               | 1,068      | 24                    |
| 48.221                      | 2006         | Valley River   | Valley River       | Male   | 454               | 786        | 26                    |
| 48.271                      | 2006         | Valley River   | Valley River       | Female | 505               | 1,270      | 15                    |
| 48.352                      | 2006         | Valley River   | Nottely River      | Male   | 485               | 1,086      | 7                     |
| 48.371                      | 2006         | Valley River   | Brasstown Creek    | Male   | 505               | 1,256      | 11                    |
| 48.422                      | 2006         | Valley River   | Valley River       | Female | 486               | 1,224      | 28                    |
| 48.731                      | 2006         | Hiwassee River | Valley River       | Male   | 510               | 986        | 15                    |
| 48.751                      | 2006         | Valley River   | Valley River       | Male   | 460               | 934        | 17                    |
| 48.771                      | 2006         | Valley River   | Valley River       | Male   | 501               | 1,082      | 22                    |
| 48.791                      | 2006         | Valley River   | Valley River       | Female | 512               | 1,228      | 24                    |
| 48.811                      | 2006         | Hiwassee River | Valley River       | Male   | 458               | 896        | 25                    |
| 48.832                      | 2006         | Valley River   | Valley River       | Male   | 495               | 1,098      | 16                    |
| 48.871                      | 2006         | Valley River   | Valley River       | Female | 489               | 1,004      | 21                    |
| 48.891                      | 2006         | Valley River   | Valley River       | Male   | 434               | 785        | 26                    |
| 48.931                      | 2006         | Valley River   | Valley River       | Female | 493               | 1,130      | 18                    |
| 48.951                      | 2006         | Valley River   | Valley River       | Female | 560               | 1,564      | 9                     |
| 48.971                      | 2006         | Valley River   | Valley River       | Male   | 492               | 960        | 27                    |
| 48.991                      | 2006         | Valley River   | Valley River       | Female | 494               | 1,252      | 24                    |
| 48.011                      | 2007         | Hiwassee River | Valley River       | Female | 541               | 1,300      | 12                    |
| 48.031                      | 2007         | Hiwassee River | Valley River       | Female | 523               | 1,386      | 11                    |
| 48.051                      | 2007         | Hiwassee River | Valley River       | Male   | 479               | 993        | 10                    |
| 48.071                      | 2007         | Hiwassee River | Brasstown Creek    | Female | 511               | 1,360      | 12                    |
| 48.081                      | 2007         | Hiwassee River | Brasstown Creek    | Male   | 553               | 1,588      | 7                     |
| 48.122                      | 2007         | Hiwassee River | Unknown            | Female | 560               | 1,410      | 12                    |
| 48.251                      | 2007         | Hiwassee River | Valley River       | Male   | 550               | 1,396      | 13                    |
| 48.911                      | 2007         | Hiwassee River | Unknown            | Female | 533               | 1,265      | 10                    |
| 48.951                      | 2007         | Hiwassee River | Unknown            | Female | 472               | 993        | 12                    |
| Mean                        |              |                |                    |        | 502               | 1,155      | 16.9                  |
| SD                          |              |                |                    |        | 32                | 212        | 6.7                   |

Table 2.—Tributaries where no radio-tagged or observed sicklefin redhorse were found.

| Waterbody radio-tracked | Receiving waterbody | Date      | Season       | Distance from confluence or description (km) |
|-------------------------|---------------------|-----------|--------------|--|
| Cook Creek              | Beaver Dam Creek    | 14-May-07 | Spawning     | 1.3  |
| Copper Creek            |                     | 14-May-07 | Spawning     | 3.7  |
| Radford Creek           |                     | 14-May-07 | Spawning     | 2.2  |
| Little Brasstown Creek  | Brasstown Creek     | 9-Jun-07  | Postspawning | 1.5  |
| Pinelog Creek           |                     | 30-Apr-07 | Spawning     | 1.0  |
| West Prong Grape Creek  | Grape Creek         | 20-May-07 | Spawning     | 2.3  |
| Owl Creek               | Hanging Dog Creek   | 20-May-06 | Spawning     | 3.0  |
|                         |                     | 24-May-06 | Spawning     | 3.0  |
|                         |                     | 30-May-06 | Spawning     | 3.0  |
|                         |                     | 12-Jun-06 | Postspawning | 3.0  |
|                         |                     | 20-Jun-06 | Postspawning | 3.0  |
|                         |                     | 26-Jun-06 | Postspawning | 3.0  |
|                         |                     | 3-Apr-07  | Spawning     | 3.0  |
|                         |                     | 10-Apr-07 | Spawning     | 3.0  |
|                         |                     | 22-Apr-07 | Spawning     | 3.0  |
|                         |                     | 1-May-07  | Spawning     | 3.0  |
|                         |                     | 14-May-07 | Spawning     | 3.0  |
|                         |                     | 29-May-07 | Spawning     | 3.0  |
|                         |                     | 5-Jun-07  | Postspawning | 5.5  |
| Grape Creek             | Hiwassee Lake       | 20-May-07 | Spawning     | 3.2  |
| Martin Creek            | Hiwassee River      | 20-Apr-07 | Spawning     | 8.5  |
|                         |                     | 20-May-07 | Spawning     | 8.5  |
| Peachtree Creek         |                     | 7-Apr-07  | Spawning     | 4.4  |
| Dooley Creek            | Nottely River       | 29-Apr-07 | Spawning     | 1.5  |
| Owenby Creek            |                     | 29-Apr-07 | Spawning     | 1.0  |
| Rapier Mill Creek       |                     | 29-Apr-07 | Spawning     | 1.0  |
|                         |                     | 20-May-07 | Spawning     | 4.5  |
| Hyatt Creek             | Valley River        | 1-Jun-06  | Postspawning | 0.6  |
| Junaluska Creek         |                     | 26-Jun-06 | Postspawning | 3.0  |
|                         |                     | 23-Apr-07 | Spawning     | 3.0  |
| Tatham Creek            |                     | 22-Apr-07 | Spawning     | 0.8  |
| Vengence Creek          |                     | 24-May-07 | Spawning     | 1.2  |
| Hiwassee Dam tailspill  | Apalachia Lake      | 14-May-07 | Spawning     | Below Hiwassee Dam                           |
| Hiwassee Lake           | Apalachia Lake      | 13-May-06 | Spawning     | Upper Hiwassee Lake                          |
|                         |                     | 16-May-06 | Spawning     | Upper Hiwassee Lake                          |
|                         |                     | 22-May-06 | Spawning     | Upper Hiwassee Lake                          |
|                         |                     | 29-May-06 | Spawning     | Upper Hiwassee Lake                          |
|                         |                     | 12-Jun-06 | Postspawning | Upper Hiwassee Lake                          |
|                         |                     | 24-Jun-06 | Postspawning | Entire Hiwassee Lake                         |
|                         |                     | 30-Jun-06 | Postspawning | Upper Hiwassee Lake                          |
|                         |                     | 7-Jul-06  | Postspawning | Upper Hiwassee Lake                          |

Table 2.—(Continued).

| Waterbody radio-tracked | Receiving waterbody | Date      | Season       | Distance from confluence or description (km) |
|-------------------------|---------------------|-----------|--------------|--|
| Hiwassee Lake           | Apalachia Lake      | 17-Apr-07 | Spawning     | Upper Hiwassee Lake                          |
|                         |                     | 7-May-07  | Spawning     | Upper Hiwassee Lake                          |
|                         |                     | 22-May-07 | Spawning     | Upper Hiwassee Lake                          |
|                         |                     | 9-Jun-07  | Postspawning | Entire Hiwassee Lake                         |
|                         |                     | 15-Jan-08 | Winter       | Upper Hiwassee Lake                          |

Table 3.—Summarized microhabitat use and availability for all Hiwassee Basin streams where a sicklefin redhorse was documented.

| Variable and statistic  | Reproductive behavior |              | Season       |              |               | Available    |
|-------------------------|-----------------------|--------------|--------------|--------------|---------------|--------------|
|                         | Courting              | Spawning     | Spawning     | Postspawning | Winter        |              |
| Temperature (C°)        |                       |              |              |              |               |              |
| <i>N</i>                | 17                    | 31           | 357          | 279          | 20            |              |
| Mean                    | 17.9                  | 17.6         | 16.7         | 20.4         | 8.3           |              |
| SE                      | 0.55                  | 0.52         | 0.17         | 0.18         | 0.48          |              |
| Min – max               | 14.5 – 21.1           | 11.0 – 21.1  | 7.8 – 23.7   | 11.7 – 24.5  | 5.5 – 12.8    |              |
| Dissolved oxygen (mg/L) |                       |              |              |              |               |              |
| <i>N</i>                | 17                    | 31           | 357          | 279          | 20            |              |
| Mean                    | 8.77                  | 8.72         | 9.87         | 9.09         | 11.54         |              |
| SE                      | 0.19                  | 0.11         | 0.05         | 0.04         | 0.05          |              |
| Min – max               | 7.70 – 10.68          | 7.70 – 10.00 | 7.70 – 12.23 | 7.87 – 11.01 | 11.13 – 11.91 |              |
| Depth (m)               |                       |              |              |              |               |              |
| <i>N</i>                | 17                    | 31           | 353          | 260          | 20            | 1815         |
| Mean                    | 0.34                  | 0.26         | 0.53         | 0.45         | 0.75          | 0.37         |
| SE                      | 0.02                  | 0.01         | 0.01         | 0.01         | 0.07          | 0.01         |
| Min – max               | 0.18 – 0.46           | 0.15 – 0.44  | 0.09 – 1.88  | 0.08 – 1.66  | 0.40 – 1.50   | 0.00 – 2.00  |
| Bottom velocity (m/s)   |                       |              |              |              |               |              |
| <i>N</i>                | 17                    | 31           | 353          | 260          | 20            | 1815         |
| Mean                    | 0.16                  | 0.19         | 0.17         | 0.14         | 0.21          | 0.10         |
| SE                      | 0.04                  | 0.02         | 0.01         | 0.01         | 0.03          | 0.00         |
| Min – max               | 0.00 – 0.42           | 0.02 – 0.55  | 0.00 – 0.75  | 0.00 – 0.90  | 0.03 – 0.58   | 0.00 – 1.23  |
| Mean velocity (m/s)     |                       |              |              |              |               |              |
| <i>N</i>                | 17                    | 31           | 353          | 260          | 20            | 1815         |
| Mean                    | 0.42                  | 0.50         | 0.44         | 0.42         | 0.51          | 0.22         |
| SE                      | 0.06                  | 0.03         | 0.01         | 0.01         | 0.05          | 0.01         |
| Min – max               | 0.03 – 0.84           | 0.24 – 0.90  | 0.00 – 1.50  | 0.00 – 1.46  | 0.16 – 1.02   | 0.00 – 1.50  |
| Dominant substrate      |                       |              |              |              |               |              |
| <i>N</i>                | 17                    | 31           | 357          | 266          | 20            | 1815         |
| Mode                    | SC                    | SC           | BR           | BR           | BR            | BR           |
| SE                      | 0.41                  | 0.12         | 0.16         | 0.11         | 0.73          | 0.10         |
| Min – max               | FG – SB               | VCG – SB     | SI – BR      | SA – BR      | SA – BR       | CL – BR      |
| Subdominant substrate   |                       |              |              |              |               |              |
| <i>N</i>                | 17                    | 31           | 357          | 266          | 20            | 1815         |
| Mode                    | VCG                   | VCG          | BR           | BR           | BR            | SA           |
| SE                      | 0.40                  | 0.17         | 0.19         | 0.21         | 0.85          | 0.09         |
| Min – max               | SA – SB               | CG – SB      | SI – BR      | SI – BR      | SI – BR       | CL – BR      |
| Distance to bank (m)    |                       |              |              |              |               |              |
| <i>N</i>                | 17                    | 31           | 347          | 260          | 20            | 1815         |
| Mean                    | 4.00                  | 2.60         | 7.50         | 13.53        | 21.15         | 13.43        |
| SE                      | 0.44                  | 0.26         | 0.38         | 0.48         | 3.41          | 0.30         |
| Min – max               | 1.00 – 6.00           | 0.75 – 8.00  | 0.50 – 45.00 | 1.00 – 30.00 | 4.00 – 60.00  | 0.00 – 83.00 |
| Cover                   |                       |              |              |              |               |              |
| <i>N</i>                | 17                    | 31           | 356          | 260          | 20            | 1815         |
| Mode                    | Boulder               | Boulder      | Boulder      | No cover     | No cover      | No cover     |

Table 3.—(Continued).

| Variable and statistic | Reproductive behavior |             | Season      |              |             | Available   |
|------------------------|-----------------------|-------------|-------------|--------------|-------------|-------------|
|                        | Courting              | Spawning    | Spawning    | Postspawning | Winter      |             |
| Distance to cover (m)  |                       |             |             |              |             |             |
| <i>N</i>               | 14                    | 30          | 136         | 83           | 2           | 969         |
| Mean                   | 0.84                  | 0.24        | 1.15        | 0.99         | 0.75        | 1.02        |
| SE                     | 0.19                  | 0.05        | 0.05        | 0.07         | 0.25        | 0.02        |
| Min – max              | 0.10 – 2.00           | 0.10 – 1.00 | 0.10 – 2.00 | 0.01 – 2.00  | 0.50 – 1.00 | 0.10 – 2.00 |
| River weed             |                       |             |             |              |             |             |
| <i>N</i>               | 17                    | 31          | 357         | 269          | 20          | 1815        |
| Present (%)            | 0                     | 0           | 55          | 92           | 55          | 29          |
| SE                     |                       |             | 0.03        | 0.02         | 0.11        | 0.01        |

Table 4.—Summarized microhabitat use and availability for Beaverdam Creek.

| Variable and statistic  | Reproductive behavior |          | Season      |              |        | Available |
|-------------------------|-----------------------|----------|-------------|--------------|--------|-----------|
|                         | Courting              | Spawning | Spawning    | Postspawning | Winter |           |
| Temperature (C°)        |                       |          |             |              |        |           |
| <i>N</i>                | 1                     |          | 2           |              |        |           |
| Mean                    | 18.9                  |          | 18.9        |              |        |           |
| SE                      |                       |          | 0.00        |              |        |           |
| Min – max               | 18.9 – 18.9           |          | 18.9 – 18.9 |              |        |           |
| Dissolved oxygen (mg/L) |                       |          |             |              |        |           |
| <i>N</i>                | 1                     |          | 2           |              |        |           |
| Mean                    | 8.90                  |          | 8.90        |              |        |           |
| SE                      |                       |          | 0.00        |              |        |           |
| Min – max               | 8.90 – 8.90           |          | 8.90 – 8.90 |              |        |           |
| Depth (m)               |                       |          |             |              |        |           |
| <i>N</i>                | 1                     |          | 2           |              |        |           |
| Mean                    | 0.36                  |          | 0.24        |              |        |           |
| SE                      |                       |          | 0.00        |              |        |           |
| Min – max               | 0.36 – 0.36           |          | 0.24 – 0.24 |              |        |           |
| Bottom velocity (m/s)   |                       |          |             |              |        |           |
| <i>N</i>                | 1                     |          | 2           |              |        |           |
| Mean                    | 0.05                  |          | 0.29        |              |        |           |
| SE                      |                       |          | 0.00        |              |        |           |
| Min – max               | 0.05 – 0.05           |          | 0.29 – 0.29 |              |        |           |
| Mean velocity (m/s)     |                       |          |             |              |        |           |
| <i>N</i>                | 1                     |          | 2           |              |        |           |
| Mean                    | 0.16                  |          | 0.39        |              |        |           |
| SE                      |                       |          | 0.00        |              |        |           |
| Min – max               | 0.16 – 0.16           |          | 0.39 – 0.39 |              |        |           |
| Dominant substrate      |                       |          |             |              |        |           |
| <i>N</i>                | 1                     |          | 2           |              |        |           |
| Mode                    | MG                    |          | VCG         |              |        |           |
| SE                      |                       |          | 0.00        |              |        |           |
| Min – max               | MG – MG               |          | VCG – VCG   |              |        |           |
| Subdominant substrate   |                       |          |             |              |        |           |
| <i>N</i>                | 1                     |          | 2           |              |        |           |
| Mode                    | CG                    |          | SC          |              |        |           |
| SE                      |                       |          | 0.00        |              |        |           |
| Min – max               | CG – CG               |          | SC – SC     |              |        |           |
| Distance to bank (m)    |                       |          |             |              |        |           |
| <i>N</i>                | 1                     |          | 2           |              |        |           |
| Mean                    | 3.0                   |          | 3.0         |              |        |           |
| SE                      |                       |          | 0.00        |              |        |           |
| Min – max               | 3.0 – 3.0             |          | 3.0 – 3.0   |              |        |           |
| Cover                   |                       |          |             |              |        |           |
| <i>N</i>                | 1                     |          | 2           |              |        |           |
| Mode                    | Boulder               |          | Boulder     |              |        |           |
| Distance to cover (m)   |                       |          |             |              |        |           |
| <i>N</i>                | 1                     |          | 2           |              |        |           |
| Mean                    | 0.5                   |          | 1.0         |              |        |           |
| SE                      |                       |          | 0.00        |              |        |           |
| Min – max               | 0.50 – 0.50           |          | 1.00 – 1.00 |              |        |           |

Table 4.—(Continued).

| Variable and statistic | Reproductive behavior |          | Season   |              |        | Available |
|------------------------|-----------------------|----------|----------|--------------|--------|-----------|
|                        | Courting              | Spawning | Spawning | Postspawning | Winter |           |
| River weed             |                       |          |          |              |        |           |
| <i>N</i>               | 1                     |          | 2        |              |        |           |
| Present (%)            | 0                     |          | 100      |              |        |           |
| SE                     |                       |          |          |              |        |           |

Table 5.—Summarized microhabitat use and availability for Brasstown Creek.

| Variable and statistic  | Reproductive behavior |              | Season       |              |        | Available   |
|-------------------------|-----------------------|--------------|--------------|--------------|--------|-------------|
|                         | Courting              | Spawning     | Spawning     | Postspawning | Winter |             |
| Temperature (C°)        |                       |              |              |              |        |             |
| <i>N</i>                | 2                     | 2            | 55           | 4            |        |             |
| Mean                    | 14.5                  | 14.6         | 14.5         | 20.4         |        |             |
| SE                      | 0.00                  | 3.60         | 0.56         | 0.61         |        |             |
| Min – max               | 14.5 – 14.5           | 11.0 – 18.2  | 7.8 – 22.5   | 19.3 – 21.6  |        |             |
| Dissolved oxygen (mg/L) |                       |              |              |              |        |             |
| <i>N</i>                | 2                     | 2            | 55           | 4            |        |             |
| Mean                    | 8.85                  | 9.45         | 10.14        | 8.40         |        |             |
| SE                      | 0.00                  | 0.55         | 0.17         | 0.17         |        |             |
| Min – max               | 8.85 – 8.85           | 8.90 – 10.00 | 8.10 – 12.19 | 8.08 – 8.70  |        |             |
| Depth (m)               |                       |              |              |              |        |             |
| <i>N</i>                | 2                     | 2            | 55           | 4            |        | 263         |
| Mean                    | 0.40                  | 0.40         | 0.47         | 0.26         |        | 0.28        |
| SE                      | 0.03                  | 0.01         | 0.03         | 0.03         |        | 0.01        |
| Min – max               | 0.36 – 0.43           | 0.38 – 0.41  | 0.09 – 1.02  | 0.20 – 0.32  |        | 0.00 – 0.90 |
| Bottom velocity (m/s)   |                       |              |              |              |        |             |
| <i>N</i>                | 2                     | 2            | 55           | 4            |        | 263         |
| Mean                    | 0.32                  | 0.18         | 0.12         | 0.48         |        | 0.12        |
| SE                      | 0.01                  | 0.10         | 0.02         | 0.16         |        | 0.01        |
| Min – max               | 0.31 – 0.32           | 0.08 – 0.27  | 0.00 – 0.62  | 0.15 – 0.90  |        | 0.00 – 1.23 |
| Mean velocity (m/s)     |                       |              |              |              |        |             |
| <i>N</i>                | 2                     | 2            | 55           | 4            |        | 263         |
| Mean                    | 0.52                  | 0.43         | 0.33         | 0.82         |        | 0.23        |
| SE                      | 0.02                  | 0.07         | 0.03         | 0.11         |        | 0.02        |
| Min – max               | 0.50 – 0.53           | 0.36 – 0.50  | 0.00 – 1.50  | 0.60 – 1.01  |        | 0.00 – 1.35 |
| Dominant substrate      |                       |              |              |              |        |             |
| <i>N</i>                | 2                     | 2            | 55           | 4            |        | 263         |
| Mode                    | SC                    |              | SA           | LC           |        | BR          |
| SE                      | 0.00                  | 1.00         | 0.50         | 1.03         |        | 0.27        |
| Min – max               | SC – SC               | VCG – LC     | SA – BR      | LC – BR      |        | SI – BR     |
| Subdominant substrate   |                       |              |              |              |        |             |
| <i>N</i>                | 2                     | 2            | 55           | 4            |        | 263         |
| Mode                    | VCG                   | SC           | FG           | BR           |        | SA          |
| SE                      | 0.00                  | 0.00         | 0.44         | 1.22         |        | 0.24        |
| Min – max               | VCG – VCG             | SC – SC      | SA – BR      | SC – BR      |        | SI – BR     |
| Distance to bank (m)    |                       |              |              |              |        |             |
| <i>N</i>                | 2                     | 2            | 55           | 4            |        | 263         |
| Mean                    | 3.3                   | 2.5          | 3.5          | 3.8          |        | 7.5         |
| SE                      | 0.25                  | 0.50         | 0.17         | 1.11         |        | 0.30        |
| Min – max               | 3.0 – 3.5             | 2.0 – 3.0    | 1.0 – 7.0    | 1.0 – 6.0    |        | 0.0 – 20.8  |
| Cover                   |                       |              |              |              |        |             |
| <i>N</i>                | 2                     | 2            | 55           | 4            |        | 263         |
| Mode                    | CWD                   |              | No cover     | Boulder      |        | No cover    |
| Distance to cover (m)   |                       |              |              |              |        |             |
| <i>N</i>                | 2                     | 2            | 28           | 3            |        | 118         |
| Mean                    | 1.50                  | 0.50         | 1.07         | 1.00         |        | 0.99        |
| SE                      | 0.50                  | 0.00         | 0.14         | 0.00         |        | 0.06        |
| Min – max               | 1.00 – 2.00           | 0.50 – 0.50  | 0.10 – 2.00  | 1.00 – 1.00  |        | 0.10 – 2.00 |

Table 5.—(Continued).

| Variable and statistic | Reproductive behavior |          | Season   |              |        | Available |
|------------------------|-----------------------|----------|----------|--------------|--------|-----------|
|                        | Courting              | Spawning | Spawning | Postspawning | Winter |           |
| River weed             |                       |          |          |              |        |           |
| <i>N</i>               | 2                     | 2        | 55       | 4            |        | 263       |
| Present (%)            | 0                     | 0        | 47       | 100          |        | 27        |
| SE                     |                       |          | 0.07     | 0.00         |        | 0.03      |

Table 6.—Summarized microhabitat use and availability for Hanging Dog Creek.

| Variable and statistic  | Reproductive behavior |             | Season       |              |        | Available   |
|-------------------------|-----------------------|-------------|--------------|--------------|--------|-------------|
|                         | Courting              | Spawning    | Spawning     | Postspawning | Winter |             |
| Temperature (C°)        |                       |             |              |              |        |             |
| <i>N</i>                | 1                     | 1           | 17           | 7            |        | 365         |
| Mean                    | 21.1                  | 21.1        | 14.9         | 19.4         |        | 12.3        |
| SE                      |                       |             | 0.95         | 0.63         |        | 0.31        |
| Min – max               | 21.1 – 21.1           | 21.1 – 21.1 | 10.6 – 21.1  | 18.4 – 22.7  |        | 0.1 – 21.7  |
| Dissolved oxygen (mg/L) |                       |             |              |              |        |             |
| <i>N</i>                | 1                     | 1           | 17           | 7            |        |             |
| Mean                    | 7.70                  | 7.70        | 9.60         | 8.39         |        |             |
| SE                      |                       |             | 0.19         | 0.10         |        |             |
| Min – max               | 7.70 – 7.70           | 7.70 – 7.70 | 7.70 – 10.55 | 8.01 – 8.88  |        |             |
| Depth (m)               |                       |             |              |              |        |             |
| <i>N</i>                | 1                     | 1           | 17           | 7            |        | 328         |
| Mean                    | 0.18                  | 0.16        | 0.55         | 0.30         |        | 0.30        |
| SE                      |                       |             | 0.07         | 0.07         |        | 0.01        |
| Min – max               | 0.18 – 0.18           | 0.16 – 0.16 | 0.22 – 0.94  | 0.08 – 0.50  |        | 0.00 – 0.98 |
| Bottom velocity (m/s)   |                       |             |              |              |        |             |
| <i>N</i>                | 1                     | 1           | 17           | 7            |        | 328         |
| Mean                    | 0.37                  | 0.32        | 0.20         | 0.42         |        | 0.07        |
| SE                      |                       |             | 0.05         | 0.11         |        | 0.01        |
| Min – max               | 0.37 – 0.37           | 0.32 – 0.32 | 0.03 – 0.75  | 0.07 – 0.80  |        | 0.00 – 1.20 |
| Mean velocity (m/s)     |                       |             |              |              |        |             |
| <i>N</i>                | 1                     | 1           | 17           | 7            |        | 328         |
| Mean                    | 0.65                  | 0.40        | 0.41         | 0.58         |        | 0.15        |
| SE                      |                       |             | 0.05         | 0.10         |        | 0.01        |
| Min – max               | 0.65 – 0.65           | 0.40 – 0.40 | 0.19 – 1.01  | 0.20 – 0.94  |        | 0.00 – 1.35 |
| Dominant substrate      |                       |             |              |              |        |             |
| <i>N</i>                | 1                     | 1           | 17           | 7            |        | 328         |
| Mode                    | VCG                   | SC          | LB           | MB           |        | SA          |
| SE                      |                       |             | 0.46         | 1.20         |        | 0.24        |
| Min – max               | VCG – VCG             | SC – SC     | CG – BR      | SA – LB      |        | SI – BR     |
| Subdominant substrate   |                       |             |              |              |        |             |
| <i>N</i>                | 1                     | 1           | 17           | 7            |        | 328         |
| Mode                    | SC                    | VCG         | SA           | LB           |        | SA          |
| SE                      |                       |             | 1.02         | 0.53         |        | 0.23        |
| Min – max               | SC – SC               | VCG – VCG   | SA – BR      | SC – LB      |        | SI – BR     |
| Distance to bank (m)    |                       |             |              |              |        |             |
| <i>N</i>                | 1                     | 1           | 12           | 7            |        | 328         |
| Mean                    | 1.0                   | 2.0         | 3.3          | 3.7          |        | 6.2         |
| SE                      |                       |             | 0.42         | 0.54         |        | 0.23        |
| Min – max               | 1.0 – 1.0             | 2.0 – 2.0   | 0.5 – 5.0    | 2.5 – 6.0    |        | 0.0 – 19.2  |
| Cover                   |                       |             |              |              |        |             |
| <i>N</i>                | 1                     | 1           | 17           | 7            |        | 328         |
| Mode                    | Boulder               | Boulder     | Boulder      | Boulder      |        | Boulder     |
| Distance to cover (m)   |                       |             |              |              |        |             |
| <i>N</i>                | 1                     | 1           | 9            | 7            |        | 226         |
| Mean                    | 0.10                  | 0.10        | 1.00         | 0.67         |        | 1.04        |
| SE                      |                       |             | 0.14         | 0.25         |        | 0.04        |
| Min – max               | 0.10 – 0.10           | 0.10 – 0.10 | 0.50 – 2.00  | 0.01 – 2.00  |        | 0.10 – 2.00 |

Table 6.—(Continued).

| Variable and statistic | Reproductive behavior |          | Season   |              |        | Available |
|------------------------|-----------------------|----------|----------|--------------|--------|-----------|
|                        | Courting              | Spawning | Spawning | Postspawning | Winter |           |
| River weed             |                       |          |          |              |        |           |
| <i>N</i>               | 1                     | 1        | 17       | 7            |        | 328       |
| Present (%)            | 0                     | 0        | 53       | 86           |        | 24        |
| SE                     |                       |          | 0.12     | 0.14         |        | 0.02      |

Table 7.—Summarized microhabitat use and availability for Hiwassee River.

| Variable and statistic  | Reproductive behavior |          | Season       |              |               | Available   |
|-------------------------|-----------------------|----------|--------------|--------------|---------------|-------------|
|                         | Courting              | Spawning | Spawning     | Postspawning | Winter        |             |
| Temperature (C°)        |                       |          |              |              |               |             |
| <i>N</i>                |                       |          | 92           | 223          | 19            | 365         |
| Mean                    |                       |          | 18.5         | 20.2         | 8.4           | 14.7        |
| SE                      |                       |          | 0.32         | 0.21         | 0.50          | 0.22        |
| Min – max               |                       |          | 12.7 – 23.7  | 11.7 – 24.5  | 5.5 – 12.8    | 5.0 – 22.0  |
| Dissolved oxygen (mg/L) |                       |          |              |              |               |             |
| <i>N</i>                |                       |          | 92           | 223          | 19            |             |
| Mean                    |                       |          | 9.72         | 9.15         | 11.52         |             |
| SE                      |                       |          | 0.09         | 0.04         | 0.05          |             |
| Min – max               |                       |          | 7.87 – 11.61 | 7.97 – 11.01 | 11.13 – 11.86 |             |
| Depth (m)               |                       |          |              |              |               |             |
| <i>N</i>                |                       |          | 92           | 204          | 19            | 424         |
| Mean                    |                       |          | 0.60         | 0.46         | 0.75          | 0.46        |
| SE                      |                       |          | 0.03         | 0.02         | 0.08          | 0.02        |
| Min – max               |                       |          | 0.20 – 1.88  | 0.10 – 1.66  | 0.40 – 1.50   | 0.00 – 2.00 |
| Bottom velocity (m/s)   |                       |          |              |              |               |             |
| <i>N</i>                |                       |          | 92           | 204          | 19            | 424         |
| Mean                    |                       |          | 0.07         | 0.13         | 0.21          | 0.10        |
| SE                      |                       |          | 0.01         | 0.01         | 0.03          | 0.01        |
| Min – max               |                       |          | 0.00 – 0.65  | 0.00 – 0.75  | 0.03 – 0.58   | 0.00 – 0.85 |
| Mean velocity (m/s)     |                       |          |              |              |               |             |
| <i>N</i>                |                       |          | 92           | 204          | 19            | 424         |
| Mean                    |                       |          | 0.38         | 0.42         | 0.51          | 0.27        |
| SE                      |                       |          | 0.02         | 0.02         | 0.05          | 0.01        |
| Min – max               |                       |          | 0.05 – 0.78  | 0.00 – 1.46  | 0.16 – 1.02   | 0.00 – 1.11 |
| Dominant substrate      |                       |          |              |              |               |             |
| <i>N</i>                |                       |          | 92           | 210          | 19            | 424         |
| Mode                    |                       |          | BR           | BR           | BR            | BR          |
| SE                      |                       |          | 0.18         | 0.11         | 0.75          | 0.20        |
| Min – max               |                       |          | SI – BR      | SA – BR      | SA – BR       | CL – BR     |
| Subdominant substrate   |                       |          |              |              |               |             |
| <i>N</i>                |                       |          | 92           | 209          | 19            | 424         |
| Mode                    |                       |          | BR           | BR           | BR            | SA          |
| SE                      |                       |          | 0.44         | 0.24         | 0.89          | 0.18        |
| Min – max               |                       |          | SI – BR      | SI – BR      | SI – BR       | SI – BR     |
| Distance to bank (m)    |                       |          |              |              |               |             |
| <i>N</i>                |                       |          | 91           | 204          | 19            | 424         |
| Mean                    |                       |          | 14.8         | 15.6         | 21.5          | 26.3        |
| SE                      |                       |          | 1.05         | 0.52         | 3.58          | 0.89        |
| Min – max               |                       |          | 3.0 – 45.0   | 3.0 – 30.0   | 4.0 – 60.0    | 0.0 – 83.0  |
| Cover                   |                       |          |              |              |               |             |
| <i>N</i>                |                       |          | 92           | 204          | 19            | 424         |
| Mode                    |                       |          | No cover     | No cover     | No cover      | No cover    |
| Distance to cover (m)   |                       |          |              |              |               |             |
| <i>N</i>                |                       |          | 32           | 61           | 2             | 233         |
| Mean                    |                       |          | 1.12         | 1.02         | 0.75          | 1.17        |
| SE                      |                       |          | 0.07         | 0.08         | 0.25          | 0.04        |
| Min – max               |                       |          | 0.20 – 2.00  | 0.05 – 2.00  | 0.50 – 1.00   | 0.10 – 2.00 |

Table 7.—(Continued).

| Variable and statistic | Reproductive behavior |          | Season   |              |        | Available |
|------------------------|-----------------------|----------|----------|--------------|--------|-----------|
|                        | Courting              | Spawning | Spawning | Postspawning | Winter |           |
| River weed             |                       |          |          |              |        |           |
| <i>N</i>               |                       |          | 92       | 213          | 19     | 424       |
| Present (%)            |                       |          | 96       | 96           | 58     | 56        |
| SE                     |                       |          | 0.02     | 0.01         | 0.12   | 0.02      |

Table 8.—Summarized microhabitat use and availability for Nottely River.

| Variable and statistic  | Reproductive behavior |          | Season       |              |        | Available   |
|-------------------------|-----------------------|----------|--------------|--------------|--------|-------------|
|                         | Courting              | Spawning | Spawning     | Postspawning | Winter |             |
| Temperature (C°)        |                       |          |              |              |        |             |
| <i>N</i>                |                       |          | 4            | 2            |        |             |
| Mean                    |                       |          | 15.5         | 15.3         |        |             |
| SE                      |                       |          | 0.67         | 0.00         |        |             |
| Min – max               |                       |          | 14.4 – 17.0  | 15.3 – 15.3  |        |             |
| Dissolved oxygen (mg/L) |                       |          |              |              |        |             |
| <i>N</i>                |                       |          | 4            | 2            |        |             |
| Mean                    |                       |          | 11.27        | 9.84         |        |             |
| SE                      |                       |          | 0.59         | 0.00         |        |             |
| Min – max               |                       |          | 9.85 – 12.23 | 9.84 – 9.84  |        |             |
| Depth (m)               |                       |          |              |              |        |             |
| <i>N</i>                |                       |          | 4            | 2            |        | 111         |
| Mean                    |                       |          | 0.80         | 0.39         |        | 0.53        |
| SE                      |                       |          | 0.11         | 0.01         |        | 0.04        |
| Min – max               |                       |          | 0.48 – 0.94  | 0.38 – 0.40  |        | 0.00 – 1.32 |
| Bottom velocity (m/s)   |                       |          |              |              |        |             |
| <i>N</i>                |                       |          | 4            | 2            |        | 111         |
| Mean                    |                       |          | 0.06         | 0.07         |        | 0.06        |
| SE                      |                       |          | 0.07         | 0.03         |        | 0.01        |
| Min – max               |                       |          | 0.00 – 0.19  | 0.04 – 0.09  |        | 0.00 – 0.63 |
| Mean velocity (m/s)     |                       |          |              |              |        |             |
| <i>N</i>                |                       |          | 4            | 2            |        | 111         |
| Mean                    |                       |          | 0.44         | 0.19         |        | 0.18        |
| SE                      |                       |          | 0.21         | 0.04         |        | 0.02        |
| Min – max               |                       |          | 0.22 – 1.05  | 0.15 – 0.23  |        | 0.00 – 1.06 |
| Dominant substrate      |                       |          |              |              |        |             |
| <i>N</i>                |                       |          | 4            | 2            |        | 111         |
| Mode                    |                       |          | SC           | BR           |        | SI          |
| SE                      |                       |          | 1.25         | 0.00         |        | 0.25        |
| Min – max               |                       |          | SC – BR      | BR – BR      |        | CL – MB     |
| Subdominant substrate   |                       |          |              |              |        |             |
| <i>N</i>                |                       |          | 4            | 2            |        | 111         |
| Mode                    |                       |          | FG           | BR           |        | SA          |
| SE                      |                       |          | 2.18         | 0.00         |        | 0.28        |
| Min – max               |                       |          | FG – BR      | BR – BR      |        | CL – MB     |
| Distance to bank (m)    |                       |          |              |              |        |             |
| <i>N</i>                |                       |          | 4            | 2            |        | 111         |
| Mean                    |                       |          | 3.5          | 15.0         |        | 12.7        |
| SE                      |                       |          | 0.96         | 1.00         |        | 0.77        |
| Min – max               |                       |          | 2.0 – 6.0    | 14.0 – 16.0  |        | 0.0 – 28.5  |
| Cover                   |                       |          |              |              |        |             |
| <i>N</i>                |                       |          | 4            | 2            |        | 111         |
| Mode                    |                       |          | CWD          | Boulder      |        | No cover    |
| Distance to cover (m)   |                       |          |              |              |        |             |
| <i>N</i>                |                       |          | 4            | 2            |        | 68          |
| Mean                    |                       |          | 0.63         | 0.38         |        | 0.56        |
| SE                      |                       |          | 0.13         | 0.13         |        | 0.07        |
| Min – max               |                       |          | 0.50 – 1.00  | 0.25 – 0.50  |        | 0.10 – 2.00 |

Table 8.—(Continued).

| Variable and statistic | Reproductive behavior |          | Season   |              |        | Available |
|------------------------|-----------------------|----------|----------|--------------|--------|-----------|
|                        | Courting              | Spawning | Spawning | Postspawning | Winter |           |
| River weed             |                       |          |          |              |        |           |
| <i>N</i>               |                       |          | 4        | 2            |        | 111       |
| Present (%)            |                       |          | 100      | 100          |        | 19        |
| SE                     |                       |          |          |              |        | 0.04      |

Table 9.—Summarized microhabitat use and availability for Valley River.

| Variable and statistic  | Reproductive behavior |             | Season       |              |               | Available    |
|-------------------------|-----------------------|-------------|--------------|--------------|---------------|--------------|
|                         | Courting              | Spawning    | Spawning     | Postspawning | Winter        |              |
| Temperature (C°)        |                       |             |              |              |               |              |
| <i>N</i>                | 13                    | 28          | 187          | 43           | 1             | 365          |
| Mean                    | 18.1                  | 17.6        | 16.6         | 21.4         | 7.6           | 14.6         |
| SE                      | 0.55                  | 0.51        | 0.17         | 0.39         |               | 0.33         |
| Min – max               | 15.2 – 20.8           | 14.0 – 20.8 | 10.3 – 22.6  | 14.9 – 24.5  | 7.6 – 7.6     | 1.0 – 24.9   |
| Dissolved oxygen (mg/L) |                       |             |              |              |               |              |
| <i>N</i>                | 13                    | 28          | 187          | 43           | 1             |              |
| Mean                    | 8.83                  | 8.71        | 9.88         | 8.95         | 7.60          |              |
| SE                      | 0.24                  | 0.11        | 0.05         | 0.10         |               |              |
| Min – max               | 7.97 – 10.68          | 8.10 – 9.90 | 7.97 – 11.18 | 7.87 – 10.45 | 7.60 – 7.60   |              |
| Depth (m)               |                       |             |              |              |               |              |
| <i>N</i>                | 13                    | 28          | 183          | 43           | 1             | 689          |
| Mean                    | 0.34                  | 0.25        | 0.51         | 0.48         | 0.72          | 0.37         |
| SE                      | 0.03                  | 0.01        | 0.02         | 0.03         |               | 0.01         |
| Min – max               | 0.18 – 0.46           | 0.15 – 0.44 | 0.15 – 1.27  | 0.17 – 1.10  | 0.72 – 0.72   | 0.00 – 1.80  |
| Bottom velocity (m/s)   |                       |             |              |              |               |              |
| <i>N</i>                | 13                    | 28          | 183          | 43           | 1             | 689          |
| Mean                    | 0.13                  | 0.19        | 0.23         | 0.12         | 0.14          | 0.12         |
| SE                      | 0.04                  | 0.02        | 0.01         | 0.02         |               | 0.01         |
| Min – max               | 0.00 – 0.42           | 0.02 – 0.55 | 0.00 – 0.65  | 0.00 – 0.50  | 0.14 – 0.14   | 0.00 – 0.95  |
| Mean velocity (m/s)     |                       |             |              |              |               |              |
| <i>N</i>                | 13                    | 28          | 183          | 43           | 1             | 689          |
| Mean                    | 0.41                  | 0.50        | 0.51         | 0.34         | 0.52          | 0.23         |
| SE                      | 0.07                  | 0.03        | 0.02         | 0.02         |               | 0.01         |
| Min – max               | 0.03 – 0.84           | 0.24 – 0.90 | 0.00 – 1.33  | 0.10 – 0.73  | 0.52 – 0.52   | 0.00 – 1.50  |
| Dominant substrate      |                       |             |              |              |               |              |
| <i>N</i>                | 13                    | 28          | 187          | 43           | 1             | 689          |
| Mode                    | SC                    | SC          | BR           | BR           | SC            | BR           |
| SE                      | 0.50                  | 0.12        | 0.20         | 0.39         |               | 0.15         |
| Min – max               | FG – SB               | VCG – SB    | SI – BR      | SA – BR      | SC – SC       | CL – BR      |
| Subdominant substrate   |                       |             |              |              |               |              |
| <i>N</i>                | 13                    | 28          | 187          | 42           | 1             | 689          |
| Mode                    | VCG                   | VCG         | SC           | BR           | VCG           | SA           |
| SE                      | 0.51                  | 0.19        | 0.22         | 0.48         |               | 0.13         |
| Min – max               | SA – SB               | CG – SB     | SI – BR      | SA – BR      | VCG – VCG     | CL – BR      |
| Distance to bank (m)    |                       |             |              |              |               |              |
| <i>N</i>                | 13                    | 28          | 183          | 43           | 1             | 689          |
| Mean                    | 4.42                  | 2.63        | 5.49         | 6.24         | 15.00         | 11.33        |
| SE                      | 0.49                  | 0.29        | 0.20         | 0.40         |               | 0.29         |
| Min – max               | 1.50 – 6.00           | 0.75 – 8.00 | 1.00 – 17.00 | 2.50 – 15.00 | 15.00 – 15.00 | 0.00 – 35.00 |
| Cover                   |                       |             |              |              |               |              |
| <i>N</i>                | 13                    | 28          | 186          | 43           | 1             | 689          |
| Mode                    | Boulder               | Boulder     | No cover     | No cover     | No cover      | No cover     |
| Distance to cover (m)   |                       |             |              |              |               |              |
| <i>N</i>                | 10                    | 27          | 61           | 10           | 0             | 324          |
| Mean                    | 0.81                  | 0.22        | 1.26         | 1.14         |               | 1.01         |
| SE                      | 0.22                  | 0.05        | 0.07         | 0.23         |               | 0.04         |
| Min – max               | 0.10 – 2.00           | 0.10 – 1.00 | 0.10 – 2.00  | 0.10 – 2.00  |               | 0.10 – 2.00  |

Table 9.—(Continued).

| Variable and statistic | Reproductive behavior |          | Season   |              |        | Available |
|------------------------|-----------------------|----------|----------|--------------|--------|-----------|
|                        | Courting              | Spawning | Spawning | Postspawning | Winter |           |
| River weed             |                       |          |          |              |        |           |
| <i>N</i>               | 13                    | 28       | 187      | 43           | 1      | 689       |
| Present (%)            | 0                     | 0        | 37       | 74           | 0      | 18        |
| SE                     |                       |          | 0.04     | 0.07         |        | 0.01      |

Table 10.—Stream morphology and available microhabitat characteristics obtained from microhabitat availability surveys for streams and rivers where radio-tagged sicklefin redhorse were relocated. Other than dominant and subdominant substrate, for which mode is reported, mean values are reported for all stream morphology and available microhabitat variables.

| Stream                      | N    | Stream width (m) | Bank angle (°) | Microhabitat |                       |                     |                    |                       |
|-----------------------------|------|------------------|----------------|--------------|-----------------------|---------------------|--------------------|-----------------------|
|                             |      |                  |                | Depth (m)    | Bottom velocity (m/s) | Mean velocity (m/s) | Dominant substrate | Subdominant substrate |
| Valley River                | 689  | 21.8             | 124            | 0.37         | 0.12                  | 0.23                | Bedrock            | Sand                  |
| Brasstown Creek             | 263  | 14.4             | 127            | 0.28         | 0.12                  | 0.23                | Bedrock            | Sand                  |
| Hanging Dog Creek           | 328  | 12.1             | 133            | 0.30         | 0.07                  | 0.15                | Sand               | Sand                  |
| Nottely River               | 111  | 24.6             | 104            | 0.53         | 0.06                  | 0.18                | Silt               | Sand                  |
| Hiwassee River <sup>b</sup> | 424  | 50.2             | 124            | 0.46         | 0.10                  | 0.27                | Bedrock            | Sand                  |
| Mean/mode                   | 363  | 24.6             | 122            | 0.39         | 0.09                  | 0.21                | Bedrock            | Sand                  |
| CV (%) <sup>a</sup>         | 59.2 | 61.8             | 9.1            | 27.2         | 28.6                  | 22.6                | 65.5               | 0.0                   |

<sup>a</sup> (SD/mean) × 100.

<sup>b</sup> Upstream of Valley River confluence.

Table 10.—(Continued).

| Stream                      | Microhabitat |                        |                   | 30-m Riparian land use (%) |             |           |
|-----------------------------|--------------|------------------------|-------------------|----------------------------|-------------|-----------|
|                             | Cover (%)    | River weed (% present) | Undercut bank (m) | Forest                     | Agriculture | Developed |
|                             | Valley River | 47                     | 18                | 0.08                       | 40.3        | 42.5      |
| Brasstown Creek             | 45           | 27                     | 0.20              | 51.7                       | 9.7         | 38.6      |
| Hanging Dog Creek           | 69           | 24                     | 0.10              | 69.0                       | 20.8        | 10.3      |
| Nottely River               | 61           | 19                     | 0.19              | 94.5                       | 3.0         | 2.6       |
| Hiwassee River <sup>b</sup> | 55           | 56                     | 0.09              | 34.2                       | 30.5        | 35.3      |
| Mean                        | 55           | 29                     | 0.13              | 57.9                       | 21.3        | 20.9      |
| CV (%) <sup>a</sup>         | 18.0         | 53.3                   | 44.9              | 42.1                       | 74.3        | 74.8      |

<sup>a</sup> (SD/mean) × 100.

<sup>b</sup> Upstream of Valley River confluence.

Table 11.—Characteristics of surveyed stream reaches in upper Hiwassee Basin that were determined to be utilized by radio-tagged sicklefin redhorse.

| Stream or river          | Upstream geographic coordinates (UTM) | Downstream geographic coordinates (UTM) | Stream length (km) | Number of transects | Number of survey points |
|--------------------------|---------------------------------------|---|--------------------|---------------------|-------------------------|
| Upper Brasstown Creek    | 17S 0234045<br>3876818                | 17S 0233978<br>3877149                  | 0.33               | 15                  | 221                     |
| Lower Brasstown Creek    | 17S 0231120<br>3879232                | 17S 0231116<br>3879271                  | 0.06               | 3                   | 42                      |
| Upper Hanging Dog Creek  | 16S 0768797<br>3894344                | 16S 0768730<br>3894347                  | 0.70               | 5                   | 62                      |
| Middle Hanging Dog Creek | 16S 0768661<br>3894436                | 16S 0768595<br>3894568                  | 0.16               | 10                  | 163                     |
| Lower Hanging Dog Creek  | 16S 0766981<br>3891748                | 16S 0767185<br>3891610                  | 0.28               | 10                  | 103                     |
| Upper Hiwassee River     | 17S 0232386<br>3884316                | 17S 0231923<br>3883699                  | 0.75               | 10                  | 123                     |
| Middle Hiwassee River    | 17S 0229164<br>3882949                | 17S 0228783<br>3884328                  | 1.80               | 12                  | 157                     |
| Lower Hiwassee River     | 17S 0227031<br>3886247                | 16S 0773375<br>3886070                  | 0.85               | 10                  | 144                     |
| Lower Nottely River      | 16S 0762432<br>3879447                | 16S 0762732<br>3879777                  | 0.50               | 10                  | 111                     |
| Upper Valley River       | 17S 0239185<br>3897840                | 17S 0238742<br>3897789                  | 0.52               | 15                  | 179                     |
| Middle Valley River      | 17S 0233788<br>3895812                | 17S 0233047<br>3895432                  | 1.04               | 15                  | 177                     |
| Middle Valley River (2)  | 17S 0232650<br>3895802                | 17S 0232461<br>3895627                  | 0.30               | 10                  | 137                     |
| Lower Valley River       | 16S 0771735<br>3888373                | 16S 0771493<br>3887481                  | 0.91               | 17                  | 196                     |
| Total                    |                                       |   | 7.56               | 142                 | 1,815                   |

Table 12.—Particle size categories and associated continuous variables used to visually estimate dominant and subdominant surface substrate size for all radio-tagged fish relocations, visual observations, observed spawning and courting acts, and habitat availability survey points. Categories based on a modified Wentworth scale (Bovee 1986).

| Category           | Particle size (mm) | Continuous variable |
|--------------------|--------------------|---------------------|
| Bedrock            |                    | 13                  |
| Large boulder      | >1024              | 12                  |
| Medium boulder     | 508-1024           | 11                  |
| Small boulder      | 256-508            | 10                  |
| Large cobble       | 128-256            | 9                   |
| Small cobble       | 64-128             | 8                   |
| Very coarse gravel | 32-64              | 7                   |
| Coarse gravel      | 16-32              | 6                   |
| Medium gravel      | 8-16               | 5                   |
| Fine gravel        | 2-8                | 4                   |
| Sand               | 0.062-2.0          | 3                   |
| Silt               | 0.004-0.062        | 2                   |
| Clay               | <0.004             | 1                   |

Table 13.—Summarized weight, length, elapsed time, and absolute growth characteristics for recaptured sicklefin redhorse in Valley River (Hiwassee Basin), North Carolina, during 2006 and 2007.

| Season and statistic      | Time interval<br>(d) | Weight characteristics |                  |                   | Length characteristics |                   |                    | Absolute growth<br>(g/d) |
|---------------------------|----------------------|------------------------|------------------|-------------------|------------------------|-------------------|--------------------|--------------------------|
|                           |                      | Capture<br>(g)         | Recapture<br>(g) | Difference<br>(g) | Capture<br>(mm)        | Recapture<br>(mm) | Difference<br>(mm) |                          |
| <b>Both sexes</b>         |                      |                        |                  |                   |                        |                   |                    |                          |
| Annual ( <i>N</i> = 6)    |                      |                        |                  |                   |                        |                   |                    |                          |
| Mean                      | 369.7                | 1200.8                 | 1248.7           | 47.8              | 510.3                  | 515.3             | 5.0                | 0.13                     |
| SE                        | 3.00                 | 52.06                  | 57.63            | 18.06             | 12.77                  | 13.39             | 2.84               | 0.05                     |
| Min                       | 357                  | 1080                   | 1110             | 10                | 465                    | 462               | -3                 | 0.03                     |
| Max                       | 378                  | 1442                   | 1496             | 131               | 561                    | 565               | 18                 | 0.35                     |
| Spawning ( <i>N</i> = 10) |                      |                        |                  |                   |                        |                   |                    |                          |
| Mean                      | 14.2                 | 1270.2                 | 1249.7           | -20.5             | 518.5                  | 513.1             | -5.4               | -3.92                    |
| SE                        | 5.12                 | 62.44                  | 77.89            | 21.74             | 8.68                   | 9.18              | 1.70               | 4.44                     |
| Min                       | 1                    | 1018                   | 941              | -118              | 475                    | 463               | -13                | -39.33                   |
| Max                       | 55                   | 1686                   | 1704             | 82                | 560                    | 557               | 1                  | 9.11                     |
| <b>Male</b>               |                      |                        |                  |                   |                        |                   |                    |                          |
| Annual ( <i>N</i> = 2)    |                      |                        |                  |                   |                        |                   |                    |                          |
| Mean                      | 367.5                | 1115.5                 | 1135.5           | 20.0              | 503.5                  | 515.0             | 11.5               | 0.06                     |
| SE                        | 10.50                | 35.50                  | 25.50            | 10.00             | 6.50                   | 0.00              | 6.50               | 0.03                     |
| Min                       | 357                  | 1080                   | 1110             | 10                | 497                    | 515               | 5                  | 0.03                     |
| Max                       | 378                  | 1151                   | 1161             | 30                | 510                    | 515               | 18                 | 0.08                     |
| Spawning ( <i>N</i> = 2)  |                      |                        |                  |                   |                        |                   |                    |                          |
| Mean                      | 14.5                 | 1087.5                 | 1010.0           | -77.5             | 487.5                  | 479.5             | -8.0               | -8.25                    |
| SE                        | 7.50                 | 69.50                  | 50.00            | 19.50             | 12.50                  | 16.50             | 4.00               | 5.61                     |
| Min                       | 7                    | 1018                   | 960              | -97               | 475                    | 463               | -12                | -13.86                   |
| Max                       | 22                   | 1157                   | 1060             | -58               | 500                    | 496               | -4                 | -2.64                    |

Table 13.—(Continued).

| Season and statistic     | Time interval<br>(d) | Weight characteristics |                  |                   | Length characteristics |                   |                    | Absolute growth<br>(g/d) |
|--------------------------|----------------------|------------------------|------------------|-------------------|------------------------|-------------------|--------------------|--------------------------|
|                          |                      | Capture<br>(g)         | Recapture<br>(g) | Difference<br>(g) | Capture<br>(mm)        | Recapture<br>(mm) | Difference<br>(mm) |                          |
| Female                   |                      |                        |                  |                   |                        |                   |                    |                          |
| Annual ( <i>N</i> = 4)   |                      |                        |                  |                   |                        |                   |                    |                          |
| Mean                     | 370.8                | 1243.5                 | 1305.3           | 61.8              | 513.8                  | 515.5             | 1.8                | 0.17                     |
| SE                       | 1.70                 | 68.88                  | 70.66            | 24.59             | 19.73                  | 21.18             | 1.60               | 0.07                     |
| Min                      | 366                  | 1140                   | 1155             | 15                | 465                    | 462               | -3                 | 0.04                     |
| Max                      | 374                  | 1442                   | 1496             | 131               | 561                    | 565               | 4                  | 0.35                     |
| Spawning ( <i>N</i> = 8) |                      |                        |                  |                   |                        |                   |                    |                          |
| Mean                     | 14.1                 | 1315.9                 | 1309.6           | -6.3              | 526.3                  | 521.5             | -4.8               | -2.84                    |
| SE                       | 6.33                 | 67.85                  | 84.23            | 24.51             | 8.51                   | 8.68              | 1.94               | 5.45                     |
| Min                      | 1                    | 1032                   | 941              | -118              | 492                    | 490               | -13                | -39.33                   |
| Max                      | 55                   | 1686                   | 1704             | 82                | 560                    | 557               | 1                  | 9.11                     |

Table 14.—Annual and seasonal median, mean, standard error, minimum, and maximum linear ranges for radio-tagged sicklefin redhorse during 2006 and 2007 in the Hiwassee Basin, North Carolina and Georgia.

| Season       | <i>N</i> | Median linear range (km) | Mean linear range (km) | SE   | Min – max (km) |
|--------------|----------|--------------------------|------------------------|------|----------------|
| Annual       | 40       | 19.4                     | 18.8                   | 1.72 | 0.37 – 46.3    |
| Spawning     | 40       | 17.8                     | 16.8                   | 1.60 | 0.20 – 40.5    |
| Postspawning | 35       | 0.1                      | 2.5                    | 0.98 | 0.00 – 25.1    |
| Winter       | 19       | 3.4                      | 3.6                    | 0.89 | 0.06 – 13.6    |

Table 15.—Weekly annual and seasonal median, mean, standard error, minimum, and maximum distance from the confluence of Valley River and Hiwassee River for radio-tagged sicklefin redhorse during 2006 and 2007 in the Hiwassee Basin, North Carolina and Georgia. The confluence of Valley River and Hiwassee River is a central location for the upper Hiwassee Basin bounded by Mission Dam upstream and Hiwassee Dam downstream. The distance from the confluence indicates migratory behavior associated with spawning.

| Year and season | <i>N</i> | Median distance<br>from confluence<br>(km) | Mean distance<br>from confluence<br>(km) | SE   | Min – max<br>(km) |
|-----------------|----------|--|--|------|-------------------|
| 2006            |          |  |  |      |                   |
| Annual          | 244      | 4.75                                       | 8.15                                     | 0.50 | 0.00 – 30.47      |
| Spawning        | 107      | 7.66                                       | 10.85                                    | 0.95 | 0.65 – 30.47      |
| Postspawning    | 120      | 4.75                                       | 6.40                                     | 0.46 | 0.71 – 26.62      |
| Winter          | 17       | 2.64                                       | 3.51                                     | 0.72 | 0.00 – 10.92      |
| 2007            |          |  |  |      |                   |
| Annual          | 210      | 4.79                                       | 8.93                                     | 0.49 | 0.12 – 26.24      |
| Spawning        | 174      | 4.80                                       | 9.34                                     | 0.54 | 0.12 – 26.24      |
| Postspawning    | 34       | 4.76                                       | 9.93                                     | 1.20 | 2.65 – 22.55      |
| Winter          | 2        | 4.48                                       | 4.48                                     | 0.00 | 4.48 – 4.48       |
| Total           |          |  |  |      |                   |
| Annual          | 454      | 4.75                                       | 8.72                                     | 0.35 | 0.00 – 30.47      |
| Spawning        | 281      | 4.84                                       | 9.92                                     | 0.49 | 0.12 – 30.47      |
| Postspawning    | 154      | 4.75                                       | 7.18                                     | 0.46 | 0.71 – 26.62      |
| Winter          | 19       | 2.66                                       | 3.62                                     | 0.64 | 0.00 – 10.92      |

Table 16.—Weekly annual and seasonal median, mean, standard error, minimum, and maximum directional (i.e., upstream or downstream) linear ranges for radio-tagged sicklefin redhorse during 2006 and 2007 in the Hiwassee Basin, North Carolina and Georgia.

| Season and direction | <i>N</i> | Median distance (km) | Mean distance (km) | SE   | Min – Max (km) |
|----------------------|----------|----------------------|--------------------|------|----------------|
| Annual               |          |                      |                    |      |                |
| Upstream             | 189      | 0.09                 | 1.47               | 0.24 | 0 – 21.13      |
| Downstream           | 197      | 0.09                 | 2.00               | 0.35 | 0 – 30.47      |
| Spawning             |          |                      |                    |      |                |
| Upstream             | 76       | 0.31                 | 2.12               | 0.39 | 0 – 16.30      |
| Downstream           | 75       | 0.15                 | 2.75               | 0.71 | 0 – 30.74      |
| Postspawning         |          |                      |                    |      |                |
| Upstream             | 107      | 0.04                 | 1.00               | 0.31 | 0 – 21.13      |
| Downstream           | 108      | 0.06                 | 1.20               | 0.35 | 0 – 21.80      |

Table 17.—Comparison of utilized dominant substrate particle size for all radio-tagged and visually observed sicklefin redhorse during 2006 and 2007 within the Hiwassee Basin to available dominant substrate particle size relative to the occurrence (i.e., presence or absence) of river weed. Categorical variable river weed occurrence was compared using a likelihood ratio chi-square test. Values missing are a result of river weed never being associated with substratum clay and medium gravel concurrently with utilization by a radio-tagged or visually observed sicklefin redhorse. Utilized substrate corresponding to oviposition locations were omitted from analyses because sicklefin redhorse appear to select against river weed when selecting an oviposition location and this phenomenon would likely have biased results relative to the use of very coarse gravel, small cobble, and large cobble (i.e., suitable spawning substrates) annually.

| Substrate category | <i>N</i> |         |           |         | River weed present (%) |           | $\chi^2$ | <i>P</i> |
|--------------------|----------|---------|-----------|---------|------------------------|-----------|----------|----------|
|                    | Use      |         | Available |         | Use                    | Available |          |          |
|                    | Absent   | Present | Absent    | Present |                        |           |          |          |
| Clay               | 0        | 0       | 8         | 0       |                        | 0.0       |          |          |
| Silt               | 5        | 0       | 257       | 11      | 0.0                    | 4.1       | 0.415    | 0.5194   |
| Sand               | 21       | 1       | 188       | 21      | 4.5                    | 10.0      | 0.836    | 0.3606   |
| Fine gravel        | 13       | 1       | 90        | 6       | 7.1                    | 6.3       | 0.016    | 0.8998   |
| Medium gravel      | 0        | 0       | 55        | 16      |                        | 22.5      |          |          |
| Coarse gravel      | 19       | 4       | 62        | 23      | 17.4                   | 27.1      | 0.957    | 0.3278   |
| Very coarse gravel | 11       | 3       | 53        | 25      | 21.4                   | 32.1      | 0.669    | 0.4136   |
| Small cobble       | 36       | 38      | 99        | 54      | 51.4                   | 35.3      | 5.292    | 0.0214   |
| Large cobble       | 27       | 25      | 74        | 29      | 48.1                   | 28.2      | 8.329    | 0.0039   |
| Small boulder      | 10       | 9       | 71        | 46      | 47.4                   | 39.3      | 0.435    | 0.5097   |
| Medium boulder     | 17       | 22      | 45        | 17      | 56.4                   | 27.4      | 8.473    | 0.0036   |
| Large boulder      | 4        | 28      | 86        | 58      | 87.5                   | 40.3      | 25.637   | <0.0001  |
| Bedrock            | 24       | 314     | 192       | 229     | 92.9                   | 54.4      | 153.010  | <0.0001  |

Table 18.—Annual and seasonal summary of weekly relocation, visual observation, and observed spawning microhabitat data for sicklefin redhorse and results of statistical comparisons between utilized and available microhabitat. The Komogorov-Smirnov two-sample test was applied to continuous variables, and categorical variables were compared using a likelihood-ratio chi-square test. Oviposition site microhabitat data from Beaverdam Creek ( $N = 1$ ) was excluded because microhabitat availability data was not collected from this stream.

| Season and variable      | <i>N</i> |           | Mean  |           | SE   |           | Statistic          | <i>P</i> |
|--------------------------|----------|-----------|-------|-----------|------|-----------|--------------------|----------|
|                          | Use      | Available | Use   | Available | Use  | Available |                    |          |
| <b>Annual</b>            |          |           |       |           |      |           |                    |          |
| Distance to bank (m)     | 674      | 1815      | 9.94  | 5.81      | 0.32 | 0.14      | $D = 0.273$        | <0.0001  |
| Depth (m)                | 674      | 1815      | 0.49  | 0.37      | 0.01 | 0.01      | $D = 0.289$        | <0.0001  |
| Bottom velocity (m/s)    | 674      | 1815      | 0.16  | 0.10      | 0.01 | 0.00      | $D = 0.226$        | <0.0001  |
| Mean velocity (m/s)      | 674      | 1815      | 0.44  | 0.21      | 0.01 | 0.01      | $D = 0.451$        | <0.0001  |
| Dominate substrate       | 674      | 1815      | 7.00  | 7.00      | 0.05 | 0.04      | $D = 0.321$        | <0.0001  |
| River weed (%)           | 674      | 1815      | 66.00 | 29.00     | 0.02 | 0.01      | $\chi^2 = 276.593$ | <0.0001  |
| Cover                    | 674      | 1815      | 1.00  | 1.00      | 0.14 | 0.08      | $\chi^2 = 91.984$  | <0.0001  |
| Distance to cover (m)    | 259      | 968       | 0.97  | 1.02      | 0.04 | 0.02      | $D = 0.080$        | 0.1498   |
| <b>Observed spawning</b> |          |           |       |           |      |           |                    |          |
| Distance to bank (m)     | 42       | 1084      | 2.85  | 3.73      | 0.25 | 0.09      | $D = 0.257$        | 0.0094   |
| Depth (m)                | 42       | 1084      | 0.28  | 0.32      | 0.01 | 0.01      | $D = 0.298$        | 0.0015   |
| Bottom velocity (m/s)    | 42       | 1084      | 0.21  | 0.10      | 0.02 | 0.01      | $D = 0.438$        | <0.0001  |
| Mean velocity (m/s)      | 42       | 1084      | 0.52  | 0.19      | 0.02 | 0.01      | $D = 0.734$        | <0.0001  |
| Dominate substrate       | 42       | 1084      | 8.00  | 13.00     | 0.11 | 0.12      | $D = 0.377$        | <0.0001  |
| River weed (%)           | 42       | 1084      | 0.00  | 21.00     | 0.00 | 0.01      | $\chi^2 = 19.511$  | <0.0001  |
| Cover                    | 42       | 1084      | 9.00  | 1.00      | 0.47 | 0.11      | $\chi^2 = 43.374$  | <0.0001  |
| Distance to cover (m)    | 39       | 592       | 0.36  | 1.01      | 0.07 | 0.03      | $D = 0.561$        | <0.0001  |
| <b>Spawning season</b>   |          |           |       |           |      |           |                    |          |
| Distance to bank (m)     | 350      | 1815      | 7.52  | 5.81      | 0.38 | 0.14      | $D = 0.264$        | <0.0001  |
| Depth (m)                | 350      | 1815      | 0.53  | 0.37      | 0.01 | 0.01      | $D = 0.363$        | <0.0001  |
| Bottom velocity (m/s)    | 350      | 1815      | 0.17  | 0.10      | 0.01 | 0.00      | $D = 0.222$        | <0.0001  |
| Mean velocity (m/s)      | 350      | 1815      | 0.44  | 0.21      | 0.01 | 0.01      | $D = 0.438$        | <0.0001  |
| Dominate substrate       | 350      | 1815      | 7.00  | 7.00      | 0.07 | 0.04      | $D = 0.259$        | <0.0001  |
| River weed (%)           | 350      | 1815      | 56.00 | 29.00     | 0.03 | 0.01      | $\chi^2 = 85.792$  | <0.0001  |
| Cover                    | 350      | 1815      | 1.00  | 1.00      | 0.18 | 0.08      | $\chi^2 = 48.252$  | <0.0001  |
| Distance to cover (m)    | 134      | 968       | 1.15  | 1.02      | 0.05 | 0.02      | $D = 0.199$        | 0.0002   |
| <b>Postspawning</b>      |          |           |       |           |      |           |                    |          |
| Distance to bank (m)     | 262      | 1704      | 13.47 | 5.83      | 0.48 | 0.15      | $D = 0.480$        | <0.0001  |
| Depth (m)                | 262      | 1704      | 0.45  | 0.36      | 0.01 | 0.01      | $D = 0.273$        | <0.0001  |
| Bottom velocity (m/s)    | 262      | 1704      | 0.14  | 0.10      | 0.01 | 0.00      | $D = 0.209$        | <0.0001  |
| Mean velocity (m/s)      | 262      | 1704      | 0.42  | 0.21      | 0.01 | 0.01      | $D = 0.461$        | <0.0001  |
| Dominate substrate       | 262      | 1704      | 7.00  | 7.00      | 0.05 | 0.04      | $D = 0.508$        | <0.0001  |
| River weed (%)           | 262      | 1704      | 92.00 | 30.00     | 0.02 | 0.01      | $\chi^2 = 385.900$ | <0.0001  |
| Cover                    | 262      | 1704      | 1.00  | 1.00      | 0.22 | 0.09      | $\chi^2 = 85.534$  | <0.0001  |
| Distance to cover (m)    | 84       | 900       | 0.97  | 1.06      | 0.07 | 0.02      | $D = 0.079$        | 0.7300   |

Table 18.—(Continued).

| Season and variable   | <i>N</i> |           | Mean  |           | SE   |           | Statistic         | <i>P</i> |
|-----------------------|----------|-----------|-------|-----------|------|-----------|-------------------|----------|
|                       | Use      | Available | Use   | Available | Use  | Available |                   |          |
| Winter                |          |           |       |           |      |           |                   |          |
| Distance to bank (m)  | 20       | 620       | 21.15 | 9.51      | 3.41 | 0.33      | $D = 0.411$       | 0.0028   |
| Depth (m)             | 20       | 620       | 0.75  | 0.44      | 0.07 | 0.01      | $D = 0.465$       | 0.0005   |
| Bottom velocity (m/s) | 20       | 620       | 0.21  | 0.10      | 0.03 | 0.01      | $D = 0.421$       | 0.0021   |
| Mean velocity (m/s)   | 20       | 620       | 0.51  | 0.25      | 0.05 | 0.01      | $D = 0.561$       | <0.0001  |
| Dominate substrate    | 20       | 620       | 7.00  | 7.00      | 0.30 | 0.07      | $D = 0.377$       | 0.0083   |
| River weed (%)        | 20       | 620       | 55.00 | 46.00     | 0.11 | 0.02      | $\chi^2 = 0.634$  | 0.4260   |
| Cover                 | 20       | 620       | 1.00  | 1.00      | 0.55 | 0.13      | $\chi^2 = 17.888$ | 0.0125   |
| Distance to cover (m) | 2        | 309       | 0.75  | 1.16      | 0.25 | 0.04      | $D = 0.314$       | 0.9896   |

Table 19.—Microhabitat use for adult sicklefin redhorse oviposition sites and undisturbed microhabitat immediately upstream of oviposition sites (upstream flat). Microhabitat variables between the two locations were compared using the Student's t-test.

| Variable              | Microhabitat locality |                  | Student's <i>t</i> | <i>P</i> |
|-----------------------|-----------------------|------------------|--------------------|----------|
|                       | Upstream flat         | Oviposition site |                    |          |
| Distance to bank (m)  |                       |                  |                    |          |
| <i>N</i>              | 43                    | 43               |                    |          |
| Mean                  | 2.90                  | 2.90             |                    |          |
| SE                    | 0.25                  | 0.24             |                    |          |
| Min - max             | 0.8 – 8.0             | 0.8 – 8.0        |                    |          |
| Depth (m)             |                       |                  |                    |          |
| <i>N</i>              | 43                    | 43               | -1.587             | 0.1163   |
| Mean                  | 0.25                  | 0.28             |                    |          |
| SE                    | 0.01                  | 0.01             |                    |          |
| Min - max             | 0.12 – 0.46           | 0.15 – 0.46      |                    |          |
| Bottom velocity (m/s) |                       |                  |                    |          |
| <i>N</i>              | 43                    | 43               | 1.863              | 0.0662   |
| Mean                  | 0.26                  | 0.20             |                    |          |
| SE                    | 0.03                  | 0.02             |                    |          |
| Min - max             | 0.00 – 0.53           | 0.02 – 0.55      |                    |          |
| Mean velocity (m/s)   |                       |                  |                    |          |
| <i>N</i>              | 43                    | 43               | -0.062             | 0.9510   |
| Mean                  | 0.50                  | 0.51             |                    |          |
| SE                    | 0.02                  | 0.02             |                    |          |
| Min - max             | 0.12 – 1.01           | 0.16 – 0.90      |                    |          |
| Dominant substrate    |                       |                  |                    |          |
| <i>N</i>              | 43                    | 43               | 0.532              | 0.5970   |
| Mode                  | SC                    | SC               |                    |          |
| SE                    | 0.12                  | 0.13             |                    |          |
| Min - max             | MG - SB               | MG - SB          |                    |          |
| Cover                 |                       |                  |                    |          |
| <i>N</i>              | 43                    | 43               |                    |          |
| Mode                  | Boulder               | Boulder          |                    |          |
| Distance to cover (m) |                       |                  |                    |          |
| <i>N</i>              | 40                    | 40               |                    |          |
| Mean                  | 0.39                  | 0.36             |                    |          |
| SE                    | 0.07                  | 0.07             |                    |          |
| Min - max             | 0.10 – 2.00           | 0.10 – 2.00      |                    |          |

Table 20.—Functional comparison statistics for seasonal and variable within-season microhabitat use in Hiwassee Basin, North Carolina and Georgia. The Komogorov-Smirnov two-sample test was conducted on continuous variables, and categorical variables were compared using a likelihood-ratio chi-square test.

| Variable   | Statistic          | P       |
|--|--------------------|---------|
| <b>Spawning season microhabitat use versus spawning observation microhabitat use</b> |                    |         |
| Distance to bank (m)   | $D = 0.522$        | <0.0001 |
| Depth (m)  | $D = 0.514$        | <0.0001 |
| Bottom velocity (m/s)  | $D = 0.220$        | 0.0530  |
| Mean velocity (m/s)  | $D = 0.292$        | 0.0033  |
| Dominate substrate   | $D = 0.503$        | <0.0001 |
| Cover  | $\chi^2 = 63.851$  | <0.0001 |
| Distance to cover (m)  | $D = 0.685$        | <0.0001 |
| River weed occurrence (%)  | $\chi^2 = 62.796$  | <0.0001 |
| <b>Spawning microhabitat use versus postspawning microhabitat use</b>                |                    |         |
| Distance to bank (m)   | $D = 0.425$        | <0.0001 |
| Depth (m)  | $D = 0.130$        | 0.0127  |
| Bottom velocity (m/s)  | $D = 0.085$        | 0.2346  |
| Mean velocity (m/s)  | $D = 0.081$        | 0.2731  |
| Dominate substrate   | $D = 0.404$        | <0.0001 |
| Cover  | $\chi^2 = 32.977$  | <0.0001 |
| Distance to cover (m)  | $D = 0.171$        | 0.0991  |
| River weed occurrence (%)  | $\chi^2 = 107.474$ | <0.0001 |
| <b>Spawning microhabitat use versus winter microhabitat use</b>                      |                    |         |
| Distance to bank (m)   | $D = 0.610$        | <0.0001 |
| Depth (m)  | $D = 0.390$        | 0.0063  |
| Bottom velocity (m/s)  | $D = 0.207$        | 0.3914  |
| Mean velocity (m/s)  | $D = 0.271$        | 0.1231  |
| Dominate substrate   | $D = 0.326$        | 0.0361  |
| Cover  | $\chi^2 = 9.602$   | 0.1424  |
| Distance to cover (m)  | $D = 0.313$        | 0.9903  |
| River weed occurrence (%)  | $\chi^2 = 0.004$   | 0.9502  |
| <b>Postspawning microhabitat use versus winter microhabitat use</b>                  |                    |         |
| Distance to bank (m)   | $D = 0.282$        | 0.1040  |
| Depth (m)  | $D = 0.503$        | 0.0002  |
| Bottom velocity (m/s)  | $D = 0.254$        | 0.1824  |
| Mean velocity (m/s)  | $D = 0.345$        | 0.0240  |
| Dominate substrate   | $D = 0.197$        | 0.4669  |
| Cover  | $\chi^2 = 5.487$   | 0.2409  |
| Distance to cover (m)  | $D = 0.214$        | 1.0000  |
| River weed occurrence (%)  | $\chi^2 = 17.336$  | <0.0001 |
| <b>Spawning microhabitat use versus spawning (cold front) microhabitat use</b>       |                    |         |
| Distance to bank (m)   | $D = 0.070$        | 0.9365  |
| Depth (m)  | $D = 0.342$        | <0.0001 |
| Bottom velocity (m/s)  | $D = 0.244$        | 0.0019  |
| Mean velocity (m/s)  | $D = 0.172$        | 0.0639  |
| Dominate substrate   | $D = 0.289$        | 0.0001  |
| Cover  | $\chi^2 = 8.247$   | 0.2206  |
| Distance to cover (m)  | $D = 0.167$        | 0.4908  |
| River weed occurrence (%)  | $\chi^2 = 7.229$   | 0.0072  |

Table 21.—Annual, seasonal, and oviposition site statistics and significance values from comparisons of microhabitat use and availability principal component1 and component 2 scores. The Komogorov-Smirnov two-sample test was used to compare component scores.

| Season and principal component | <i>D</i> statistic | <i>P</i> -value |
|--------------------------------|--------------------|-----------------|
| Annual                         |                    |                 |
| PC 1                           | 0.4421             | <0.0001         |
| PC 2                           | 0.0849             | 0.0017          |
| Spawning season                |                    |                 |
| PC 1                           | 0.4055             | <0.0001         |
| PC 2                           | 0.0722             | 0.0939          |
| Postspawning                   |                    |                 |
| PC 1                           | 0.5237             | <0.0001         |
| PC 2                           | 0.1825             | <0.0001         |
| Winter                         |                    |                 |
| PC 1                           | 0.6645             | <0.0001         |
| PC 2                           | 0.2548             | 0.1614          |
| Oviposition site               |                    |                 |
| PC 1                           | 0.3191             | 0.0022          |
| PC 2                           | 0.8193             | <0.0001         |

Table 22.—Annual, seasonal, and oviposition site comparison statistics and significance values from comparisons of microhabitat use principal component1 and component 2 scores. The Komogorov-Smirnov two-sample test was used to compare component scores.

| Season and principal component          | <i>D</i> statistic | <i>P</i> -value |
|---|--------------------|-----------------|
| Spawning season versus postspawning     |                    |                 |
| PC 1                                    | 0.1793             | 0.0001          |
| PC 2                                    | 0.1753             | 0.0002          |
| Spawning season versus winter           |                    |                 |
| PC 1                                    | 0.3743             | 0.0100          |
| PC 2                                    | 0.2657             | 0.1383          |
| Spawning season versus oviposition site |                    |                 |
| PC 1                                    | 0.3929             | <0.0001         |
| PC 2                                    | 0.7538             | <0.0001         |
| Postspawning versus winter              |                    |                 |
| PC 1                                    | 0.2698             | 0.1335          |
| PC 2                                    | 0.1855             | 0.5448          |
| Postspawning versus oviposition site    |                    |                 |
| PC 1                                    | 0.5210             | <0.0001         |
| PC 2                                    | 0.8212             | <0.0001         |
| Winter versus oviposition site          |                    |                 |
| PC 1                                    | 0.6722             | <0.0001         |
| PC 2                                    | 0.8167             | <0.0001         |

Table 23.—Annual principal component eigenvector values (i.e., loadings), eigenvalues, and cumulative variance explained of microhabitat use and availability for utilized sicklefin redhorse streams and rivers in Hiwassee Basin, North Carolina and Georgia.

| Variable and statistic            | PCA axis |       |
|-----------------------------------|----------|-------|
|                                   | 1        | 2     |
| Distance to bank (m)              | 0.41     | 0.38  |
| Depth (m)                         | 0.21     | 0.68  |
| Bottom velocity (m/s)             | 0.49     | -0.47 |
| Mean velocity (m/s)               | 0.58     | -0.26 |
| Dominate substrate                | 0.35     | -0.07 |
| Distance to cover (m)             | 0.31     | 0.33  |
| Eigenvalue                        | 2.26     | 1.32  |
| Cumulative variance explained (%) | 37.7     | 59.8  |

Table 24.—Spawning season principal component eigenvector values (i.e., loadings), eigenvalues, and cumulative variance explained of microhabitat use and availability for utilized sicklefin redhorse spawning tributaries in Hiwassee Basin, North Carolina and Georgia.

| Variable and statistic            | PCA axis |       |
|-----------------------------------|----------|-------|
|                                   | 1        | 2     |
| Distance to bank (m)              | 0.41     | 0.38  |
| Depth (m)                         | 0.21     | 0.68  |
| Bottom velocity (m/s)             | 0.49     | -0.47 |
| Mean velocity (m/s)               | 0.58     | -0.26 |
| Dominate substrate                | 0.35     | -0.07 |
| Distance to cover (m)             | 0.31     | 0.33  |
| Eigenvalue                        | 2.26     | 1.32  |
| Cumulative variance explained (%) | 37.7     | 59.8  |

Table 25.—Postspawning season principal component eigenvector values (i.e., loadings), eigenvalues, and cumulative variance explained of microhabitat use and availability for utilized sicklefin redhorse streams and rivers in Hiwassee Basin, North Carolina and Georgia.

| Variable and statistic            | PCA axis |       |
|-----------------------------------|----------|-------|
|                                   | 1        | 2     |
| Distance to bank (m)              | 0.42     | 0.35  |
| Depth (m)                         | 0.24     | 0.66  |
| Bottom velocity (m/s)             | 0.48     | -0.48 |
| Mean velocity (m/s)               | 0.57     | -0.28 |
| Dominate substrate                | 0.35     | -0.06 |
| Distance to cover (m)             | 0.30     | 0.35  |
| Eigenvalue                        | 2.26     | 1.32  |
| Cumulative variance explained (%) | 37.6     | 59.7  |

Table 26.—Winter season principal component eigenvector values (i.e., loadings), eigenvalues, and cumulative variance explained of microhabitat use and availability for utilized sicklefin redhorse streams and rivers in Hiwassee Basin, North Carolina and Georgia.

| Variable and statistic            | PCA axis |       |
|-----------------------------------|----------|-------|
|                                   | 1        | 2     |
| Distance to bank (m)              | 0.42     | 0.25  |
| Depth (m)                         | 0.27     | 0.65  |
| Bottom velocity (m/s)             | 0.42     | -0.57 |
| Mean velocity (m/s)               | 0.55     | -0.32 |
| Dominate substrate                | 0.36     | 0.09  |
| Distance to cover (m)             | 0.36     | 0.27  |
| Eigenvalue                        | 2.35     | 1.23  |
| Cumulative variance explained (%) | 39.1     | 59.7  |

Table 27.—Observed spawning (i.e. quivering) principal component eigenvector values (i.e., loadings), eigenvalues, and cumulative variance explained of microhabitat use and availability for utilized sicklefin redhorse streams and rivers in Hiwassee Basin, North Carolina and Georgia.

| Variable and statistic            | PCA axis |       |
|-----------------------------------|----------|-------|
|                                   | 1        | 2     |
| Distance to bank (m)              | 0.47     | 0.29  |
| Depth (m)                         | 0.30     | 0.64  |
| Bottom velocity (m/s)             | 0.48     | -0.47 |
| Mean velocity (m/s)               | 0.54     | -0.33 |
| Dominant substrate                | 0.34     | -0.03 |
| Distance to cover (m)             | 0.21     | 0.43  |
| Eigenvalue                        | 2.42     | 1.24  |
| Cumulative variance explained (%) | 40.3     | 61.1  |

Table 28.—Summary index and statistics characterizing sicklefin redhorse spawning (i.e., quivering) subsurface substrate composition for observed spawning acts in Hiwassee Basin, North Carolina and Georgia.

| Statistic or index               | Mean  | SE    | Min – max    |
|----------------------------------|-------|-------|--------------|
| Fredle index                     | 28.2  | 1.59  | 14.2 – 55.1  |
| Geometric mean diameter (mm)     | 54.0  | 2.97  | 30.5 – 107.7 |
| Median diameter (D50,mm)         | 78.7  | 6.32  | 39.0 – 230.5 |
| First quartile diameter (D25,mm) | 34.8  | 2.80  | 15.8 – 99.   |
| Third quartile diameter (D75,mm) | 128.2 | 10.35 | 66.2 – 377.9 |
| Total fines (%)                  | 3.0   | 0.38  | 0.4 – 9.7    |
| Organic fines (%)                | 2.4   | 0.16  | 1.4 – 5.7    |

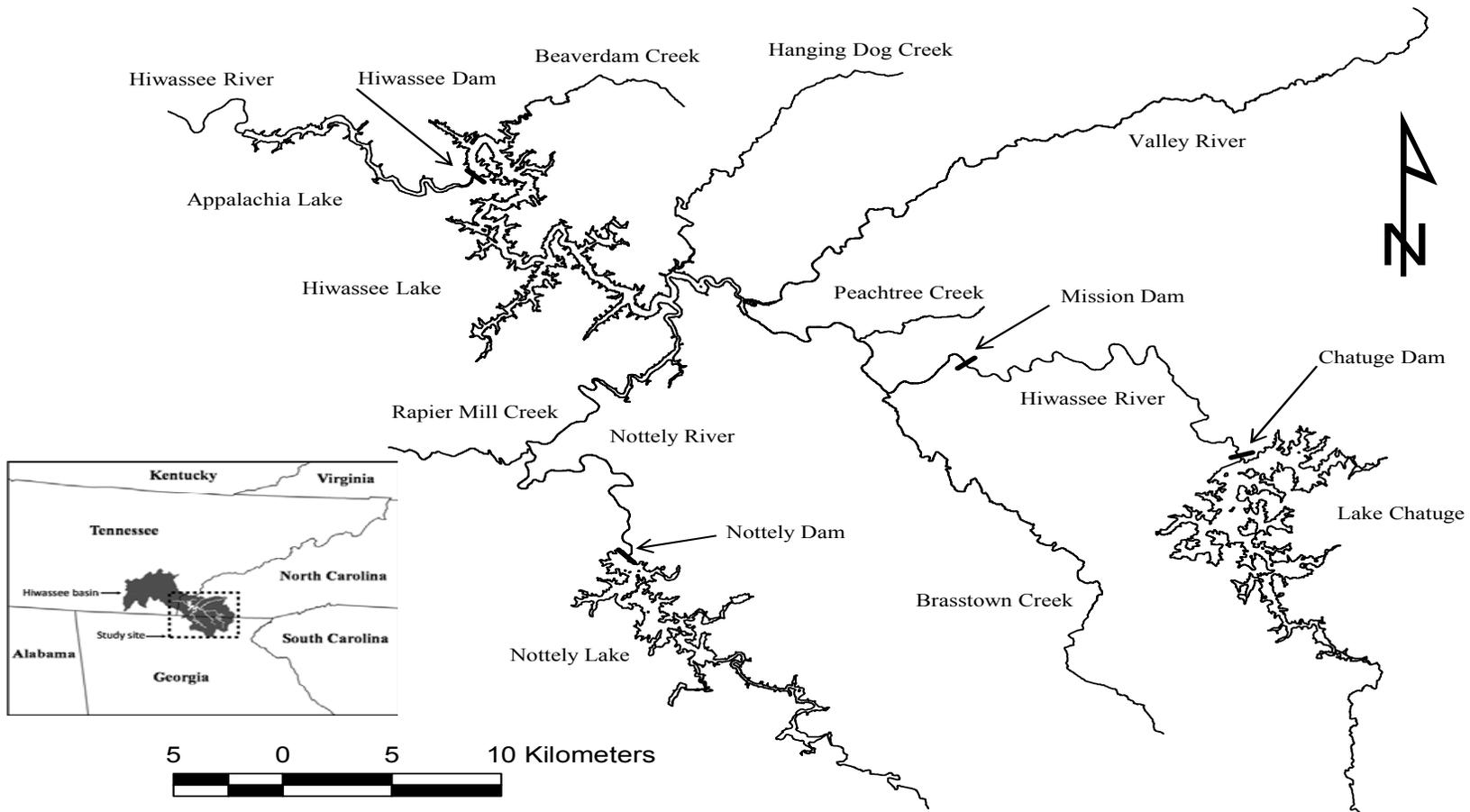


Figure 1.—Map of upper Hiwassee Basin with the study area bounded downstream by Hiwassee Dam and upstream by Mission and Nottely dams.

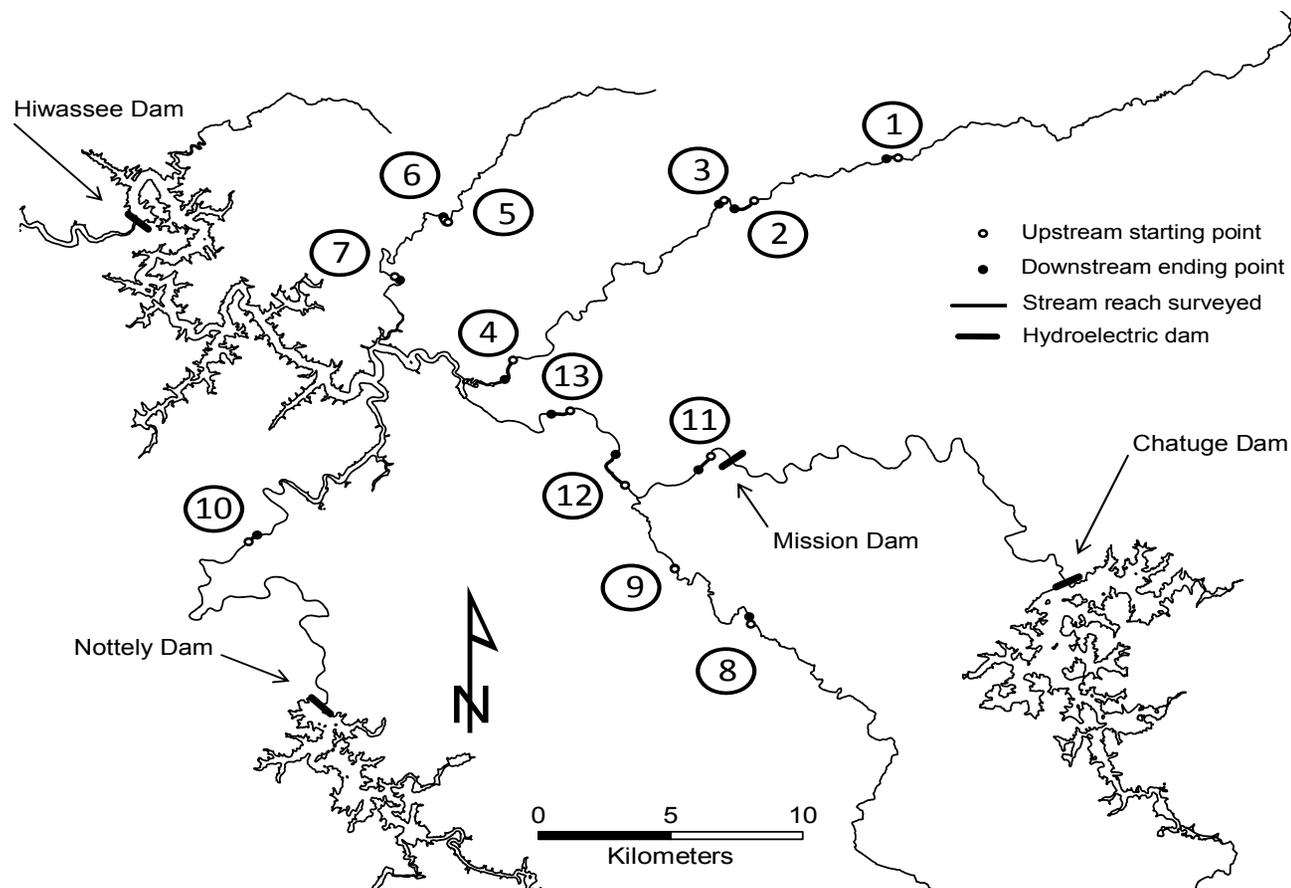


Figure 2.—Map of upper Hiwassee Basin and numbered stream reaches surveyed to quantify habitat availability. Numbered sites are (1) Upper Valley River; (2) Middle Valley River; (3) Middle Valley River; (4) Lower Valley River; (5) Upper Hanging Dog Creek; (6) Middle Hanging Dog Creek; (7) Lower Hanging Dog Creek; (8) Middle Brasstown Creek; (9) Lower Brasstown Creek; (10) Middle Nottely River; (11) Upper Hiwassee River; (12) Middle Hiwassee River; and (13) Lower Hiwassee River.

(a) Two-way resistance board weir used in 2006



(b) Two-way resistance board weir used in 2007



Figure 3.—Two modifications of two-way resistance board weirs used to sample migrating adult sicklefin redhorse during the spawning season in 2006 and 2007.

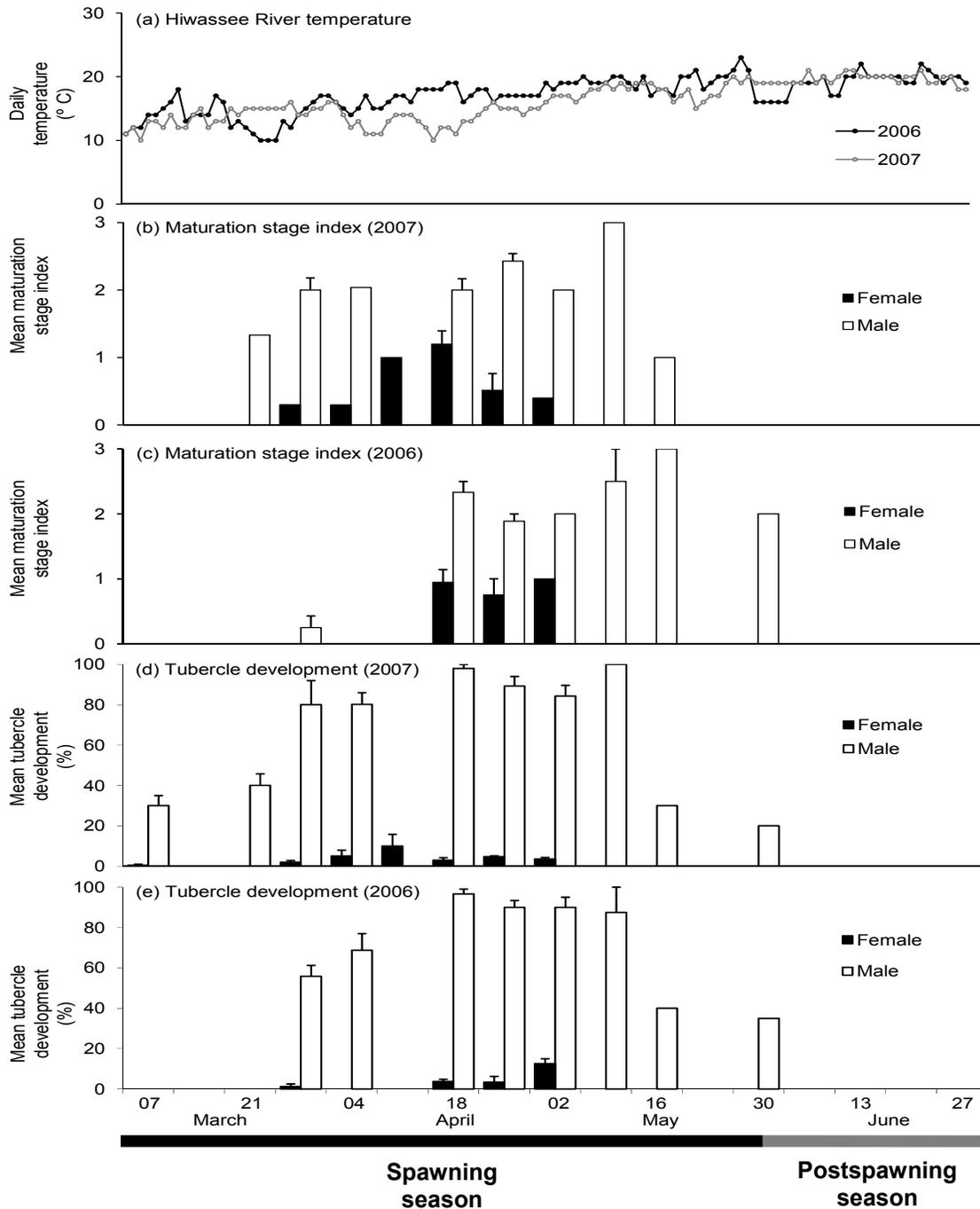


Figure 4.—Mean weekly percent tubercle development and maturation stage index (i.e., not ripe = 0; hard squeeze ripe = 1; slight squeeze ripe = 2; and running ripe = 3) for sicklefin redhorse captured in 2006 and 2007. Associated environmental variable Hiwassee River water temperature (a) is provided for comparison because adult sicklefin redhorse overwinter in lower Hiwassee River.

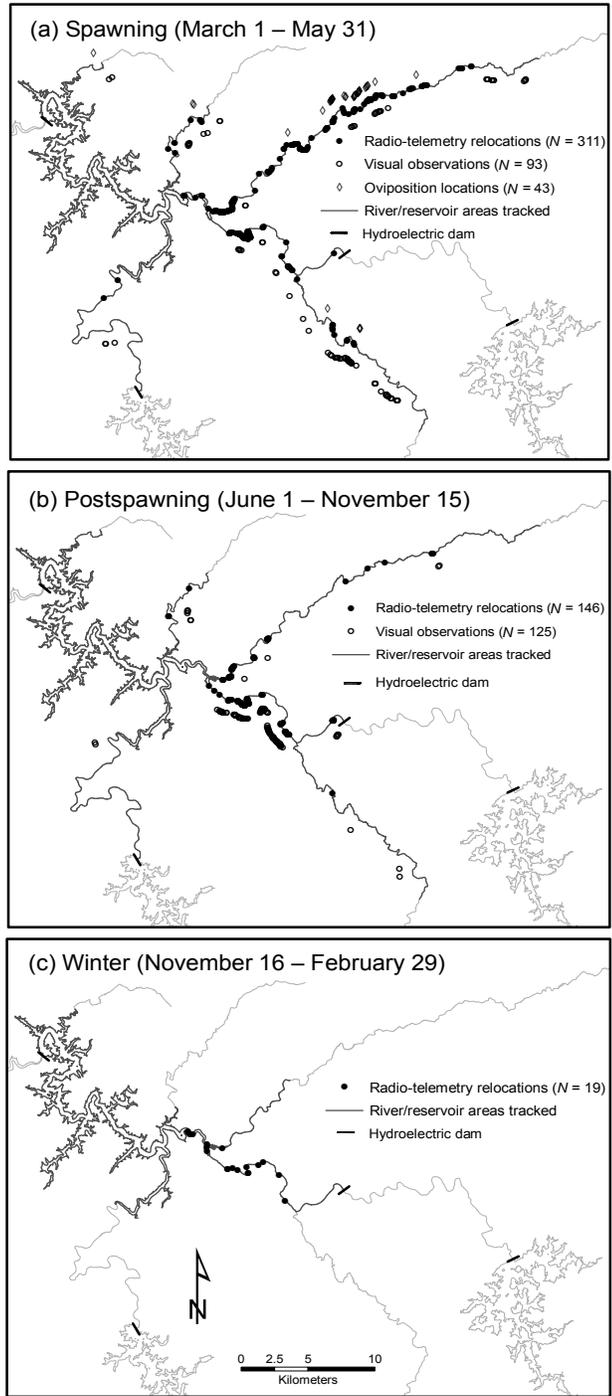


Figure 5.—Seasonal maps characterizing sicklefin redhorse relocations, visual observations, and courting/spawning visual observations for Hiwassee Basin, North Carolina and Georgia. Stream, river, and reservoir reaches tracked are denoted with a dark line.

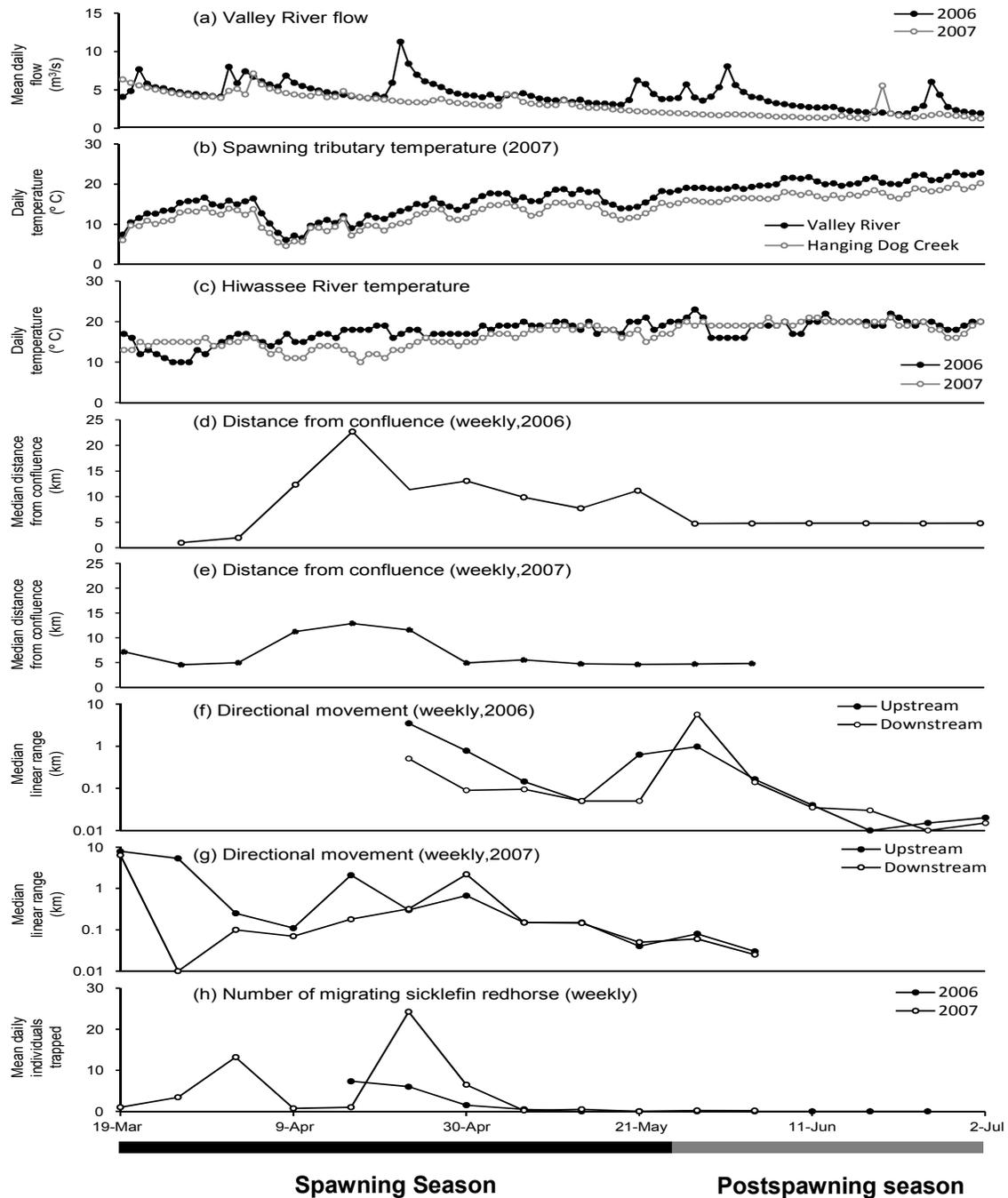


Figure 6.—Spawning migration median distance from confluence (d and e), spawning migration median directional movement (f and g), and mean daily trapped migratory sicklefin redhorse (h) per week. Associated environmental variables flow (a) and water temperature (b and c) are provided for comparison. Upstream migrations occurring late into the spawning season are a result of fish returning to and migrating up Hiwassee River.

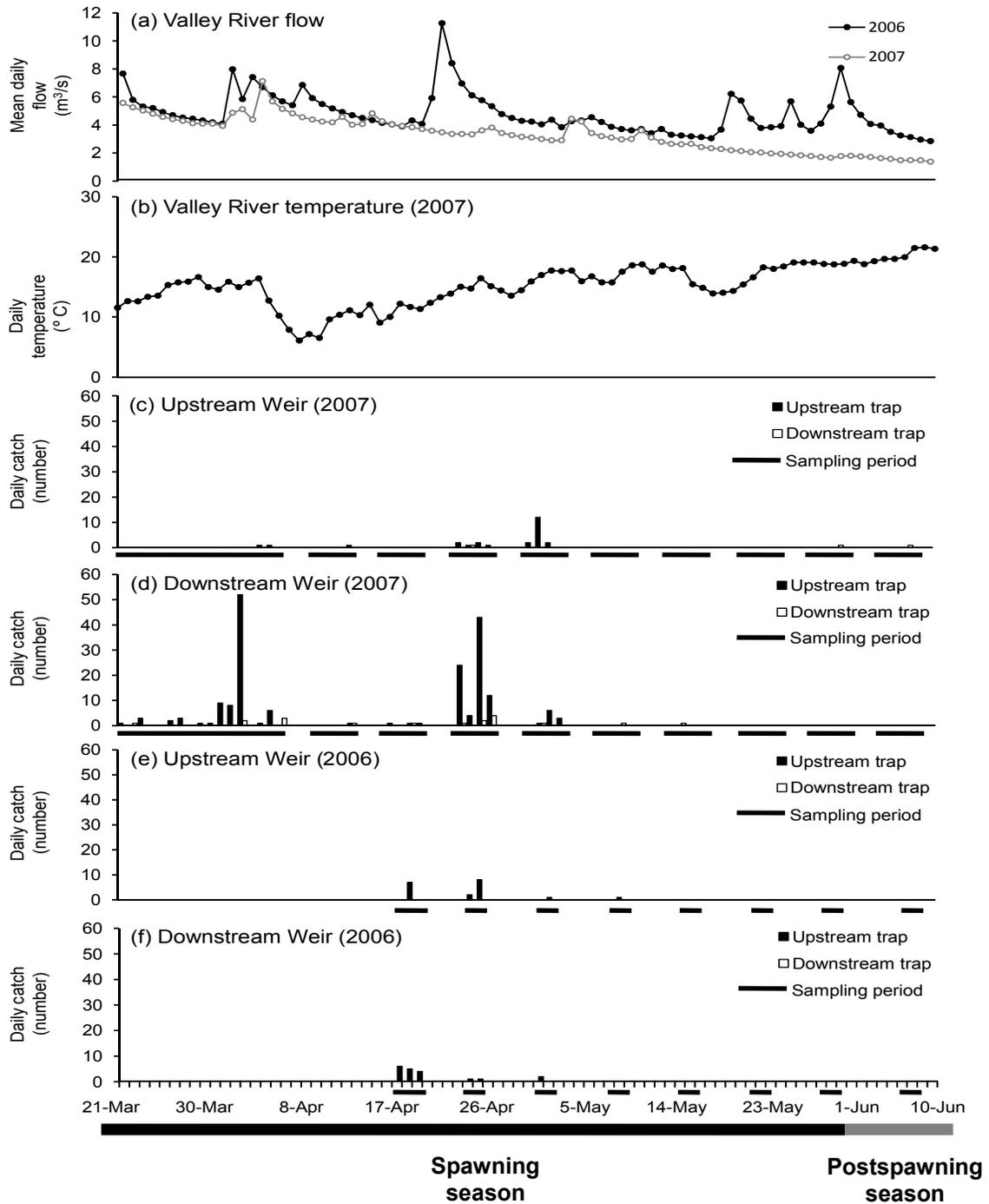


Figure 7.—Spawning season two-way resistance board weir daily directional catch for sicklefin redhorse in 2006 and 2007 (e-h). Associated environmental variables flow (a) and water temperature (b) are provided for comparison. Sampling periods are denoted by darkened x-axis segments.

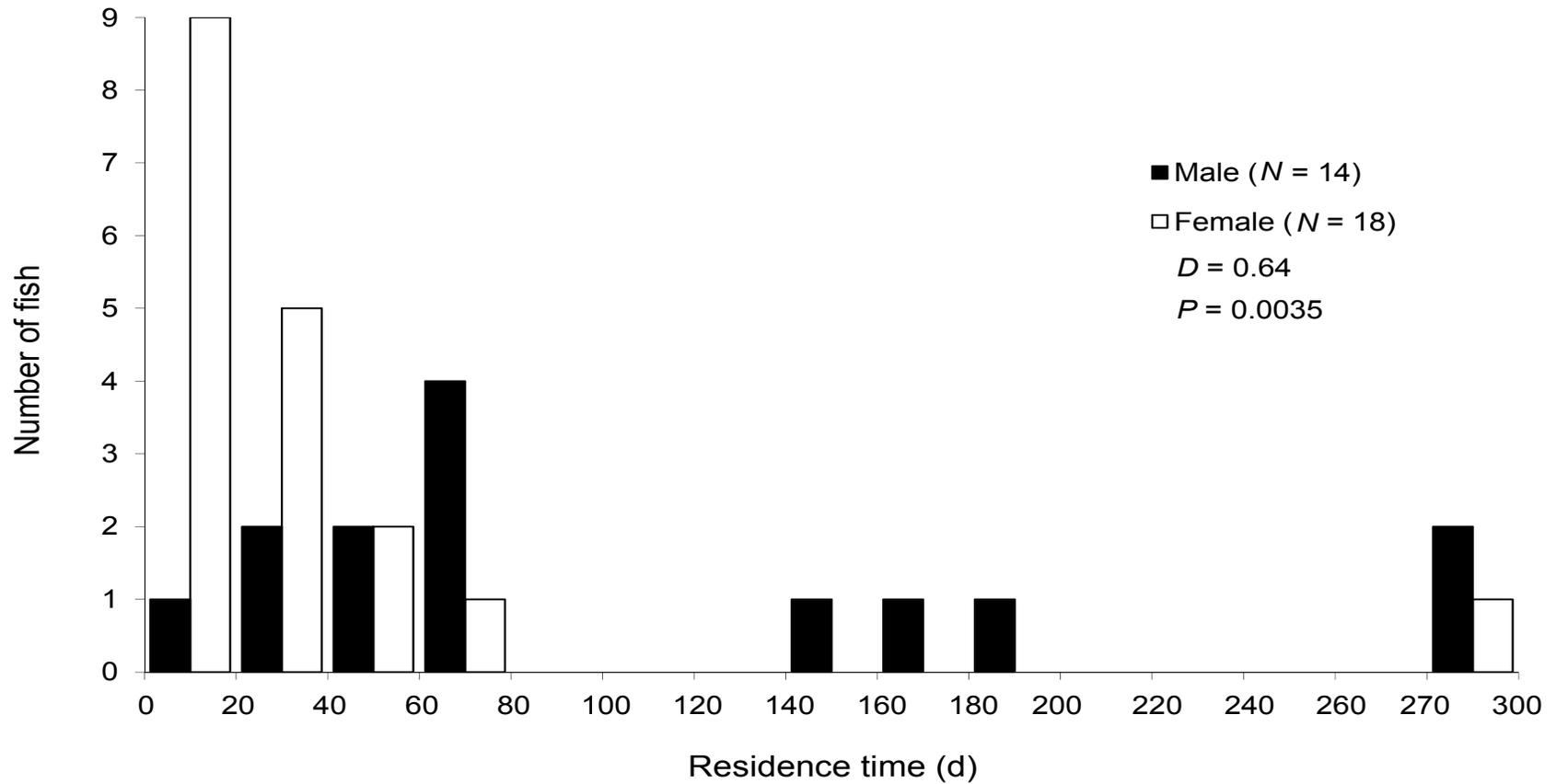


Figure 8.—Hiwassee Basin spawning tributary residence time (days) for male and female radio-tagged sicklefin redhorse that made a spawning migration in 2006 or 2007. Male and female residence times were compared using a Kolmogorov-Smirnov two-sample test.

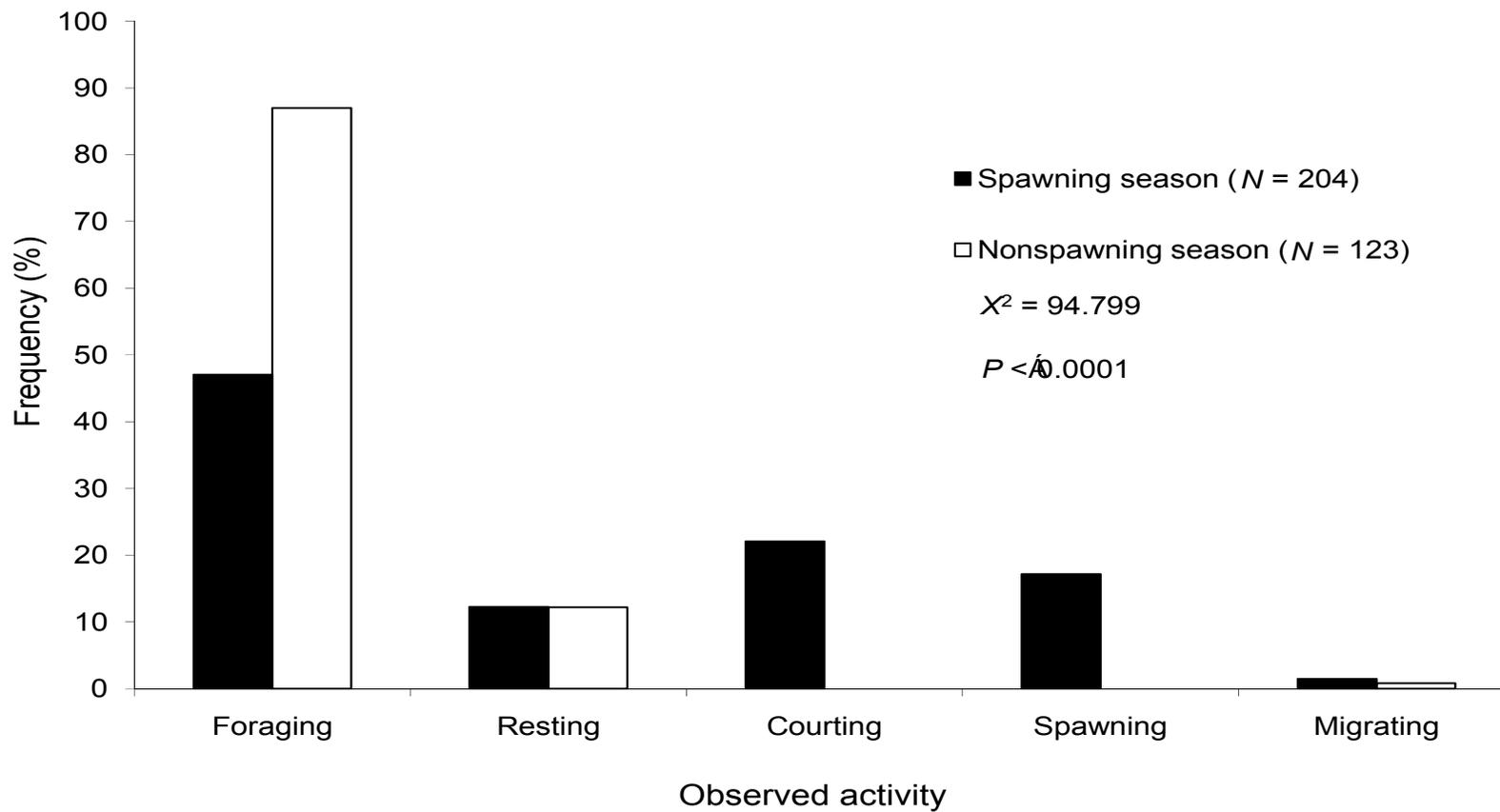


Figure 9.—Observed activity and behavior for radio-tagged and visually observed sicklefin redhorse in Hiwassee Basin, North Carolina and Georgia, during the spawning season (1 March – 31 May) and nonspawning season (1 June – 29 February) in 2006 and 2007. Spawning and nonspawning seasonal activity were compared using a likelihood-ratio chi-square test.

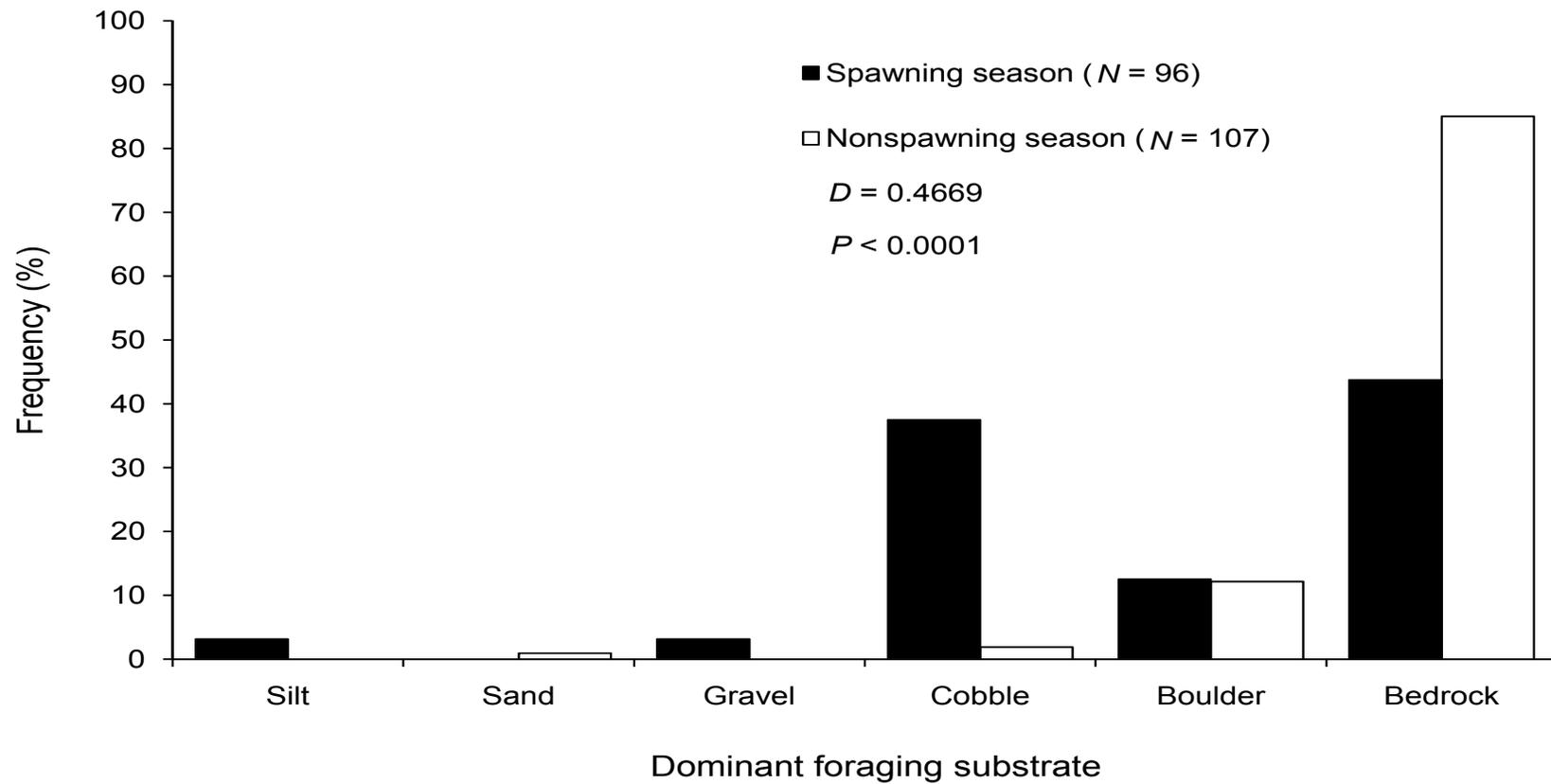


Figure 10.—Dominant foraging substrate for radio-tagged and visually observed sicklefin redhorse in Hiwassee Basin, North Carolina and Georgia, during the spawning season (1 March – 31 May) and nonspawning season (1 June – 29 February) in 2006 and 2007. Spawning and nonspawning foraging substrates were compared using a Kolmogorov-Smirnov two-sample test.

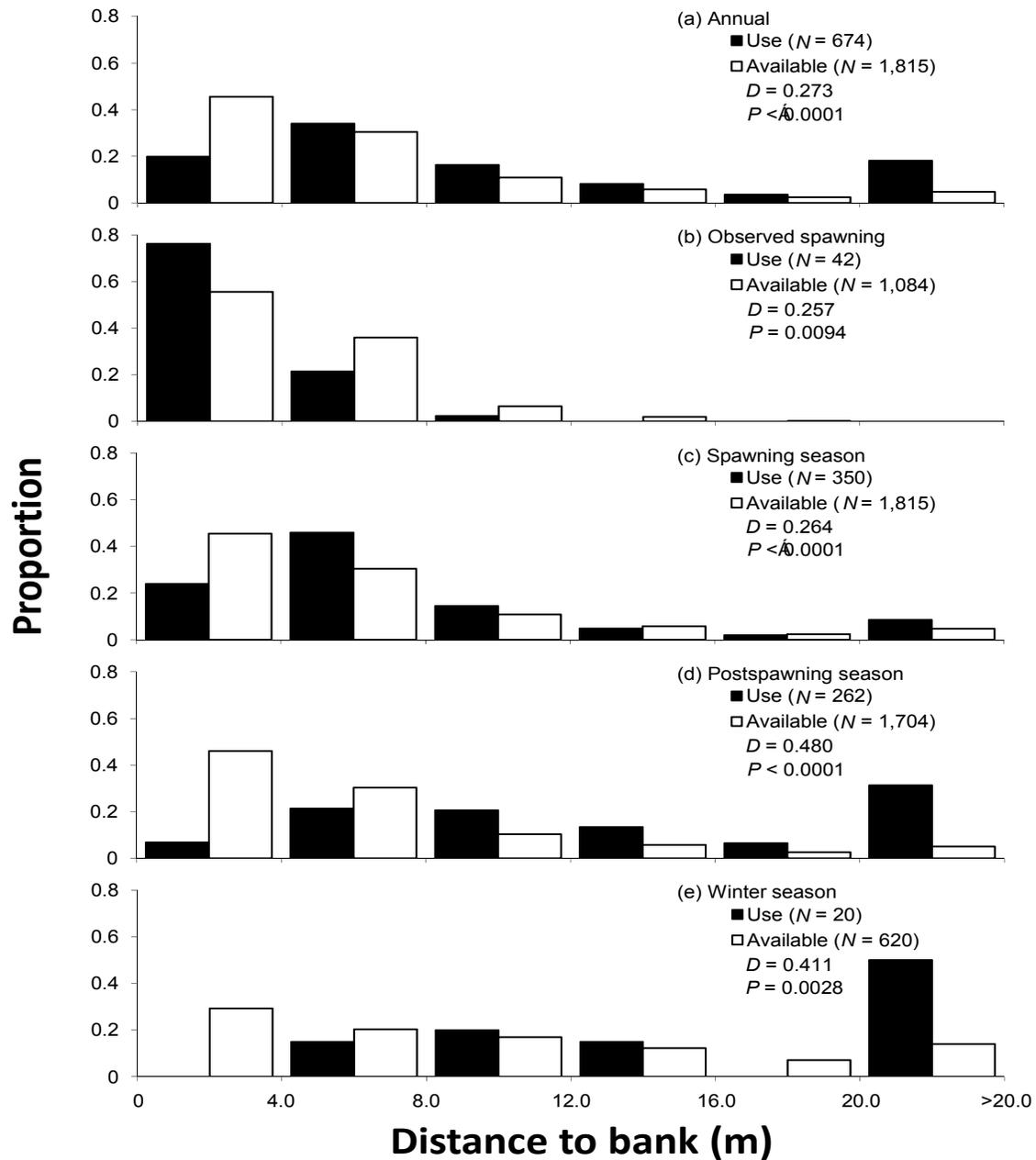


Figure 11.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal microhabitat use and availability frequency distributions and associated statistics for distance to bank in Hiwassee Basin, North Carolina and Georgia. Use and availability data were compared using a Kolmogorov-Smirnov two-sample test.

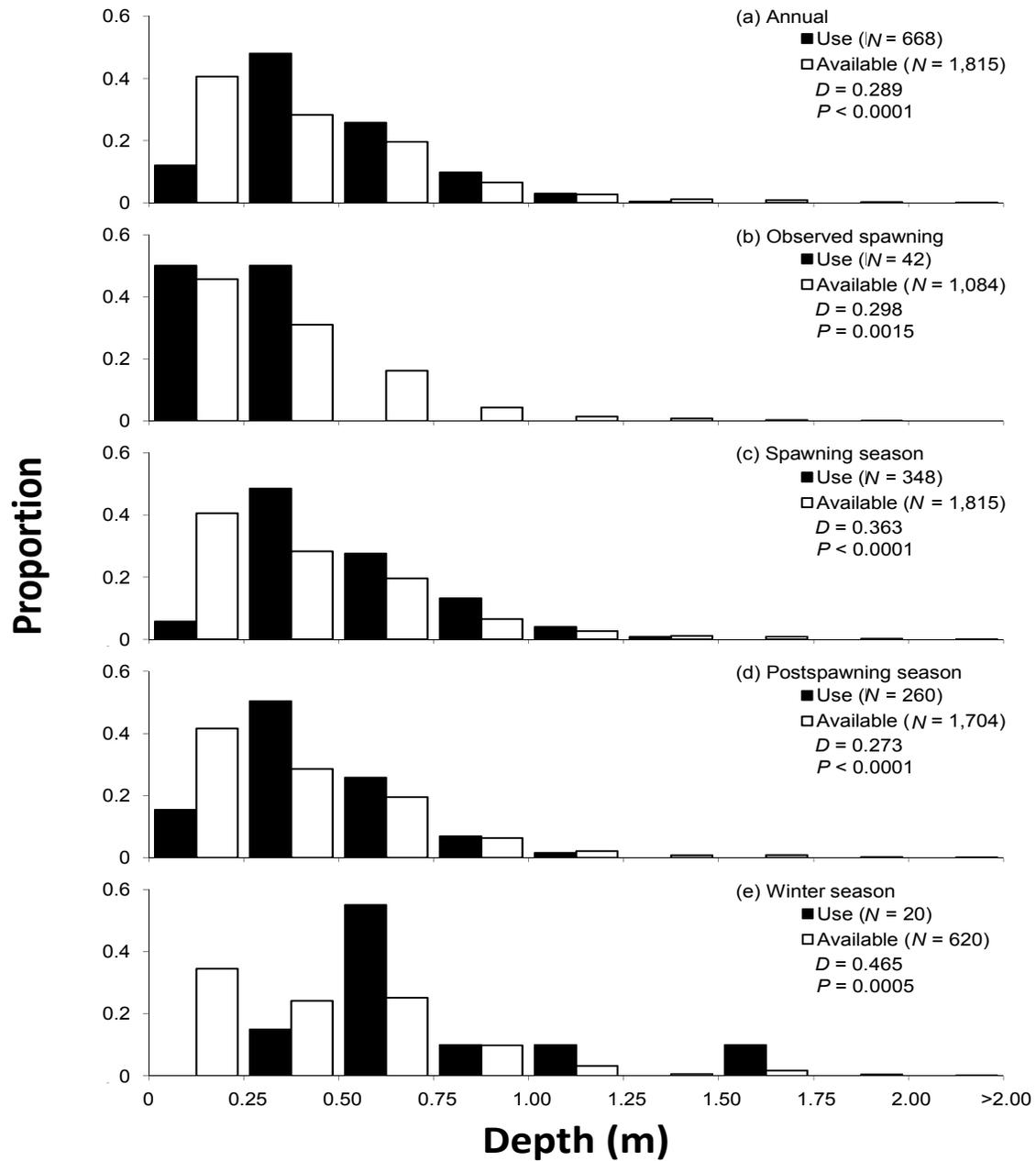


Figure 12.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal microhabitat use and availability frequency distributions and associated statistics for depth in Hiwassee Basin, North Carolina and Georgia. Use and availability data were compared using a Kolmogorov-Smirnov two-sample test.

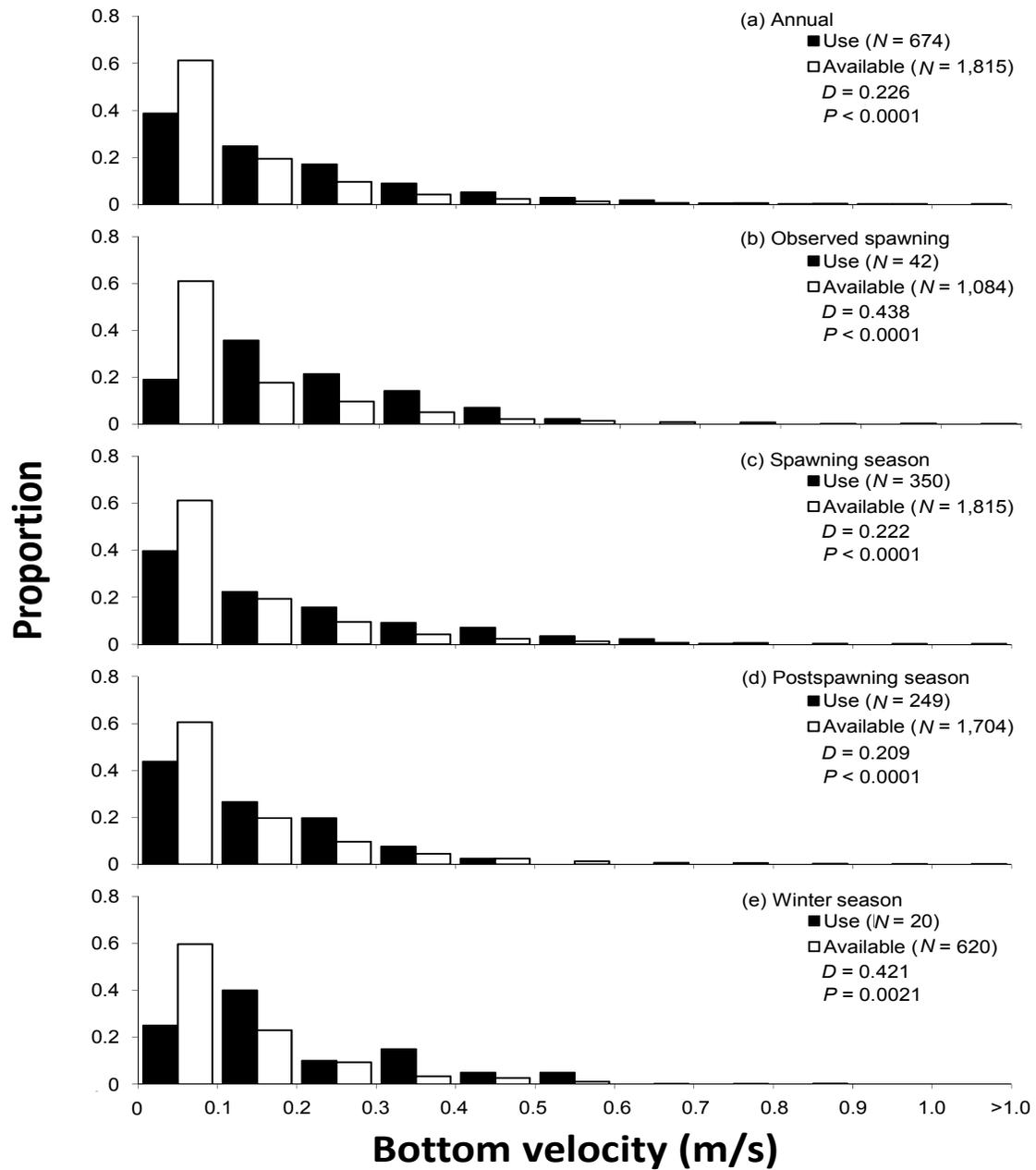


Figure 13.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal microhabitat use and availability frequency distributions and associated statistics for bottom velocity in Hiwassee Basin, North Carolina and Georgia. Use and availability data were compared using a Kolmogorov-Smirnov two-sample test.

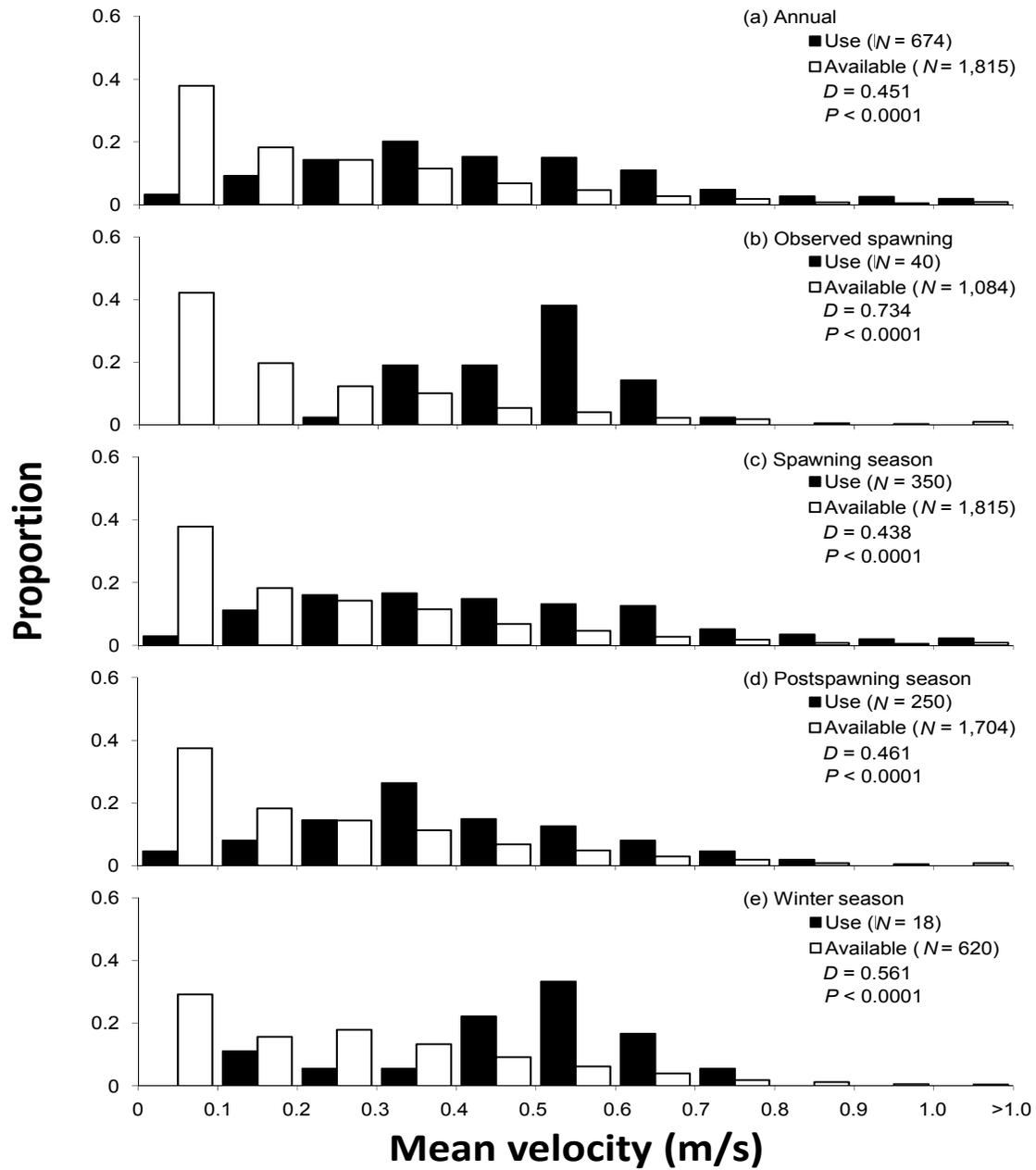


Figure 14.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal microhabitat use and availability frequency distributions and associated statistics for mean column velocity in Hiwassee Basin, North Carolina and Georgia. Use and availability data were compared using a Kolmogorov-Smirnov two-sample test.

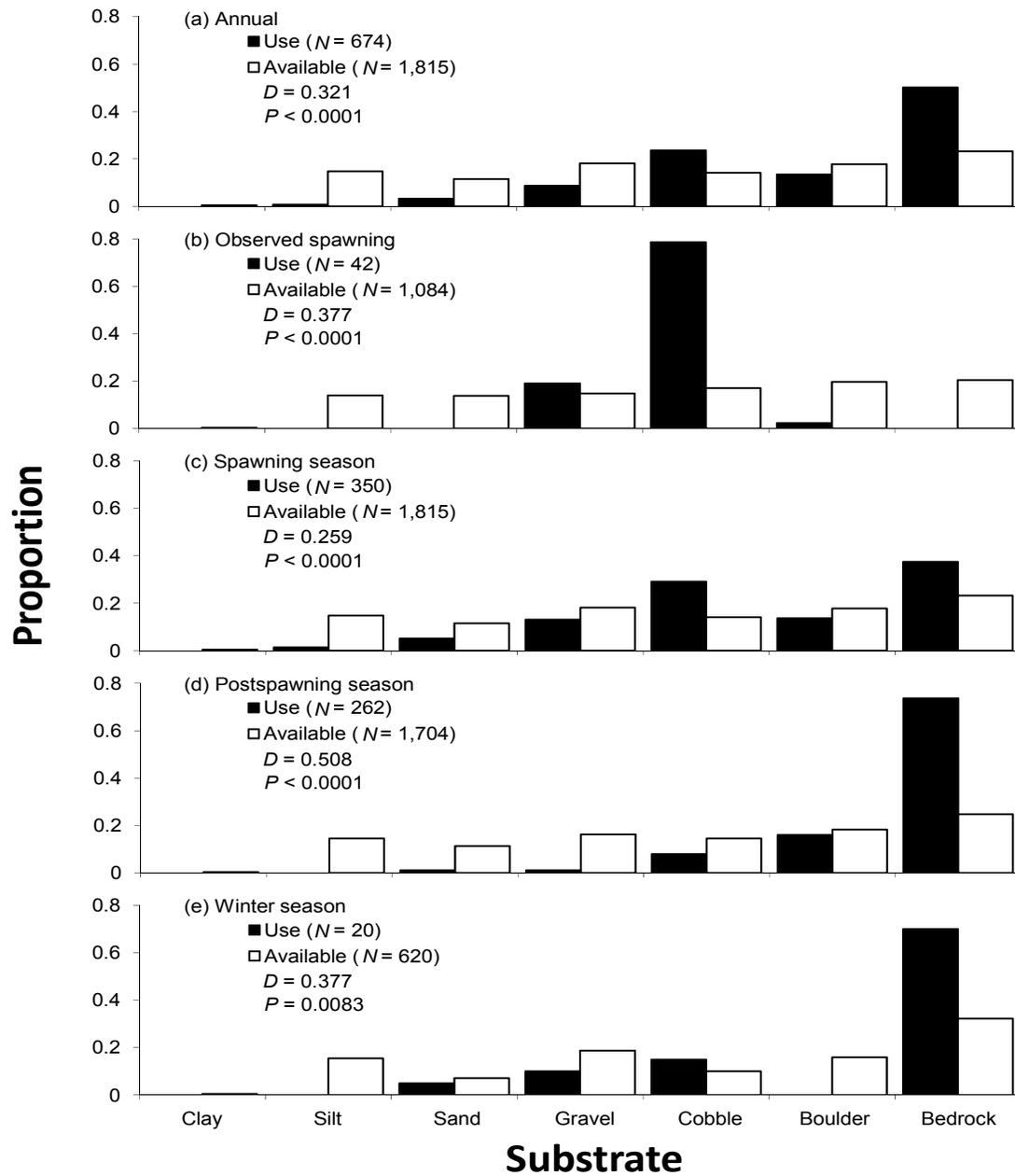


Figure 15.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal microhabitat use and availability frequency distributions and associated statistics for dominant substrate in Hiwassee Basin, North Carolina and Georgia. Use and availability data were compared using a Kolmogorov-Smirnov two-sample test.

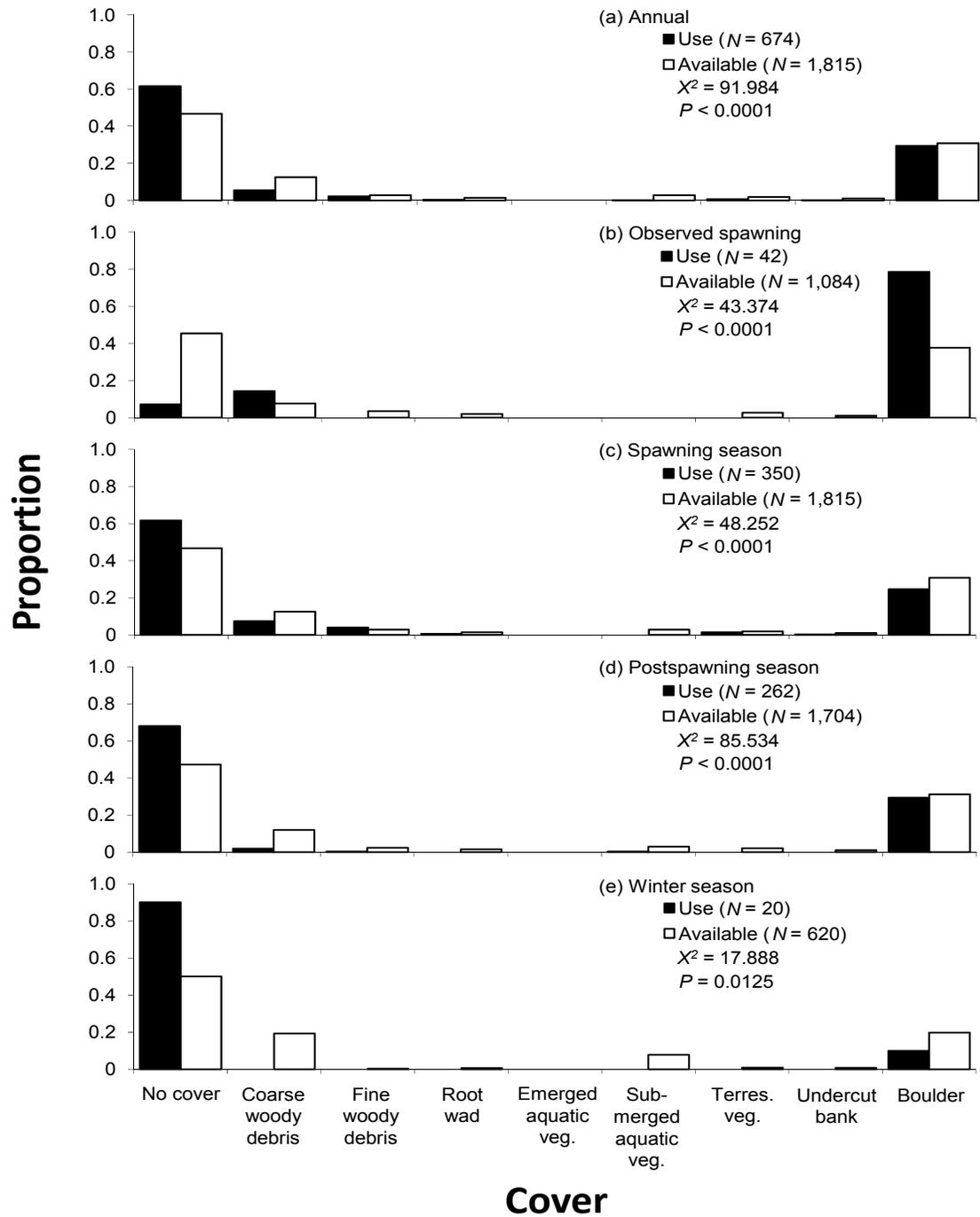


Figure 16.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal microhabitat use and availability frequency distributions and associated statistics for cover in Hiwassee Basin, North Carolina and Georgia. Use and availability data were compared using a likelihood-ratio chi-square test.

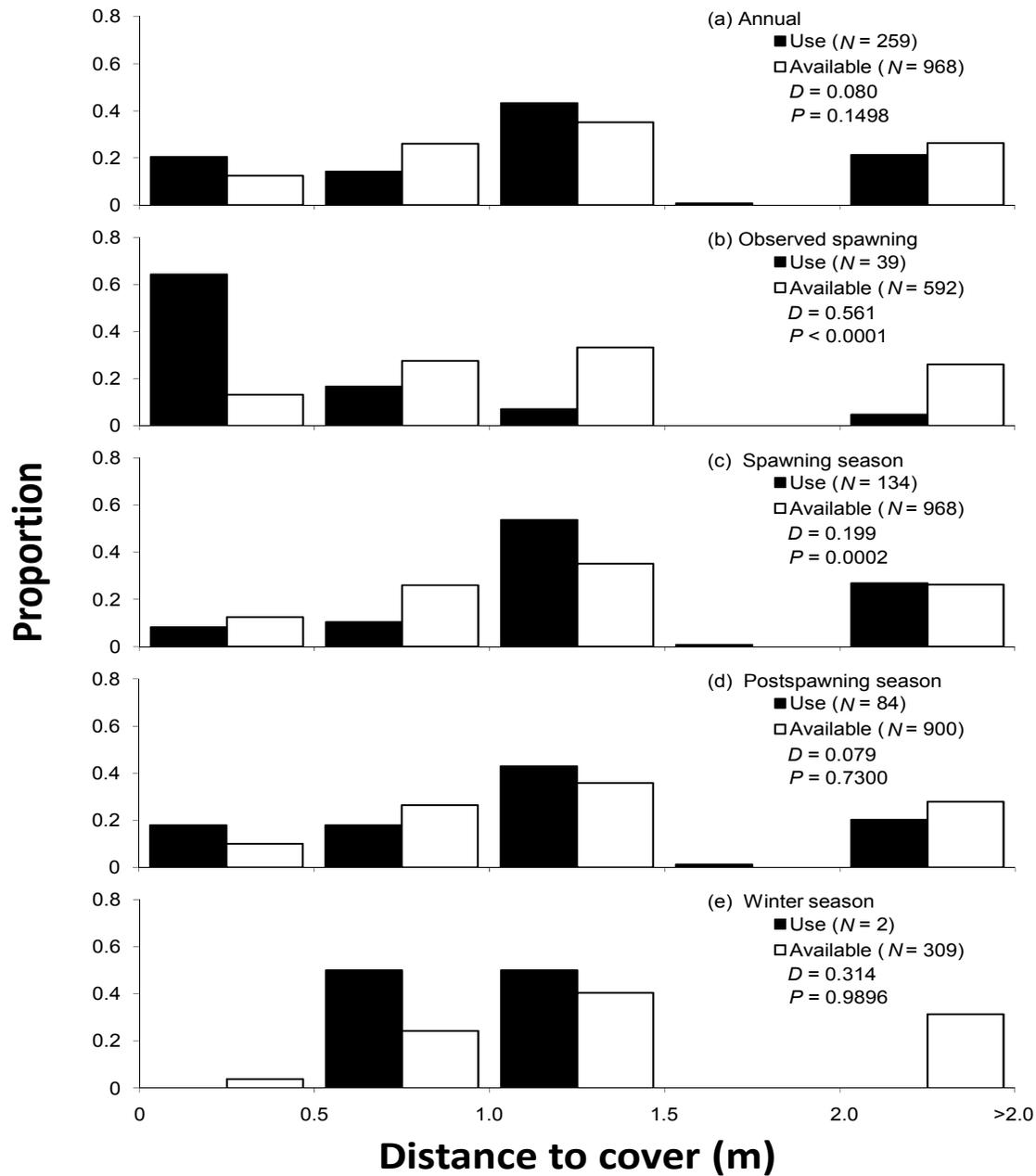


Figure 17.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal microhabitat use and availability frequency distributions and associated statistics for distance to cover in Hiwassee Basin, North Carolina and Georgia. Use and availability data were compared using a Kolmogorov-Smirnov two-sample test.

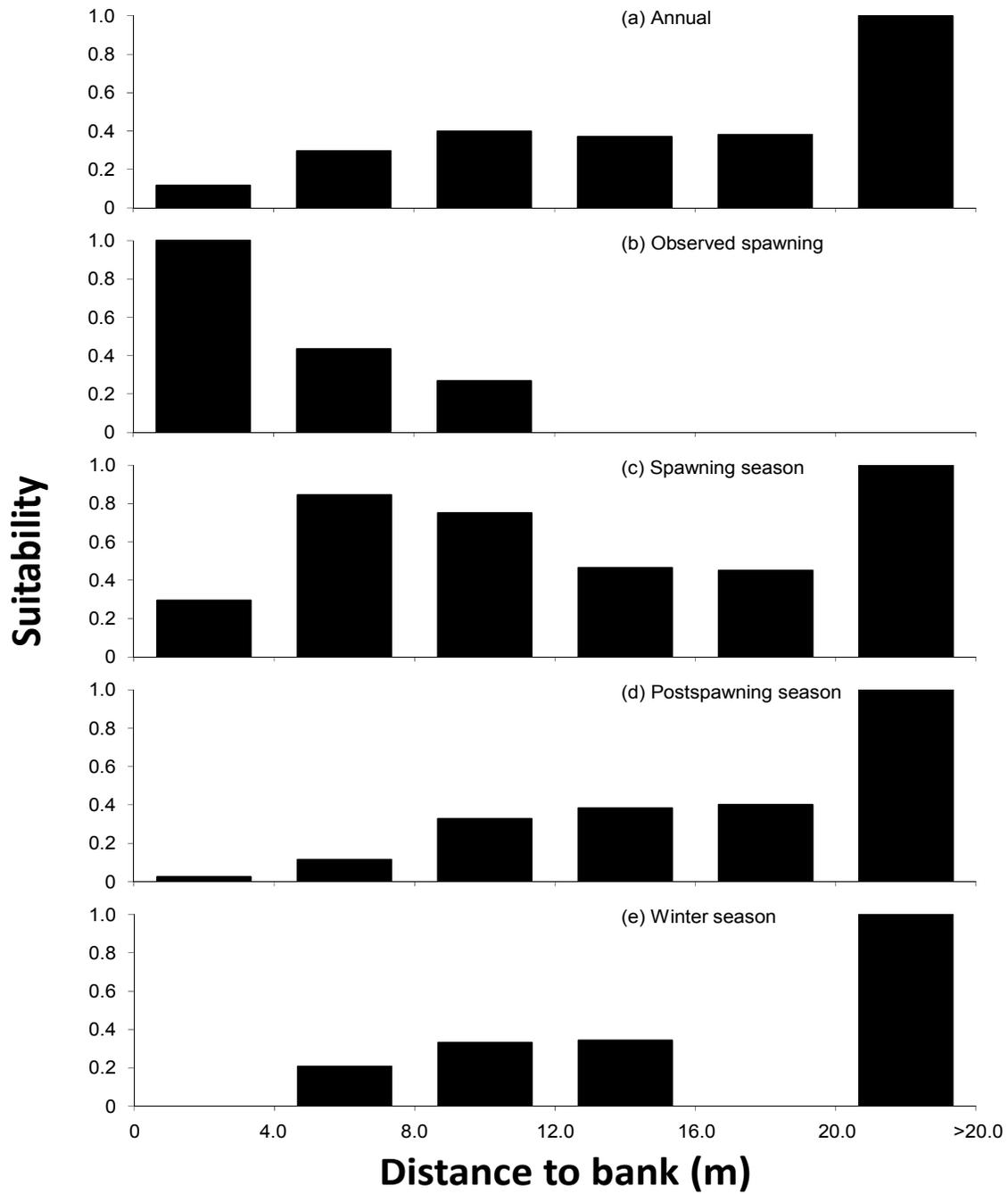


Figure 18.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal distance to bank microhabitat suitabilities for Hiwassee Basin, North Carolina and Georgia.

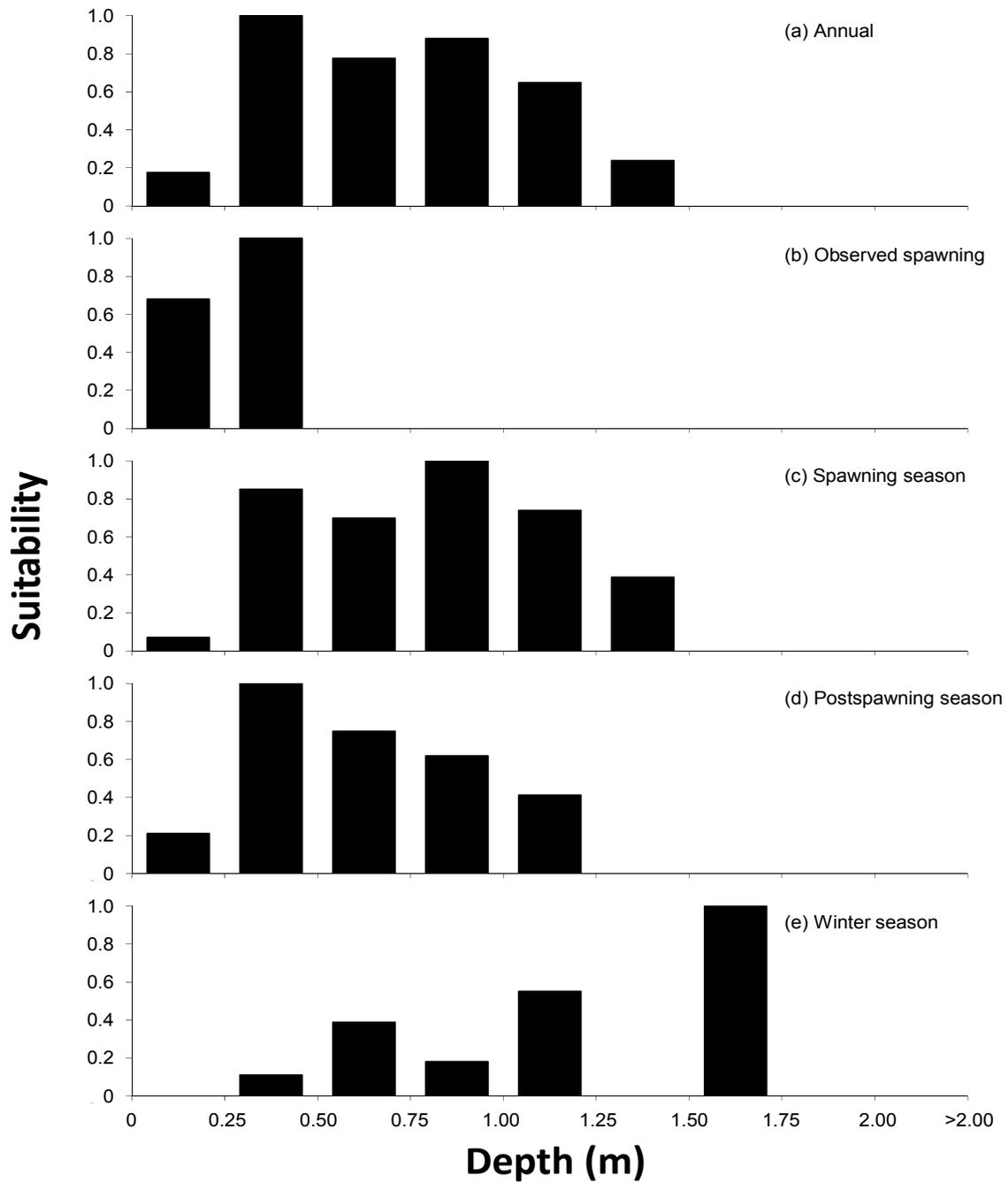


Figure 19.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal depth microhabitat suitabilities for Hiwassee Basin, North Carolina and Georgia.

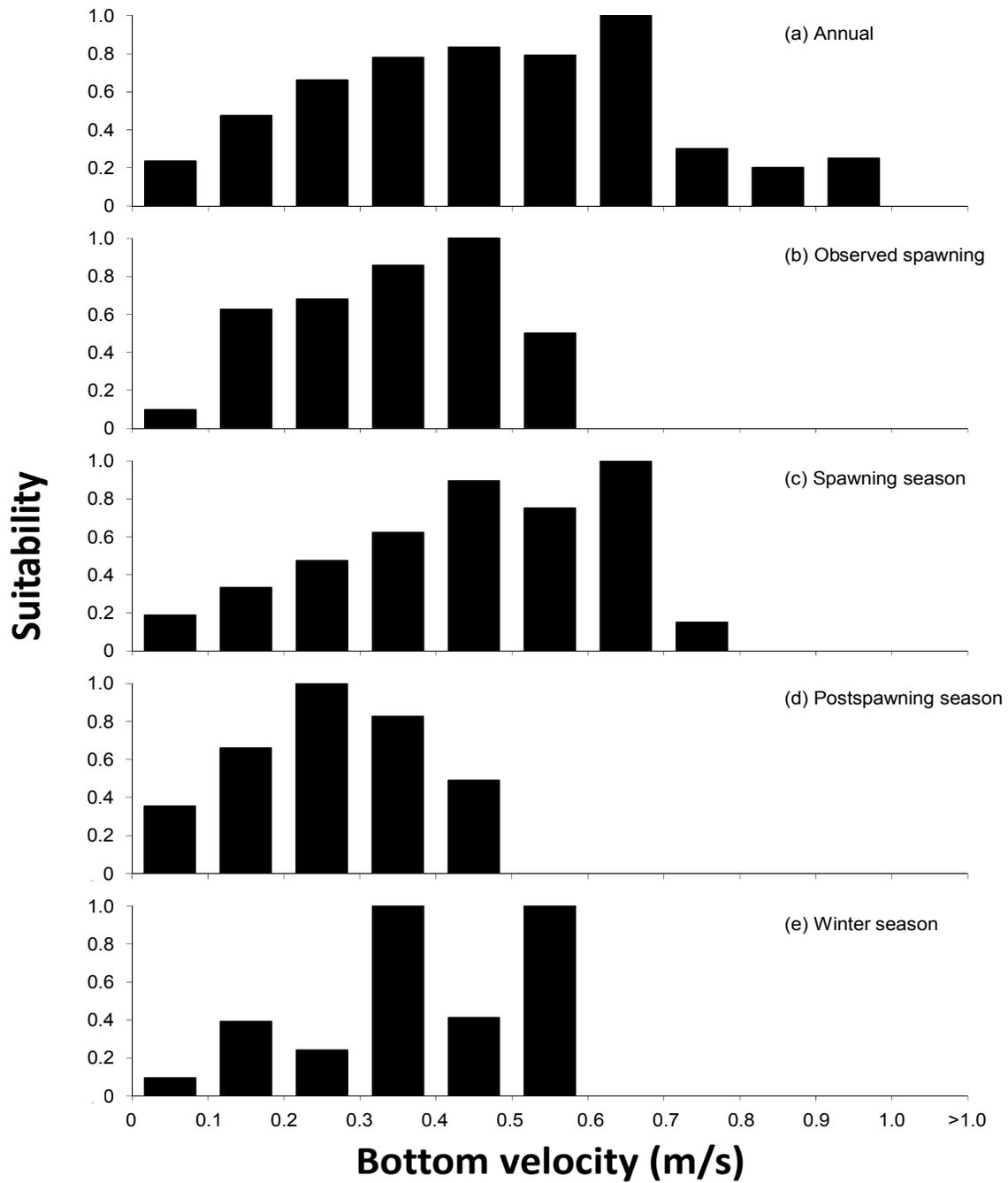


Figure 20.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal bottom velocity microhabitat suitabilities for Hiwassee Basin, North Carolina and Georgia.

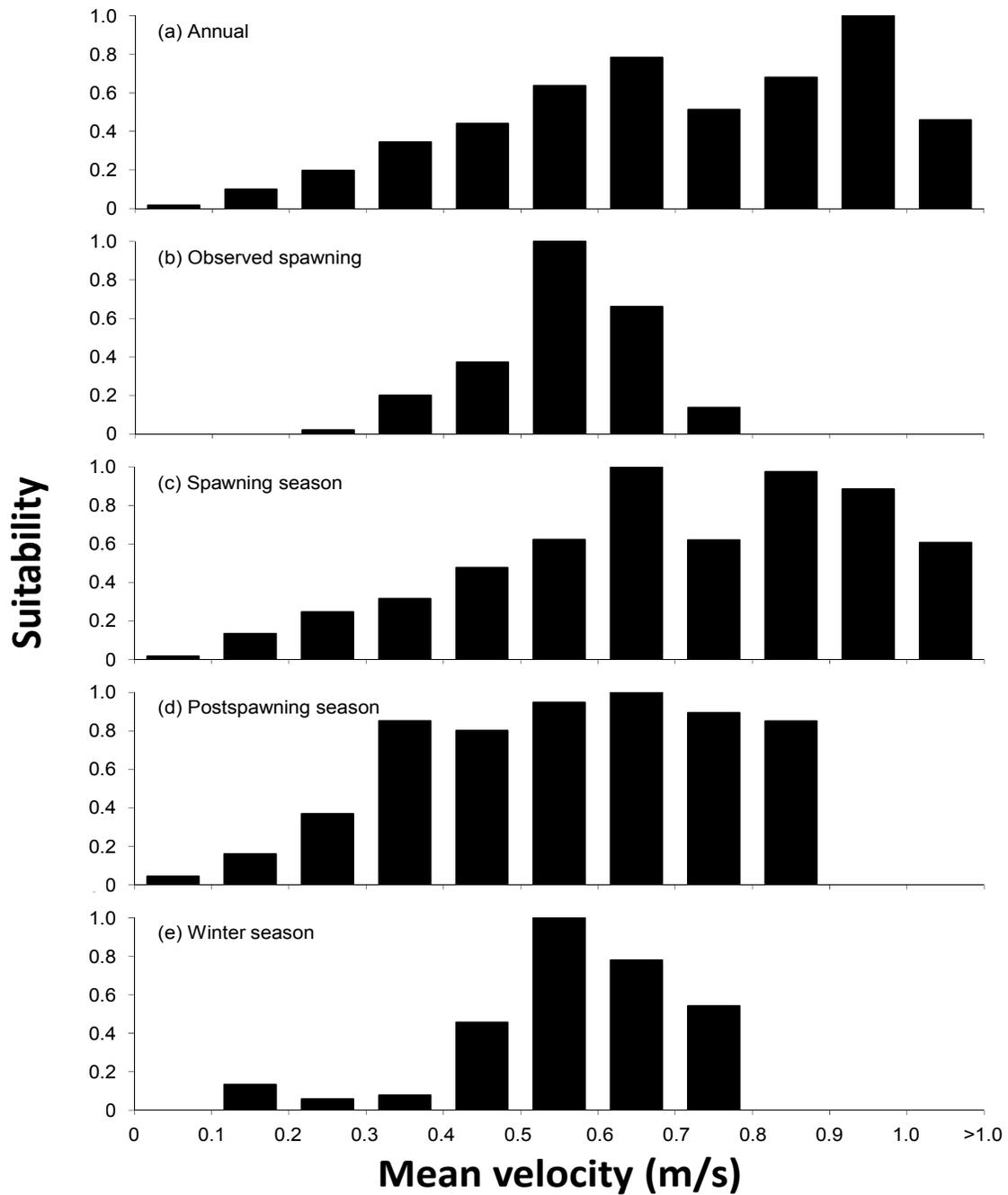


Figure 21.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal microhabitat mean column velocity suitabilities for Hiwassee Basin, North Carolina and Georgia.

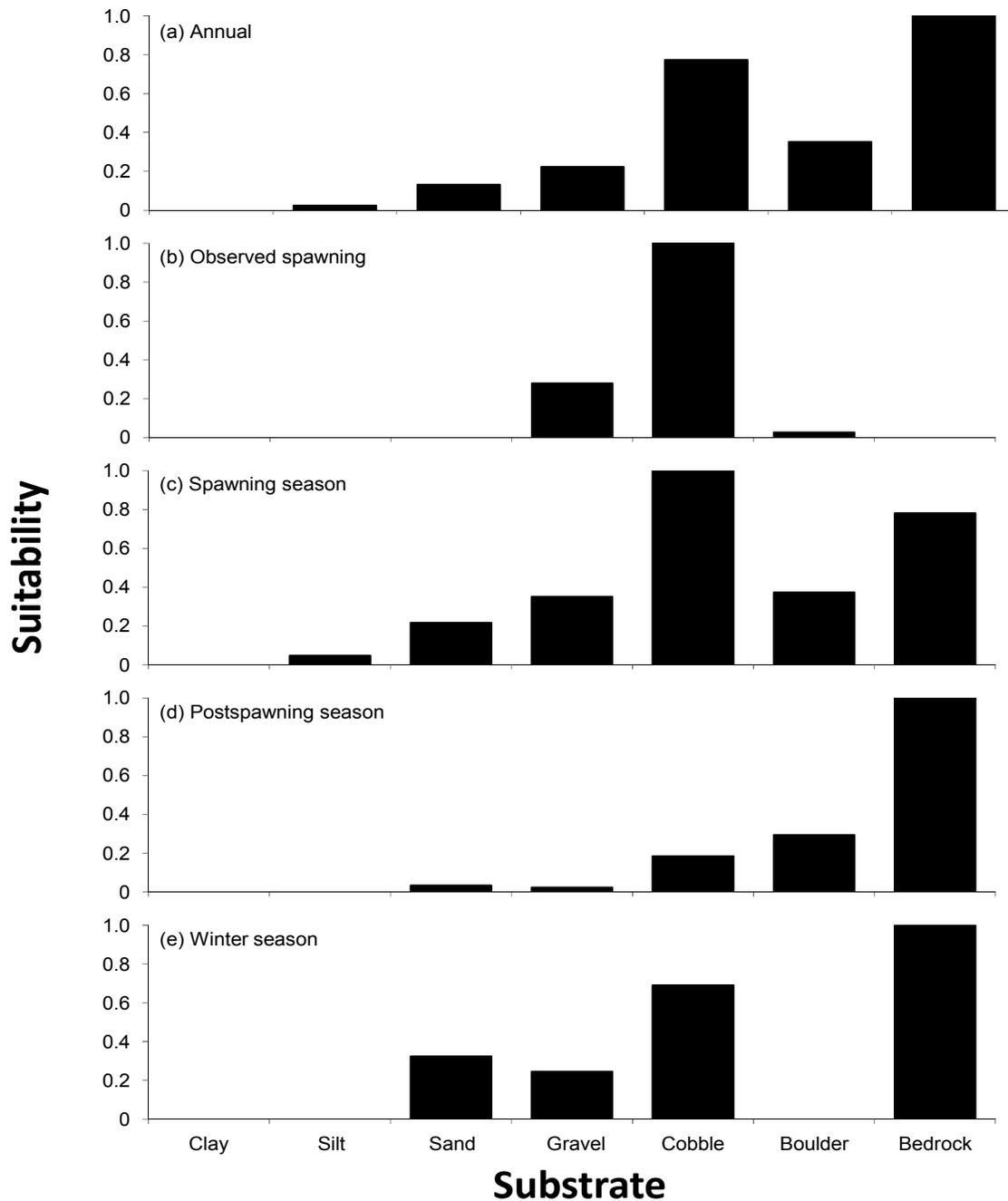


Figure 22.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal microhabitat dominant substrate suitabilities for Hiwassee Basin, North Carolina and Georgia.

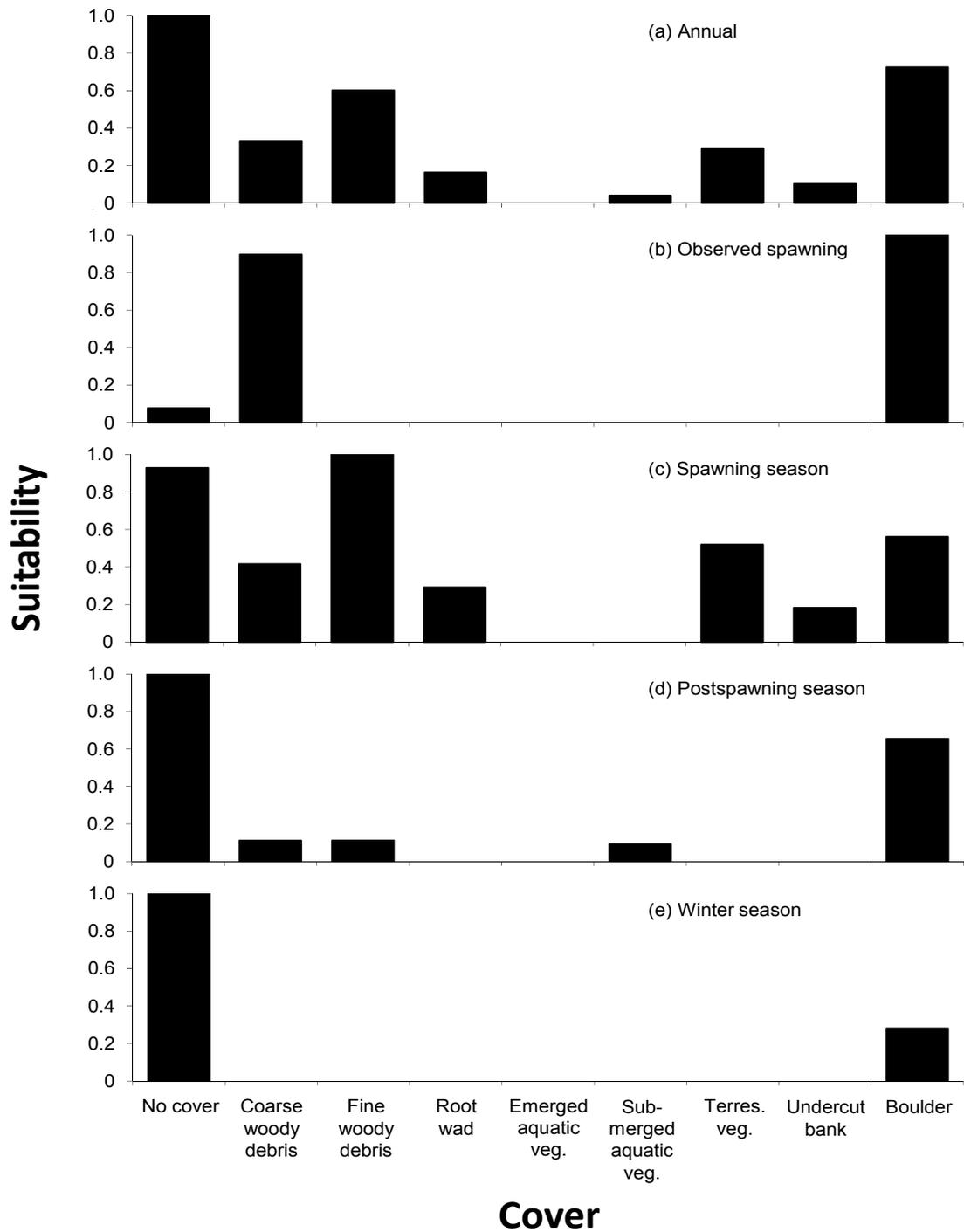


Figure 23.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal cover microhabitat suitabilities for Hiwassee Basin, North Carolina and Georgia.

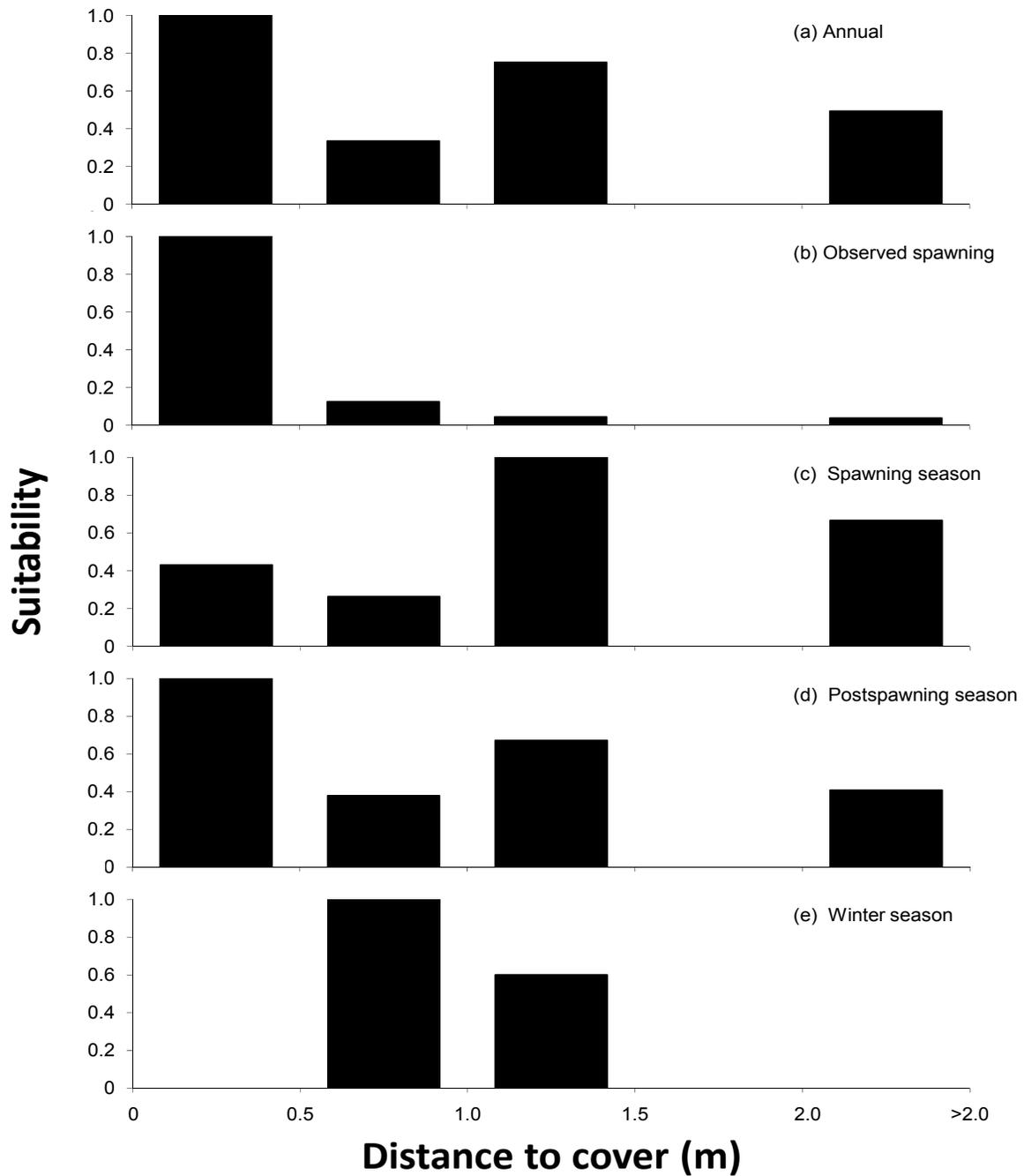


Figure 24.—Sicklefin redhorse annual (a), observed spawning (b), spawning (c), postspawning (d), and winter (e) seasonal distance to cover microhabitat suitabilities for Hiwassee Basin, North Carolina and Georgia.

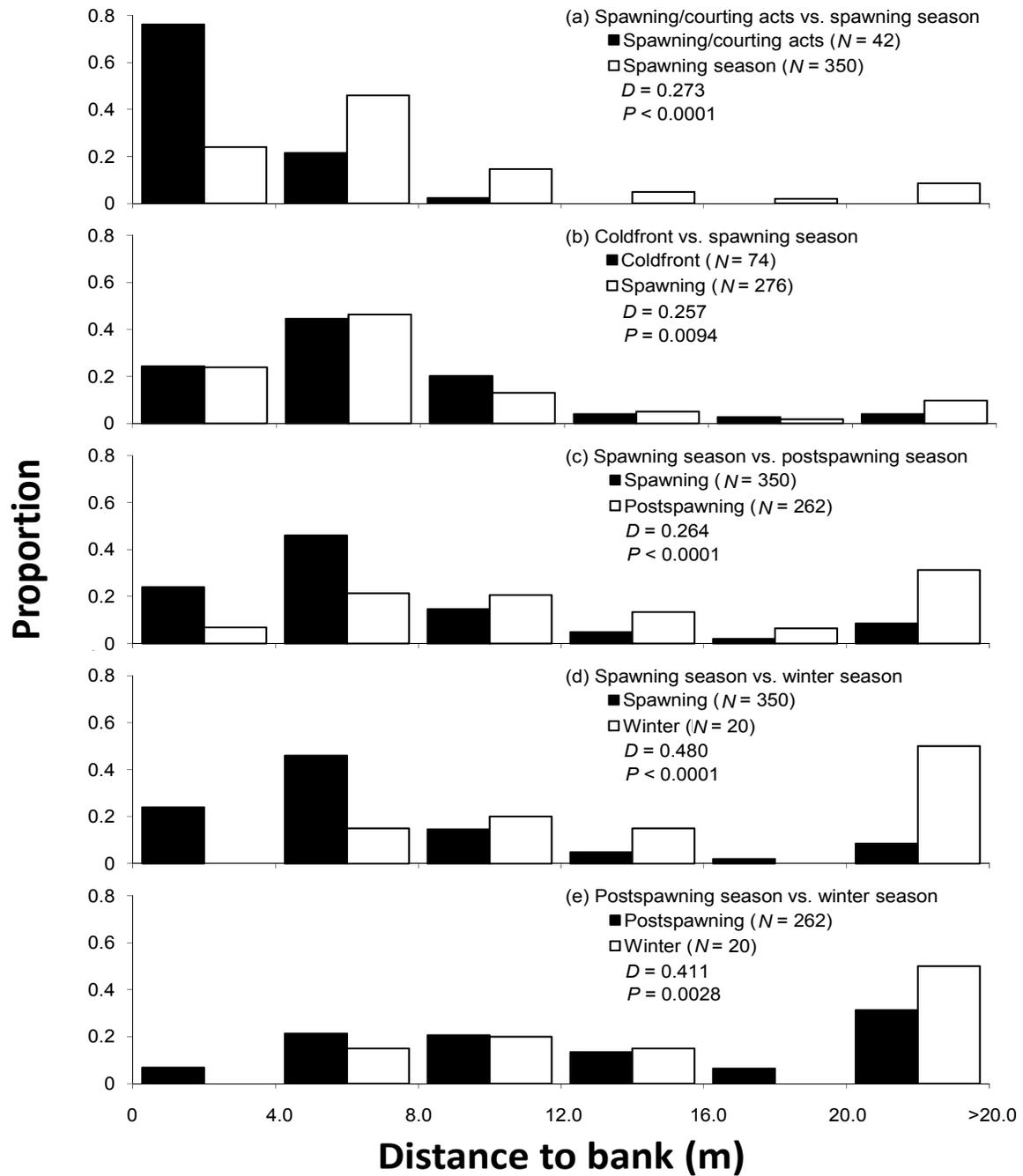


Figure 25.—Sicklefin redhorse seasonal comparisons of microhabitat use and associated statistics for distance to bank in Hiwassee Basin, North Carolina and Georgia. Seasonal microhabitat use data were compared using a Kolmogorov-Smirnov two-sample test.

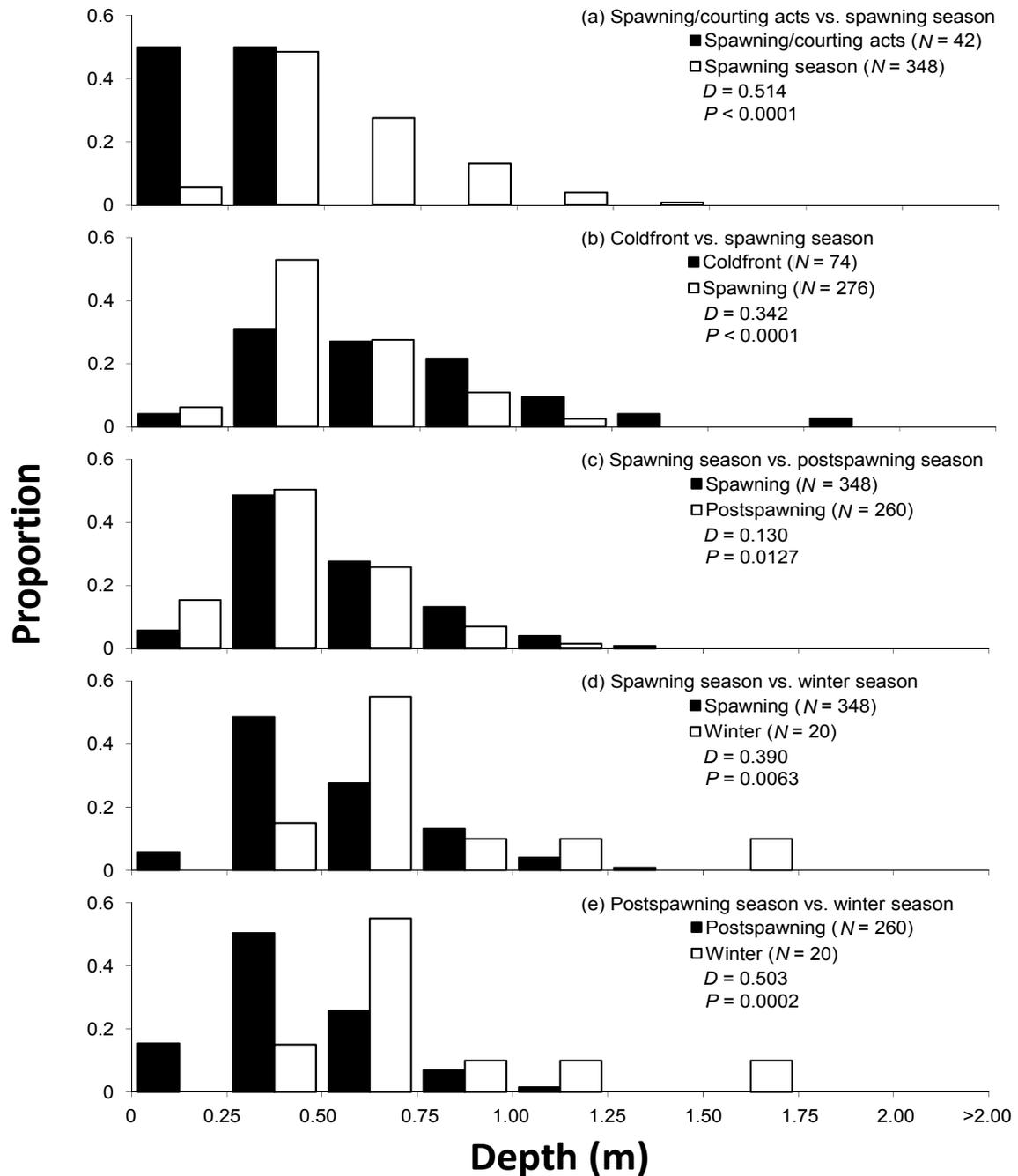


Figure 26.—Sicklefin redhorse seasonal comparisons of microhabitat use and associated statistics for depth in Hiwassee Basin, North Carolina and Georgia. Seasonal microhabitat use data were compared using a Kolmogorov-Smirnov two-sample test.

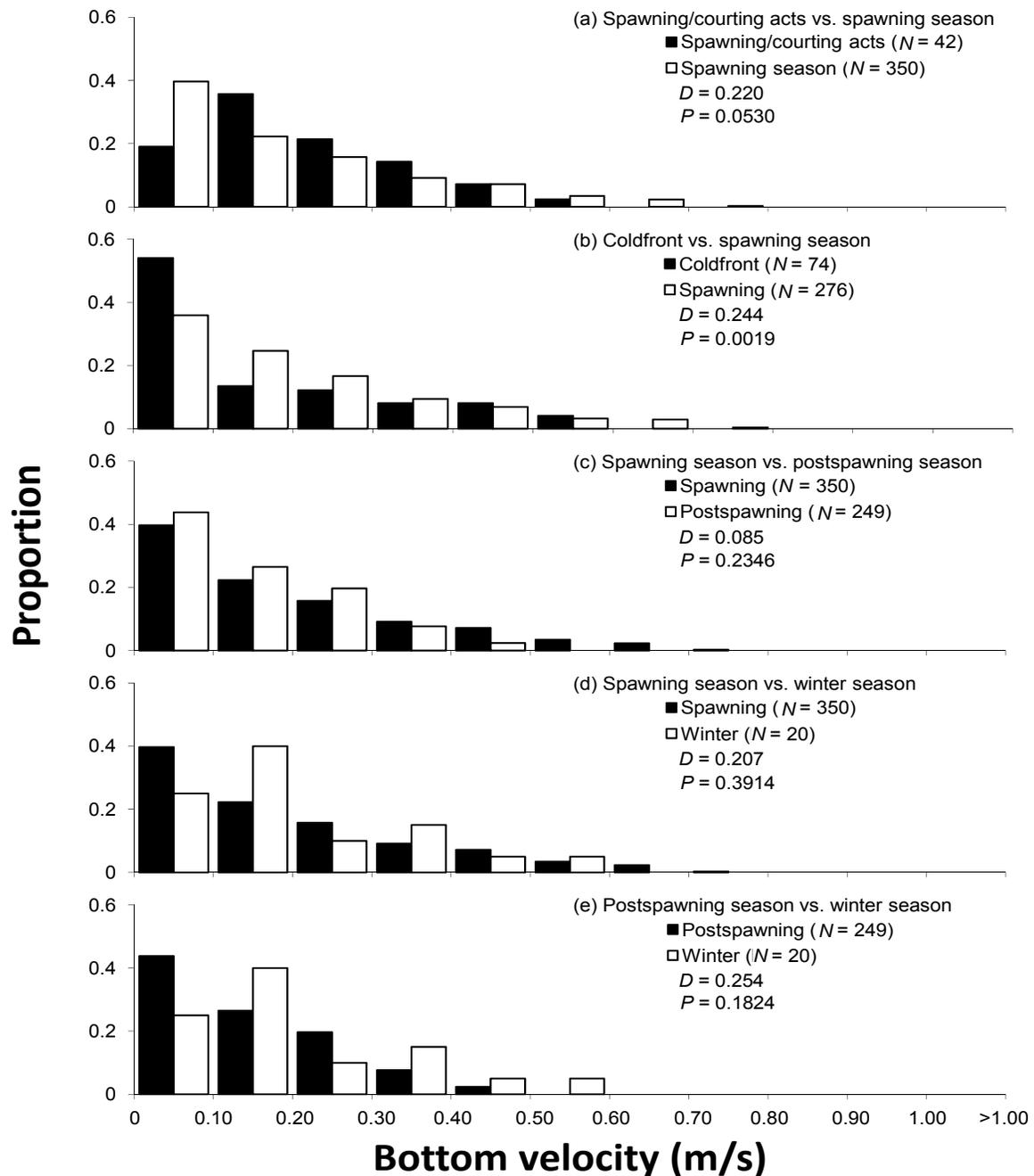


Figure 27.—Sicklefin redhorse seasonal comparisons of microhabitat use and associated statistics for bottom velocity in Hiwassee Basin, North Carolina and Georgia. Seasonal microhabitat use data were compared using a Kolmogorov-Smirnov two-sample test.

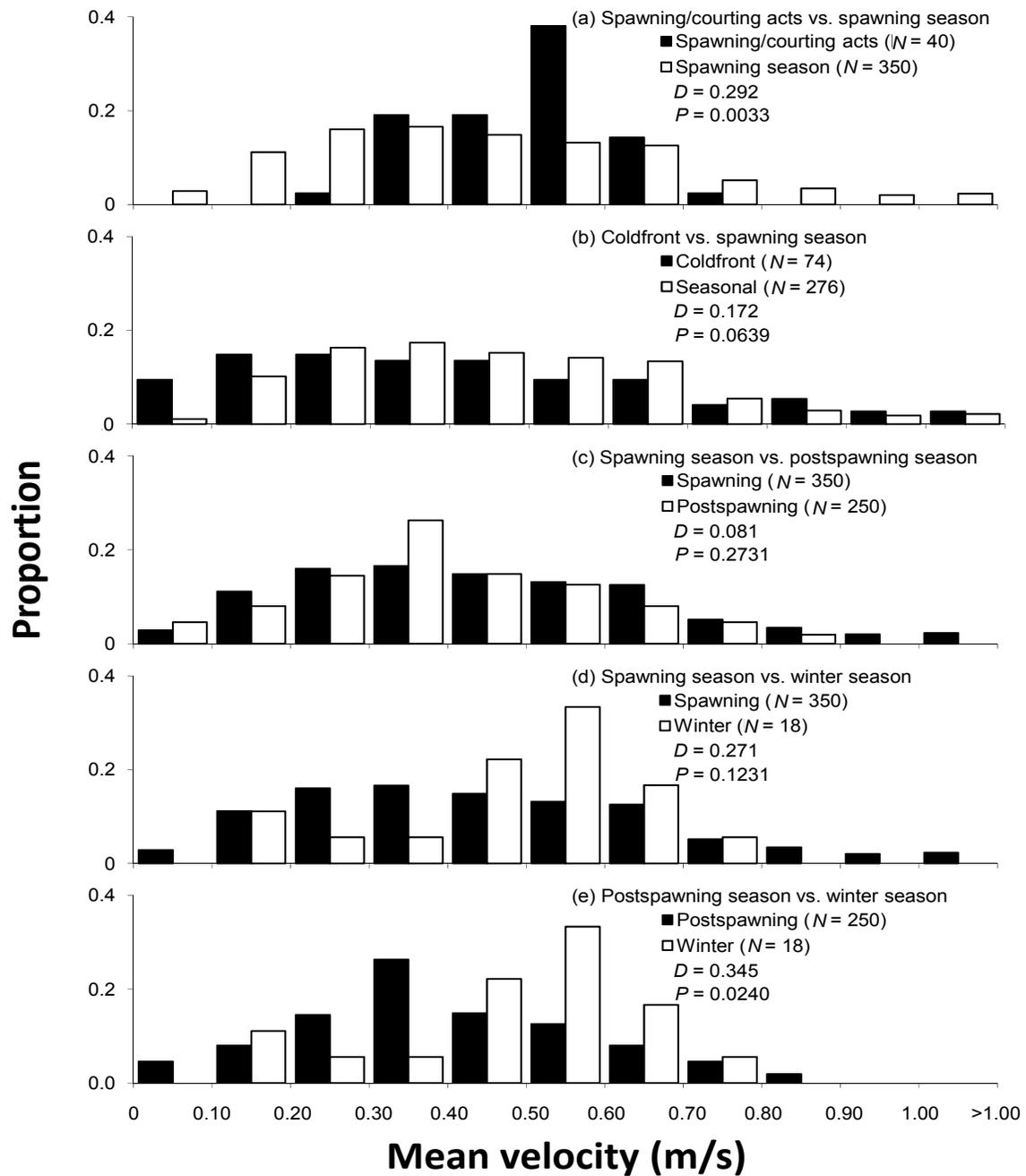


Figure 28.—Sicklefin redhorse seasonal comparisons of microhabitat use and associated statistics for mean column velocity in Hiwassee Basin, North Carolina and Georgia. Seasonal microhabitat use data were compared using a Kolmogorov-Smirnov two-sample test.

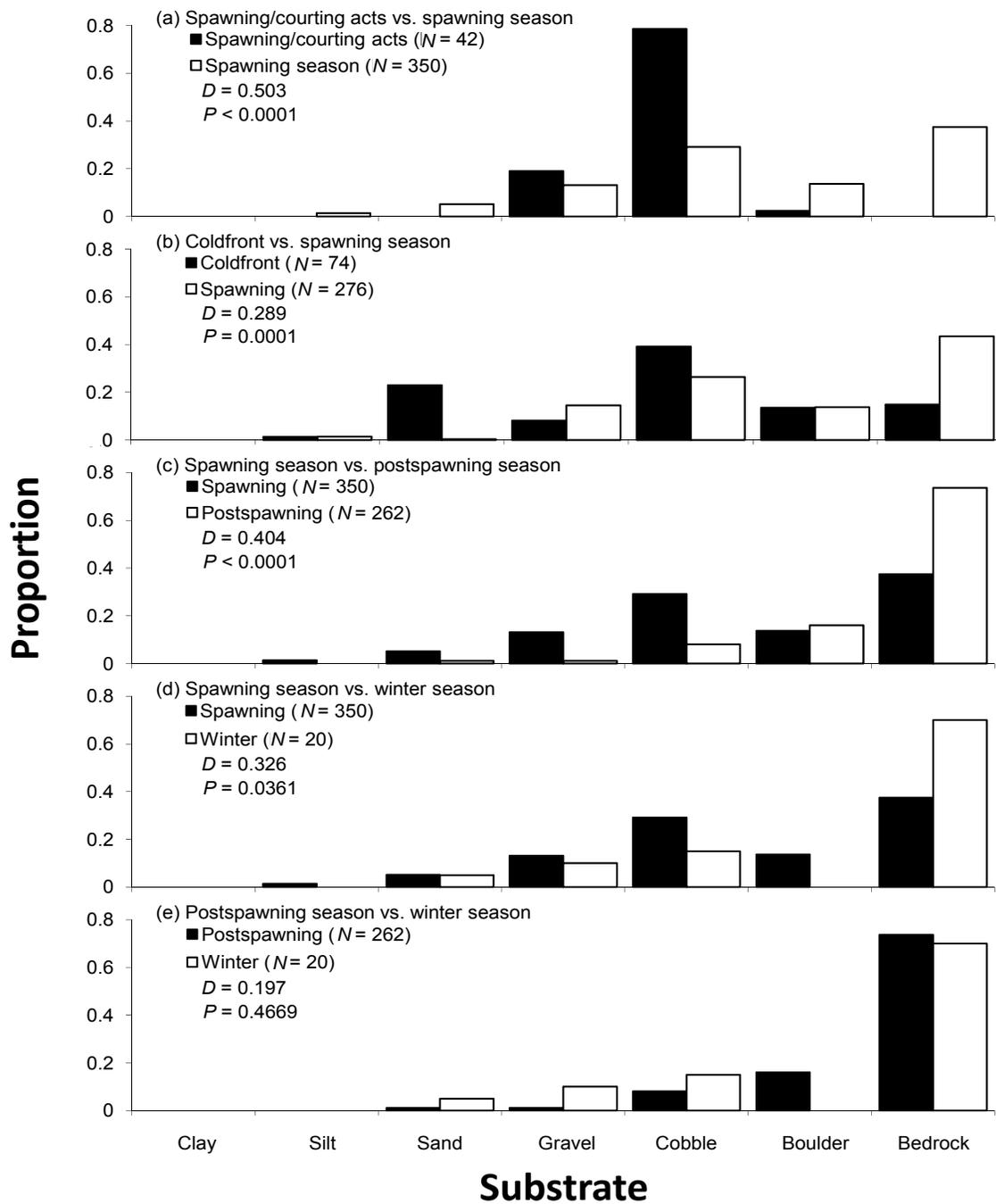


Figure 29.—Sicklefin redhorse seasonal comparisons of microhabitat use and associated statistics for dominant substrate in Hiwassee Basin, North Carolina and Georgia. Seasonal microhabitat use data were compared using a Kolmogorov-Smirnov two-sample test.

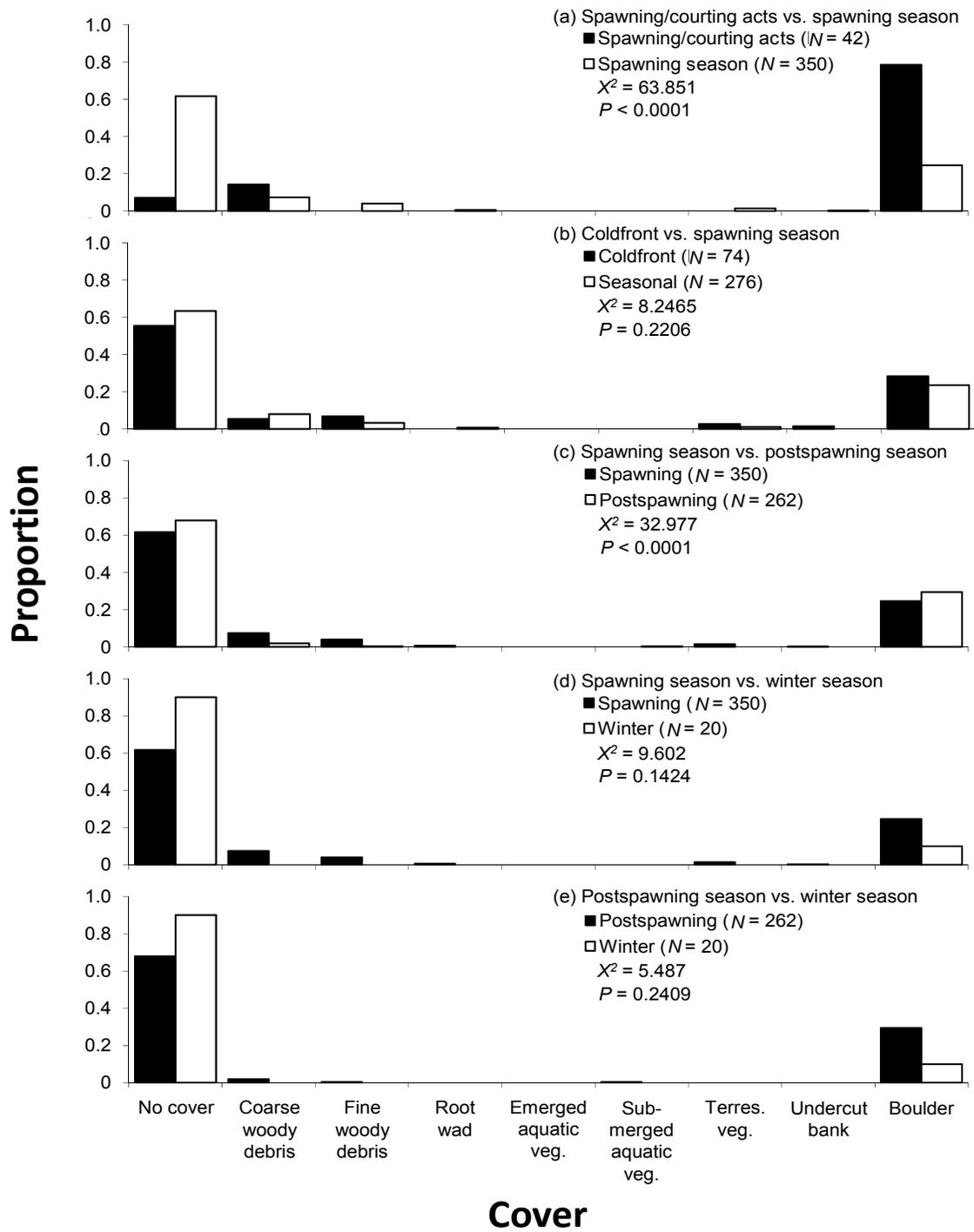


Figure 30.—Sicklefin redhorse seasonal comparisons of microhabitat use and associated statistics for cover in Hiwassee Basin, North Carolina and Georgia. Seasonal microhabitat use data were compared using a likelihood-ratio chi-square test.

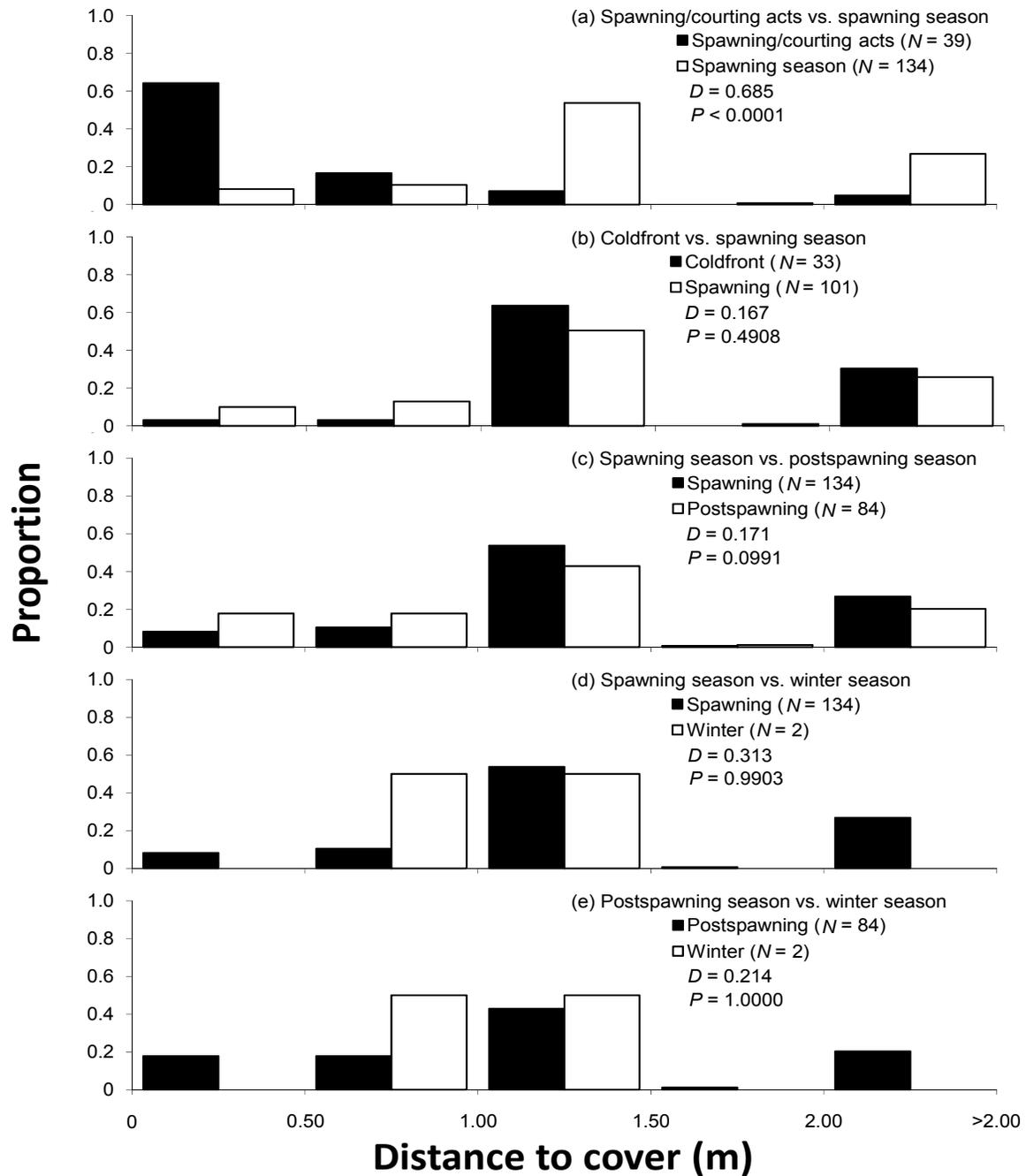


Figure 31.—Sicklefin redhorse seasonal comparisons of microhabitat use and associated statistics for distance to cover in Hiwassee Basin, North Carolina and Georgia. Seasonal microhabitat use data were compared using a Kolmogorov-Smirnov two-sample test.

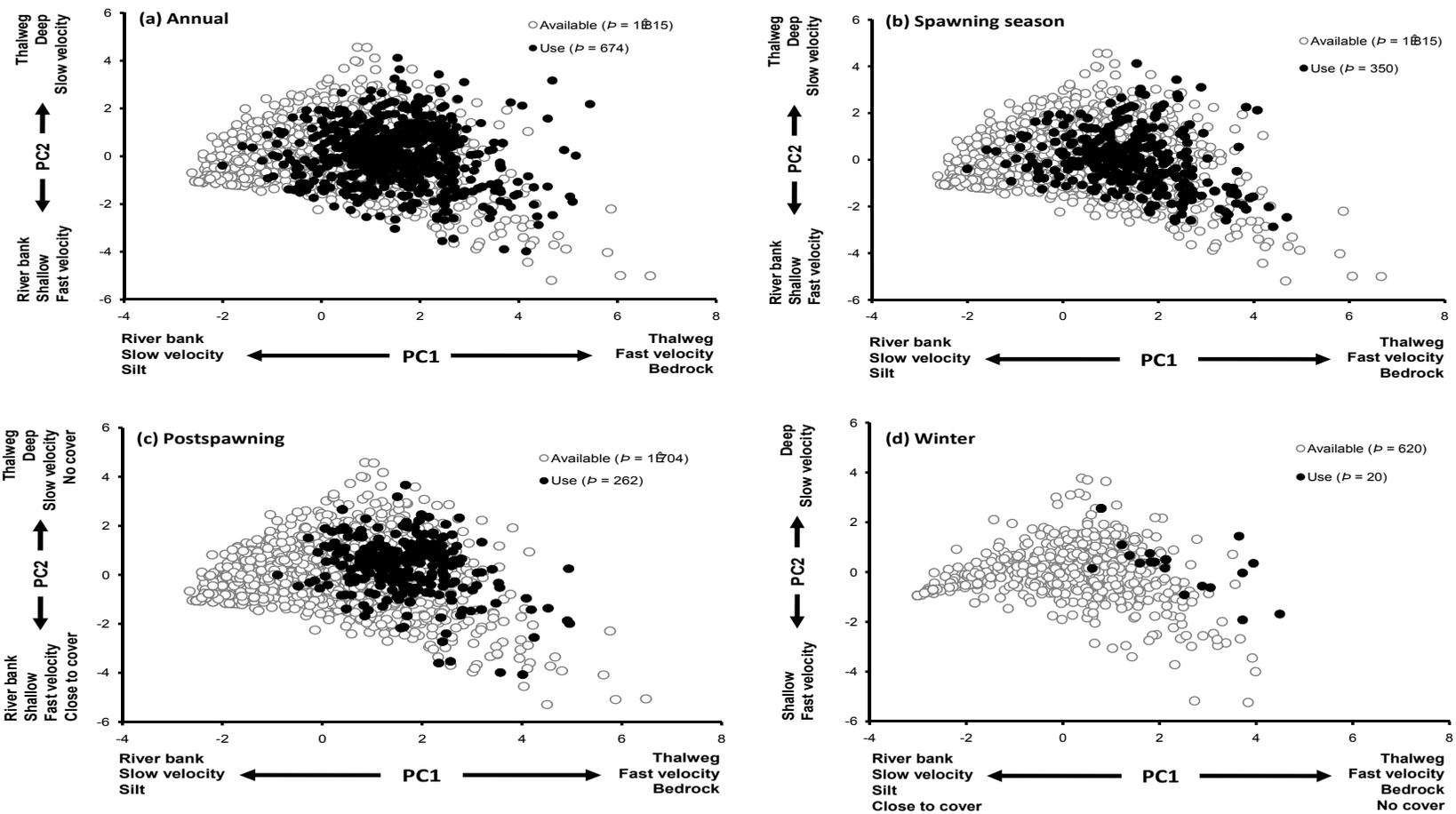


Figure 32.—Plots of annual (a), spawning (b), postspawning (c), and winter (d) sicklefin redhorse component scores for microhabitat use and availability, describing microhabitat variable combinations most important in defining suitable macrohabitat.

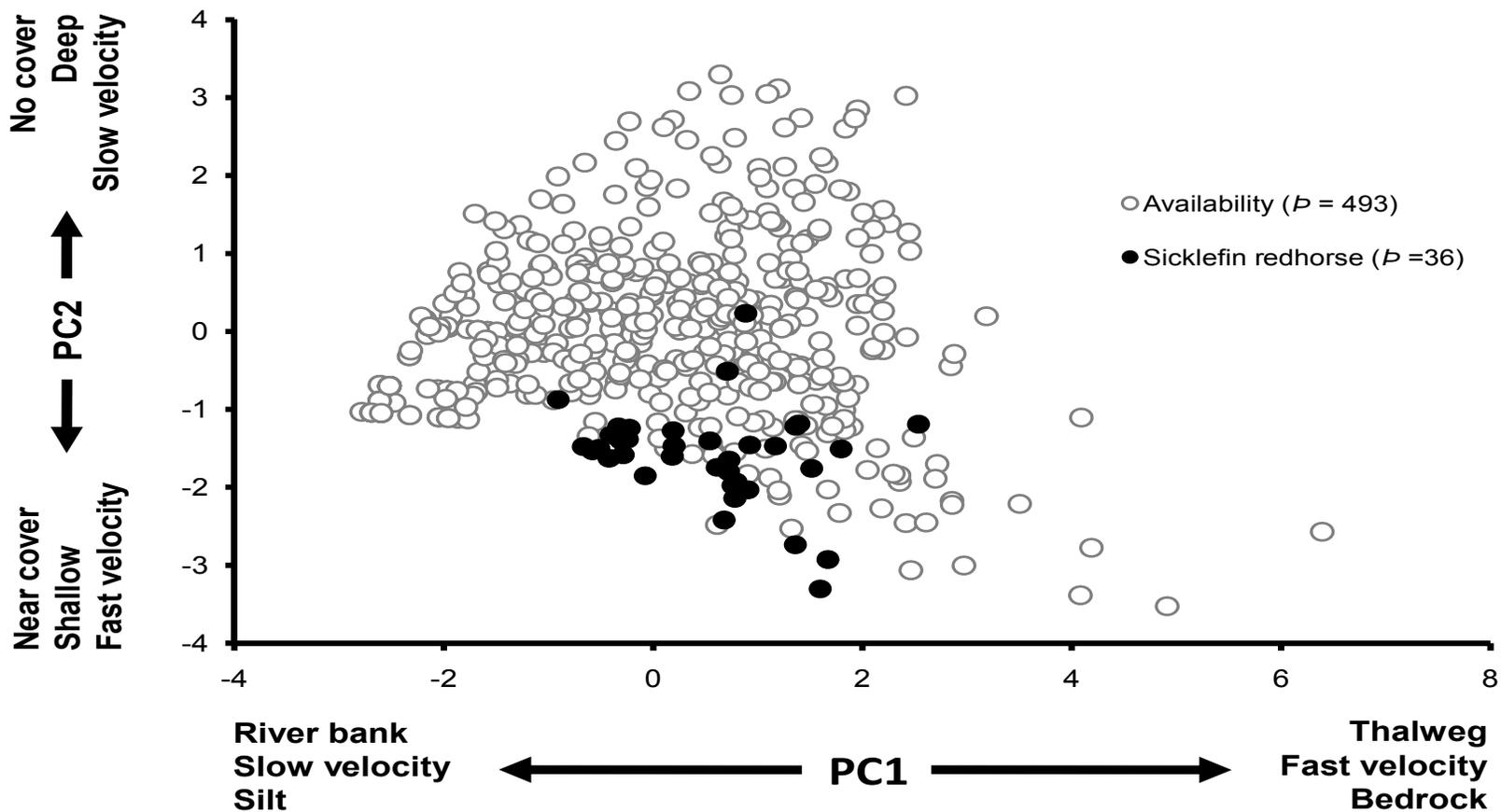
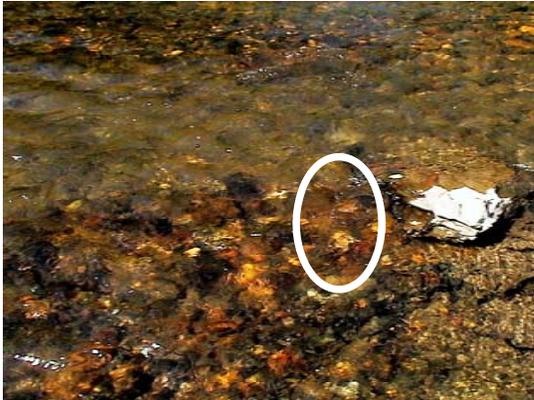


Figure 33.—Plots of observed spawning sicklefin redhorse component scores for microhabitat use and availability, describing microhabitat variable combinations most important in defining oviposition macrohabitat.

(a) Oviposition site 1



(b) Oviposition site 2



(c) Oviposition site 3



(d) Oviposition site 4



(e) Oviposition site 5



(f) Oviposition site 6



Figure 34.—Hiwassee Basin sicklefin redhorse typical oviposition sites from Valley River, North Carolina. Oviposition sites 1 – 3 were constructed in close proximity to boulders. Oviposition site 4 was adjacent to a partially embedded log. Oviposition sites 5 – 6 were positioned immediately upstream from or adjacent to small boulders.

**Chapter II – Reproductive Isolating Mechanisms Among Five Redhorses in the  
Hiwassee River Basin of the Southern Appalachian Mountains**

## Abstract

The biological species concept (BSC) defines a species as a group of organisms that can interbreed naturally and is segregated from sympatric assemblages by reproductive isolating mechanisms. Reproductive isolating mechanisms can be removed or rendered inoperative as a result of anthropomorphic alterations (e.g., altered thermal regimes). I studied the reproductive ecology of five redhorses (*Moxostoma* spp.) in Hiwassee Basin, North Carolina and Georgia, to identify and characterize functional reproductive isolating mechanisms currently acting to segregate these sympatric species. The spawning migration of black redhorse *Moxostoma duquesnei*, golden redhorse *Moxostoma erythrurum*, river redhorse *Moxostoma carinatum*, sicklefin redhorse *Moxostoma* sp., and silver redhorse *Moxostoma anisurum* was sampled with resistance board weirs and prepositioned areal electrofishers (PAEs) during the springs of 2006 and 2007 in Valley River, North Carolina. Smallmouth redhorse *Moxostoma breviceps* occupies Hiwassee Basin; however, it exhibits minimal spawning migration up Valley River.

A total of 977 adult redhorses were collected at the upstream and downstream resistance board weirs during 2006 and 2007, and 156 redhorse spawning sites were located during 2007. Spawning was observed for all five redhorse species in 2007 at water temperatures generally ranging 8–21 °C and mean daily Valley River flows ranging 1.7–4.5 m<sup>3</sup>/s. Silver redhorse migrated and spawned first, followed by black redhorse, sicklefin redhorse, golden redhorse, and river redhorse with considerable overlap occurring. Male golden redhorse and river redhorse displayed agonistic behavior and spawning site fidelity,

while female sicklefin redhorse displayed site fidelity. Silver redhorse and river redhorse were not significantly different ( $P > 0.05$ ) in size (i.e., length (TL) and weight), while the other redhorse species were ( $P < 0.05$ ). All five redhorse species exhibited a sexual dichotomy in tubercle development and maturation stage index, such that male tubercle development was considerably greater than that of females and males expressed gametes sooner and longer than females.

Golden redhorse was the only species observed to develop complete body tubercles, and golden redhorse and river redhorse were the only species observed to develop head and snout tubercles. The spawning migration chronology between males and females was significantly different ( $P < 0.05$ ) for black redhorse and sicklefin redhorse, but not for the other three species; however, spawning migration temporal bounds for both sexes of all species were similar. Downstream migrating redhorses were captured during intraspecific spawning observations or shortly thereafter, and generally in the same chronology as observed for upstream migration; however, catch rates were considerably lower.

All five species selected spawning microhabitat nonrandomly; however, redhorse spawning microhabitat characteristics were significantly different ( $P < 0.05$ ) due to deviation of one or two species, rather than all redhorses using unique microhabitats. Black redhorse and golden redhorse selected shallower spawning sites than the other redhorses. Golden redhorse and sicklefin redhorse exhibited a broad range of optimal bottom velocities, while the other species selected more specific ranges. Silver redhorse and golden redhorse selected swifter mean column velocities than the other redhorses. Black redhorse and golden redhorse

found coarse gravel as an optimal spawning substrate, while the other three redhorses selected small cobble. Black redhorse and sicklefin redhorse were the only two species to spawn in association with cover; however, sicklefin redhorse was the only species to select spawning sites immediately adjacent to cover. My findings suggest that seasonal, habitat, and ethological reproductive isolating mechanisms are functioning within Hiwassee Basin; however, anthropogenic activities and alterations have the ability to diminish or remove these reproductive barriers.

## **Introduction**

The biological species concept (BSC) defines a species as an assemblage of organisms (reproductive, ecological, and genetic unit) that currently or potentially interbreed naturally and is reproductively isolated from sympatric assemblages by reproductive isolating mechanisms (Dobzhansky 1937; Huxley 1942; Mayr 1942, 1969, 1971; and Mayr and Ashlock 1991). Mayr (1971) stated that “the reproductive isolation of a species is a protective device that guards against the breaking up of its well-integrated, coadapted gene system” and that this process typically is composed of several independent reproductive isolation barriers. Genetic deviations of sympatric populations and subsequent isolating mechanisms are diverse and generally divided into pre- and post-mating mechanisms (Mecham 1961; Mayr 1971). Pre-mating mechanisms consist of seasonal isolation (i.e., time), habitat isolation (i.e., space), ethological isolation (e.g., morphology and behavior), and mechanical isolation (e.g., spawning lacking gamete expression), while post-mating

mechanisms are typically characterized as gamete mortality, zygote mortality, unviable offspring, or infertile viable offspring (Mayr 1971). Ethological isolating mechanisms have been widely reported and are considered paramount in limiting hybridization among sympatric species (Liley 1966; Mayr 1971; Futuyma 1998; Yamazaki and Goto 2000). Dissimilar from spatial (e.g., habitat) and temporal (e.g., seasonal) isolating mechanisms that are regularly altered by humans, ethological isolating mechanisms (e.g., courting behavior, auditory recognition, pheromone reception) are typically less prone to manipulation by disturbance (e.g., habitat heterogeneity) and are therefore not excessively susceptible to breakdown (Hubbs 1961).

Such environmental factors that encumber pre-mating isolating mechanisms, as a function of space and time, have been broadly documented (Hubbs et al. 1943; Hubbs 1955, 1961; Nelson 1973) and are generally at an elevated risk to hindrance by human environmental disturbances compared to factors that may breakdown ethological isolating mechanisms. Aberrant habitat alteration and degradation has resulted in the breakdown of isolating mechanisms and interbreeding of fishes (Hubbs 1955) resulting in hybridization and subsequent disharmonious integrated genetic systems with no definite and specific niche (Mayr 1971). Ethological isolating mechanisms are crucial to speciation and reproductive segregation and will likely maintain function if isolating mechanisms associated with space and time persists. Catostomidae, Centrarchidae, and Cyprinidae are freshwater fish families considered highly susceptible to interspecific hybridization (Hubbs 1955), and thus are excellent models for field study of reproductive isolating mechanisms.

Redhorses *Moxostoma* spp. (Catostomidae) are potamodromous sympatric fishes that typically use upstream reaches of streams and rivers specifically for reproduction and lower river sections during post-spawning and overwintering periods (Curry and Spacie 1984; Page and Johnston 1990; Grabowski and Isely 2006; see Chapter 1). Over much of the range of the genus, several redhorse species inhabit the same river basin and occupy common upstream river reaches during the spring spawning season; however, redhorse interbreeding and subsequent hybridization are rarely reported (Jenkins 1970) suggesting highly specific and effective reproductive isolating mechanisms (e.g., time, space, morphology, behavior) (Kwak and Skelly 1992; Grabowski et al. 2008).

Isolating mechanisms efficiently segregating sympatric species fully develop or are refined as a result of natural selection advancing efficient isolating mechanisms by eliminating genotypes that are prone to yielding inferior interspecific hybrids relative to the two parental types (Dobzhansky 1940, 1951; Liley 1966). Dobzhansky (1940, 1951) referred to isolating mechanism strengthening or refining under circumstances of sympatry as “reinforcement” and suggested that reinforced reproductive isolating mechanisms are far more efficient than unreinforced reproductive barriers. Hubbs (1955, 1961) demonstrated that interspecific hybridization readily occurs between introduced allopatric fishes, and Liley (1966) suggested that this phenomenon is a result of inefficient isolating mechanisms that have not been subjected to reinforcement.

Spatial and temporal isolating mechanisms are regulated by environmental cues, which humans can alter by means of migration barriers, deforestation, agriculture, and water

withdrawal (Bryan and Rutherford 1993), while morphology and behavior as isolating mechanisms are generally species specific and less prone to manipulation or interference by anthropogenic influence (Hubbs 1961). Migration barriers (i.e., dams) alter environmental cues by primarily creating unnatural discharges and thermal regimes that manipulate downstream conditions (Yeager 1993) that migratory fishes use as indicators that coordinate spawning migration chronology. Deforestation contributes to abnormal flow regimes, increased sedimentation, and anomalous water temperatures (Filipek 1993). Agricultural practices contribute to sediment loading as well as elevating water temperatures, especially if cultivation or grazing practices incorporate the riparian zone (Fajen and Layzer 1993). Water withdrawals result in reduced discharges and subsequently elevated water temperatures (Zale et al. 1993). Altered discharge, water temperature, and sediment loading can result in hydrologic and microhabitat conditions that drastically deviate from those that historically facilitated effective isolating mechanisms that promoted the coexistence of numerous sympatric redhorse species that depended on common spawning tributaries to complete their life cycle.

Given the intensive and extensive microhabitat and hydrological degradation that many nongame potamodromous fishes have experienced in North America, acquiring detailed knowledge of the reproductive ecology of little known and managed fishes that face numerous risks is critical (Cooke et al. 2005). Research on reproductive ecology, relative to suitable spawning microhabitat and spawning migration hydrologic cues, is essential to

ensure that tailored management and restoration plans are conceived and executed with the highest likelihood of population enhancement possible.

Redhorses have historically been unappreciated by the public and have had a low management priority by state and federal resource agencies as these species are perceived as providing limited recreational or economical value (i.e., trash fish). Recently, implemented policy and legislation (e.g., Endangered Species Act (ESA), Committee on the Status of Wildlife in Canada, and the Species at Risk Act (SARA), Fisheries Act in Canada) have afforded imperiled nongame fishes in the United States and Canada an equal level of protection as fishes that are economically important, regardless of perceived societal value (Cooke et al. 2005). In addition, holistic management on an ecosystem or watershed scale is becoming more prevalent partially due to the realization that nongame fishes can be beneficial to game fish as well as the entire ecosystem including terrestrial species and an increased emphasis on overall fish community health. However, the reality is that management and conservation initiatives are rarely undertaken until it is determined that a nongame fish species or population is severely degraded or imperiled (Cooke et al. 2005). If a serious and concerted effort is going to be implemented and undertaken by fisheries and natural resource management entities to maintain and enhance fish diversity, understanding the isolating mechanisms among species is of critical importance because “isolating mechanisms are among the most important biological properties of species” (Mayr 1971).

## **Objectives**

The objective of this study was to identify and describe the reproductive isolating mechanisms of several sympatric freshwater redhorse fish species native to an anthropogenically altered montane river basin (Hiwassee Basin) in the southern Appalachian Mountains of North Carolina, Georgia, and Tennessee. Given the unique status of Hiwassee Basin's fish community including six redhorse species and current level of anthropogenic alterations, I desired to obtain crucial information on the reproductive ecology of the redhorse species within the basin. Spawning microhabitat use and availability and resulting microhabitat suitability for each species observed were quantified to identify spatial isolating mechanisms. Redhorse spawning chronology was quantified to identify temporal isolating mechanisms. Fish morphology and behavioral isolating mechanisms were also studied to identify interspecific variation. Specifically, my objectives were to characterize reproductive isolating mechanisms by comparing (1) spawning microhabitat; (2) migration and spawning chronology; (3) morphological characteristics; and (4) reproductive behavior.

## **Methods**

### *Site Description*

The Hiwassee River Basin is unique relative to the basins that support fishes of the genus *Moxostoma*, as it supports six species of redhorse. Only the upper Wabash River drainage in Indiana and Little Tennessee River drainage in North Carolina contain as many redhorses and none contain greater than six species (Jenkins 1999). The Hiwassee River

Basin supports black redhorse *Moxostoma duquesnei*, golden redhorse *Moxostoma erythrurum*, river redhorse *Moxostoma carinatum*, sicklefin redhorse *Moxostoma* sp., silver redhorse *Moxostoma anisurum*, and smallmouth redhorse *Moxostoma breviceps*. Hiwassee Basin is one of only two river basins that support the undescribed rare and imperiled sicklefin redhorse with the other river basin being the northerly adjacent Little Tennessee River Basin of North Carolina and Tennessee. Hiwassee Basin is fragmented by hydroelectric dams operated and maintained by the Tennessee Valley Authority (TVA) and Duke Energy and is altered in other ways by human activities such as siltation and exotic species (e.g., blueback herring *Alosa aestivalis*).

My study was conducted in the upper Hiwassee Basin of the southern Blue Ridge Province in the southern Appalachian Mountains, North Carolina and Georgia (Figure 1). Redhorse spawning was observed in unregulated tributaries between Mission Dam and Hiwassee Dam, with the majority of spawning observations being obtained from Valley River, North Carolina. Valley River is an unregulated seventh-order, moderate-gradient tributary of Hiwassee River and drains approximately 303 km<sup>2</sup>. Other tributaries in which spawning was observed were Brasstown Creek, Hanging Dog Creek, Peachtree Creek, Rapier Mill Creek, and Beaverdam Creek.

#### *Spawning Migration Sampling*

Migratory redhorses were sampled during the 2006 (20 April – 30 June) and 2007 (27 March – 6 June) spring spawning migrations. The redhorse spawning migration of Valley

River was sampled primarily with a pair of modified two-way resistance board weirs, including fish chutes and live traps that were constructed and installed following the general designs of Torbin (1994), Mogen (1996), and Stewart (2002, 2003). The two weirs were installed at downstream and upstream sites that were located 11.8 and 22.4 river km upstream from the mouth of Valley River, respectively. The same downstream and upstream sites were sampled during 2006 and 2007 and were 23.5 m and 21.5 m wide, respectively. In 2006, modified fyke nets functioned as the upstream and downstream live traps, but were periodically prone to perforation and debris congestion and were replaced with angle iron and metal conduit live boxes and fish chutes in 2007 (Stewart 2003). Both weirs were sampled concurrently over a 24-h period during 2006 and 2007. Weirs were set and collected at sunrise two days per week in 2006 and four days per week in 2007. Weirs were not sampled continuously throughout the spawning season to ensure the river remained relatively unimpeded for potamodromous fishes that might not pass through the weirs.

A gap width of 3.8 cm was the spacing interval between each picket in all weir panels, as well as each live trap and fish chute. This spacing interval was designed to sample even the most laterally compressed adult redhorse (sicklefin redhorse and black redhorse) that occupy the middle and upper reaches of Valley River (Jenkins 1999). In addition, a conservative stringer spacing interval (Torbin 1994) of 0.66 m was employed to ensure that all weir pickets and associated gaps were steadfast and nonyielding to fish passage attempts and significant flow.

Captured fish were placed in an instream recovery container, located in laminar flow before processing. Species, weight (g), total length (mm), migration direction, maturation stage, tubercle development (%), and condition prior to release were obtained for each fish collected. Captured redhorses were sexed, tested for maturation stage, and evaluated for percent tubercle development following criteria from Jenkins and Burkhead (1993) and R. E. Jenkins (Roanoke College, personnel communication). Percent tubercle development was visually and tactilely estimated, while maturation stage was gauged based on the magnitude of abdominal pressure that was required to achieve gamete expression. Maturation stages for both sexes were not ripe (NR), slight squeeze ripe (SSR), hard squeeze ripe (HSR), and running ripe (RR). After each fish was processed, it was released in the direction in which it was moving. Water temperature ( $^{\circ}\text{C}$ ), dissolved oxygen (mg/L), depth (m), bottom velocity (m/s) and mean column velocity (m/s) were also measured at each weir during each fish sample.

Prepositioned areal electrofishers (PAEs) were employed as a secondary technique to collect migratory redhorses from Valley River. PAEs were constructed and sampled similar to procedures described by Fisher and Brown (1993) and Grabowski and Isely (2005). PAE samples were collected concurrently with resistance board weir samples during 2006 and 2007. Two PAE samples were collected at each resistance board weir site; one was collected approximately 100 m upstream of each weir, and one was approximately 100 m downstream of each weir. Both PAE samples collected upstream of each weir were from a riffle, while PAE samples collected downstream of each weir were from pool habitat. Fish processing

followed identical methods as those for fish collected by resistance board weirs. Individuals captured and data collected were only applied to describe migratory redhorse tubercle development and maturation stage index. In addition, on 9 March 2007, 33 sicklefin redhorse were sampled via boat electrofishing associated with related research. Data collected for these individuals included sex, percent tubercle development, and maturation stage.

#### *Microhabitat Characterization*

Valley River was surveyed 2 – 3 times per week for actively spawning and courting redhorses from 27 March to 31 May 2007 and surveyed daily from 15 April to 15 May 2007. In addition, two sicklefin redhorse courting observations were obtained from Valley River on 4 May 2006 and included in the data set. Hiwassee River was surveyed weekly; Brasstown Creek, Hanging Dog Creek, and Nottely River were surveyed on a biweekly schedule from 27 March to 31 May 2007. Other tributaries within the upper Hiwassee Basin were irregularly surveyed for spawning and courting redhorses. Generally, effort was devoted to searching for spawning fish from sunrise until sunset when environmental conditions were favorable (e.g., suitable water clarity and temperature). From 15 April to 15 May 2007, surveys were conducted by foot along the river bank employing polarized glasses and binoculars. Prior to and following this period, spawning and courting redhorses were located by canoe as stream size and conditions allowed. Typically, from the bow of a canoe, spawning redhorses could be observed 30 – 50 m downstream due to thrashing of protruding

dorsal and caudal fins during quivering. Spawning sites were identified as silt-free oval depressions in the river substratum and were only included in this study if actual quivering was observed or spawning site fidelity displayed.

Once spawning or courting redhorses were observed by canoe or foot, the stream reach being used for spawning was approached from the bank. Fish were identified to species according to field characters described by R. E. Jenkins (personal communication). In addition to species identification, number of individuals involved in the spawning/courting act, quiver time if spawning was observed, and any pre- or post-spawning behavior was recorded. When possible, courting and spawning behavior was video taped for subsequent detailed analysis. At each site, spawning or courting was typically observed for a minimum of 1 h or until a definite spawning site (i.e., redd) could be identified and associated data collected.

Following spawning site identification and conclusion of behavioral observations, microhabitat characteristics were measured at the spawning site. Microhabitat data were collected from the bottom of the spawning site (i.e., pit) and immediately upstream of the spawning site (i.e., flat). Pit data described exact spawning microhabitat, while data from the flat described the site prior to disturbance by spawning activity (Reiser and Wesche 1977; Thurow and King 1994). Flat microhabitat data were not collected for silver redhorse spawning sites. Microhabitat variables measured were water temperature ( $^{\circ}\text{C}$ ), dissolved oxygen (mg/L), depth (m), bottom velocity (m/s), mean column velocity (m/s), dominant and subdominant substrate, cover type, distance to cover (m), distance to bank (m), and presence

or absence of river weed *Podostemum ceratophyllum*. Geographic coordinates were also obtained for each spawning site.

A top set-wading rod was used to measure depth to the nearest centimeter. A Marsh-McBirney flow meter (Model 2000) was used to measure bottom and mean column velocity (m/s). Mean column velocity was measured at a location in the water column 60% from the surface in depths of 0.75 m or less. In depths over 0.75 m, velocity was measured at locations in the water column 20% and 80% from the surface and averaged as mean column velocity (McMahon et al. 1996). Dominant and subdominant substrates were visually determined by estimating percent composition of surface substrates and classified based on a modified Wentworth classification system (Table 1; Bovee 1986). Nearest dominant cover type was visually determined by establishing the presence or absence of cover and then determining the distance between the spawning site and the associated cover. Cover types included in this study include no cover, coarse woody debris, fine woody debris, root wad, emersed aquatic vegetation, submersed aquatic vegetation, terrestrial vegetation, undercut bank, and boulder. River weed and cover were considered associated with spawning sites when they occurred within 2 m of the spawning site. Distance to bank (m) was visually estimated for all documented spawning sites.

In addition to measuring water temperature at each spawning site, continual hourly temperature data were collected using HOBO Water Temp Pro v2 Loggers (Onset Computer Corporation) from 11 January 2007 to 10 January 2008 for lower Valley River. Mean daily

discharge was obtained from the U. S. Geological Survey (USGS) gauging station 03550000 on Valley River (available online at <http://waterdata.usgs.gov/nwis/uv? 03550000>).

Line-transect surveys were conducted to characterize available microhabitat in Valley River, Brasstown Creek, and Hanging Dog Creek, as these tributaries were most commonly used for spawning by redhorses (Figure 2). Microhabitat availability data were collected when flow conditions were similar to those associated with microhabitat data collected at spawning sites. The line-transect survey method was chosen because it has minimal measurement error (McMahon et al. 1996) and is more repeatable than visual techniques (Stanfield and Jones 1998). Microhabitat availability surveys were conducted in stream reaches where spawning was observed (Figures 2-3), and surveyed reaches spanned beyond spawning sites in upstream and downstream directions, with transects evenly spaced two mean stream widths (MSW) apart. Microhabitat availability data were collected from lower, middle, and upper Valley River, from 57 transects and 689 survey points. Availability surveys were also conducted in middle and upper Brasstown Creek at 18 transects and 263 survey points, and in middle and upper Hanging Dog Creek at 25 transects and 328 survey points. All available microhabitat data were obtained employing identical sampling techniques as those for spawning microhabitat use.

### *Statistical Analysis*

Spawning Migration Chronology and Morphology.—Redhorse species have been broadly described to display a sexual dichotomy in spawning migration chronology where

males ascend spawning tributaries or mainstem spawning reaches prior to females (Hackney et al. 1967; Page and Johnston 1990). Therefore, the 2006 and 2007 resistance board weir catches were combined and mean catch per day was computed by sex for each species. Kolmogorov-Smirnov (K-S) two-sample tests were performed comparing male and female catches for each redhorse species to assess if an intraspecific sexual difference in migration chronology occurred. The nonparametric K-S test is effective with low sample sizes and non-normal distributions, and is sensitive to variation in location, dispersion, and skewness (Sokal and Rohlf 1981).

To assess variation in length and weight among the five redhorse species captured by resistance board weirs, a nonparametric Kruskal-Wallis test was performed. A nonparametric test was used because the majority of the length and weight samples were not normally distributed. A modified Nemenyi multiple comparison test (Dunn test) was performed when the Kruskal-Wallis test was significant (Zar 1996; Furlong et al. 2000).

Spawning Microhabitat.—After a majority of the variables were confirmed to be normally distributed, a Student's t test was performed on continuous microhabitat variables to identify differences between pit and flat microhabitats. Differences in spawning pit microhabitat characteristics for water temperature, depth, bottom velocity, mean column velocity, dominant substrate, distance to bank, and distance to cover were determined by employing the Kruskal-Wallis test. If the Kruskal-Wallis test rejected the null hypothesis, then nonparametric multiple comparisons were performed utilizing a modified Nemenyi test (Dunn test; Zar 1996). Differences in spawning pit cover type were determined by

contingency analysis. The average expected frequency among cells was calculated for each contingency table and examined for bias (Roscoe and Byars 1971; Zar 1996). I utilized the Pearson chi-square as apposed to the likelihood ratio chi-square, as the Pearson form yields a better approximation to table values (Ott and Longnecker 2001).

Spawning microhabitat frequency distributions were compared to corresponding microhabitat availability distributions. For each redhorse species, spawning microhabitat data that was obtained from middle and upper Valley River, Brasstown Creek, and Hanging Dog Creek and corresponding available microhabitat data were pooled to develop composite frequency distributions for microhabitat use and availability. I used a K-S test to compare the frequency distribution of spawning microhabitat to that of corresponding available microhabitat for continuous microhabitat variables to test for habitat selectivity. Dominant substrate was categorized and assigned a sequential number, ranging from one (clay) to 13 (bedrock), to compare dominant substrate use during observed spawning acts. An analogous chi-square likelihood ratio test was performed on categorical variables (cover and river weed occurrence) to test for nonrandom microhabitat use.

Microhabitat suitability was calculated for each variable by dividing percent microhabitat use by percent microhabitat available for each interval of the corresponding frequency distribution and dividing the resulting quotients by the maximum quotient of all intervals. Microhabitat suitability ranges from 0 to 1, with 0 indicating unsuitable microhabitat and 1 representing optimal microhabitat (Waters 1976; Bovee 1986).

Transferability of suitability functions may be limited to stream reaches similar to those from

which the use and available microhabitat were obtained (i.e., Category II) (Bovee 1986). To reduce bias associated with Category II criteria, influence of rare available microhabitat was eliminated from suitability analyses by omitting it and thereby yielding Category III criteria (Bovee 1986). The purpose of this modification was to enhance transferability of the suitability criteria among streams (e.g., Little Tennessee Basin) that may diverge from those where suitability criteria originated (i.e., Hiwassee Basin).

Most fishes, including redhorses, select spawning sites based on a suite of related microhabitat variables, rather than on a univariate basis. Thus, I performed principal components analysis (PCA, O'Rourke et al. 2007) on spawning and available microhabitat data obtained from Valley River. The PCA matrix included all continuous microhabitat variables to describe selectivity for spawning macrohabitat for all five redhorse species. First, PCA functions were developed from microhabitat availability data. Habitat availability scoring coefficients were then used to calculate principle component scores for microhabitats used for spawning. Principle components with eigenvalues greater than 1.0 were retained for interpretation (Kaiser 1960; Stevens 1996; Kwak and Peterson 2007). For each species, a K-S two-sample test was performed on scores of each retained principal component to test for significantly different frequency distributions between microhabitat use and availability, and between each redhorse species for microhabitat use. Statistical software packages SAS/STAT 9.1 and JMP 7.0 (SAS Institute, Cary, North Carolina) were used to conduct all statistical analyses. A significance level ( $\alpha$ ) of 0.05 was applied to all tests.

## Results

Discharge within upper Hiwassee Basin was generally similar between 2006 and 2007. Mean Valley River discharge during March through June 2006 was 4.50 m<sup>3</sup>/s (S.E., 0.14), and 3.79 m<sup>3</sup>/s (S.E., 0.23) in 2007. However, the Valley River hydrograph during 2006 was more variable than 2007 due to frequent precipitation events and subsequent discharge pulses in 2006 (March 21; April 1, 3, 8, and 22; May 21 and 27; and June 1). Precipitation and discharge pulses during 2007 were considerably less frequent (March 16; April 4; and May 5), resulting in reduced Valley River discharges during June 2007 compared to 2006.

Valley River water temperatures were generally similar during the redhorse spawning seasons of 2006 and 2007 with water temperatures increasing in mid to late-March to approach 20 °C in late-April and May. The most significant water temperature discrepancy between 2006 and 2007 occurred during early-April in 2007, when a cold front reduced average mean daily water temperature to 10.8 °C (6.8 – 13.9 °C) between 5 April and 21 April. Average mean daily water temperatures one week before and after the cold front were 16.2 °C and 15.6 °C, respectively.

### *Morphology, Maturation Stage, and Migration Chronology*

A combined total of 977 adult redhorses of five species were collected at the upstream and downstream resistance board weirs during 2006 (169 fish) and 2007 (808 fish) (Table 2). The considerable increase in redhorse catch from 2006 to 2007 may be attributed

to increased sampling effort and addition of more efficient angle iron and metal conduit live traps in 2007 that replaced the modified fyke nets employed in 2006. Overall, sicklefin redhorse was the most abundant redhorse species captured ( $N = 267$ ), while the river redhorse was the least abundant ( $N = 25$ ). Smallmouth redhorse were not collected in either weir in 2006 or 2007 likely because that species spawns in lower reaches of Valley River, downstream of either weir (Jenkins 1999). Generally, with the exception of the river redhorse, female fish outnumbered males (Table 2). Female black redhorse (76.9%), sicklefin redhorse (69.7%), golden redhorse (62.4%), and silver redhorse (53.4%) comprised the majority of the catch. Females of all species were larger and heavier than males (Table 2). The silver redhorse and river redhorse were the largest species captured with mean weights of 1,700.3 g and 1,604.6 g, respectively. The black redhorse and golden redhorse were the smallest with mean weights of 921.2 g and 1,008.9 g, respectively. The smallest redhorse captured by a resistance board weir was a male black redhorse that was 370 g and 362 mm; the largest redhorse was a female silver redhorse 2,718 g and 613 mm.

A Kruskal-Wallis test detected significant differences among redhorse species in both total length and weight ( $P \leq 0.0001$ ). Multiple comparison tests revealed significant differences ( $P \leq 0.05$ ) between most species; however, river redhorse were not significantly different from sicklefin redhorse or silver redhorse in length, nor were black redhorse and golden redhorse from each other. Silver redhorse and river redhorse were the only species that were not significantly different in weight ( $P \geq 0.05$ ).

A sexual dichotomy was observed in tubercle development for all five species sampled by weir and PAE. Males typically exhibited moderately to fully developed tubercles on the caudal and anal fins, while females generally displayed no to slight tubercle development (Figure 4). My redhorse tubercle development and location observations are in agreement with those detailed by Jenkins and Burkhead (1993). Black redhorse, silver redhorse, and sicklefin redhorse exhibited prominent tubercle development on the anal fin and lower lobe of the caudle fin. Similarly, the river redhorse displayed anal and caudle fin tubercles, but also develops prominent snout tubercles. Golden redhorse exhibited tubercles similar to the black redhorse, river redhorse, sicklefin redhorse, and silver redhorse, but also displayed body tubercles that developed on nearly every scale as well as the head.

All male redhorse species sampled initiated tubercle development before or during early-March. Silver redhorse peak tubercle development occurred during April and early-May and approached full tubercle reabsorbtion in May and June (Figure 4). Black redhorse, golden redhorse, sicklefin redhorse, and river redhorse attained full tubercle development from mid-April to mid-May and complete tubercle reabsorbtion from late-May through June (Figure 4). Female redhorse mean peak tubercle development and duration of exhibition appeared to coincide with that of males; however, was never greater than 20% developed (Figure 4).

Maturation stage (i.e., ripeness) also differed between sexes for all five redhorse species collected. Males sampled by weir were generally ripe to some degree (i.e., expressed milt), while females were typically not ripe while migrating upstream regardless of the

timing in the spawning season (Figure 5). In general, females reached a higher degree of maturation toward the end of the migration, while males were typically at a high maturation stage throughout the upstream spawning migration (Figure 5).

Migration and spawning chronology appears to initiate with silver redhorse, followed by sicklefin redhorse, black redhorse, golden redhorse, and river redhorse (Figure 6). Silver redhorse upstream migration likely commenced prior to weir installation during both years, but spawning migration neared completion in early to mid-May with peak migration in late-March and early-April. Black redhorse, golden redhorse, and sicklefin redhorse upstream migration started in late-March (Figure 6). The spawning migration of black redhorse and sicklefin redhorse peaked in late-April and early-May and approached completion in mid-May, although capture of solitary migratory individuals was common until late-May for black redhorse and early-June for sicklefin redhorse. Golden redhorse spawning migration climaxed during mid-May and fully terminated by late-May and the first week of June. The spawning migration of river redhorse was from late-April to mid-June (Figure 6).

The spawning migration chronology of sicklefin redhorse and black redhorse was statistically significantly different between sexes ( $P < 0.0006$ ); golden redhorse, river redhorse, and silver redhorse chronologies were not statistically significantly different between sexes (Figure 6;  $P > 0.05$ ). Despite a sexual dichotomy in the spawning migration of both black redhorse and sicklefin redhorse ( $P < 0.0001$  and  $P = 0.0005$ , respectively), the bounds of migration timing were similar between sexes. A nearly equal ratio of migratory male and female golden redhorse were captured from mid-March to late-April when females

became more frequently represented in weir samples in early-May; however, male golden redhorse became more common than females during the peak and end of the migration in mid and late-May (Figure 6). The spawning migration of male and female river redhorse and silver redhorse not only appear to commence and terminate simultaneously, but also appear to occur at equivalent magnitudes throughout the spawning migration ( $P = 0.9080$  and  $P = 0.5601$ , respectively). The general trend among these five redhorse species is that males and females start and end spawning migrations simultaneously, but no common sexual dichotomy occurs among all of these potamodromous redhorse populations.

Downstream migration was not documented well for all redhorse species during 2006, but sampling efficiency of downstream migrants improved in 2007. Passive capture and retention of downstream migrating redhorses during 2006 with modified fyke nets was inefficient, as fish were reluctant to enter the traps and fish that did enter the nets could escape due to their upstream orientation (i.e., facing the trap opening). During 2007, angle iron and metal conduit live traps were employed, and golden redhorse ( $N = 15$ ), river redhorse ( $N = 4$ ), sicklefin redhorse ( $N = 21$ ), and silver redhorse ( $N = 79$ ) downstream migration was captured. The majority of downstream migrating fish was sampled in late-March and April during high to moderate flows and catches reduced as discharge decreased in late-April and May.

In 2007, downstream migration chronology resembled that of upstream migration chronology during 2006 and 2007. Silver redhorse were consistently captured migrating downstream from 23 March – 4 May, with peak rates of 19 and 10 fish occurring on 5 and 6

April, respectively. Sicklefin redhorse were sporadically captured within the downstream trap of the downstream weir from 22 March – 8 June with peak capture rates occurring on 2 and 6 April and 26 and 27 April. Golden redhorse were consistently captured from 10 May – 23 May with a peak sampling of 6 fish occurring on 17 May. Downstream migrating river redhorse were captured on 26 April, 18 May, and 30 May. No black redhorse were captured migrating downstream during 2007.

#### *Spawning Chronology, Water Temperature, and Discharge*

The spawning chronology of redhorses within upper Hiwassee Basin was generally similar to their migration chronology in Valley River (Figure 7). Silver redhorse spawning occurred and ended prior to the commencement of any other redhorse spawning during 2007. Sicklefin redhorse, black redhorse, golden redhorse, and river redhorse spawning all co-occurred within the same river reaches, but was staggered over time (Figure 7).

Silver redhorse spawning sites were located the earliest, associated with the highest discharge volumes (Figure 7). Silver redhorse spawning was observed during late-March when mean daily water temperatures were approximately 16 – 18 °C and mean daily discharge was 4.1 – 4.3 m<sup>3</sup>/s (Figure 7). Generally, observation dates of spawning sicklefin redhorse and black redhorse were synonymous, during mid to early-May. Mean daily water temperatures and daily discharges that corresponded to sicklefin redhorse and black redhorse spawning were 16 – 19 °C and 2.9 – 4.5 m<sup>3</sup>/s, respectively. Golden redhorse spawned during early to mid-May and were encountered in abundance simultaneously with spawning

sicklefin redhorse and black redhorse. Golden redhorse spawned with mean daily water temperatures of 18 – 20 °C and mean daily discharges of 3.0–3.6 m<sup>3</sup>/s. River redhorse spawning was relatively brief, predominately occurring during late-May at mean daily water temperatures of 17 – 20 °C and mean daily discharges of 1.7 – 2.7 m<sup>3</sup>/s and overlapped with the conclusion of golden redhorse spawning (Figure 7).

### *Spawning Sites and Microhabitat*

The majority of redhorse spawning occurred within middle and upper reaches of Valley River. However, black redhorse, golden redhorse, and sicklefin redhorse were also observed spawning in Beaverdam Creek, Peachtree Creek, Brasstown Creek, or Rapier Mill Creek (Figure 3). All river redhorse and silver redhorse spawning sites were observed in Valley River. Overall, Valley River yielded 114 (73.1%) spawning sites among the five redhorse species observed (Figure 3).

Redhorse pit and flat spawning microhabitat variable comparisons were significantly different for 9 of 16 comparisons (Table 3;  $P < 0.05$ ). Thus, pit microhabitat measurements were used for all resulting analyses as this point was actually occupied during the quivering process. However, all river redhorse pit and flat spawning microhabitat variable comparisons were significantly different ( $P < 0.05$ ), and all sicklefin redhorse pit and flat spawning microhabitat variable comparisons were not significantly different (Table 3;  $P > 0.05$ ). This finding indicates consistent differences in pit and flat microhabitat characteristics, but they were not substantial in magnitude for most variables (Table 4).

Except for silver redhorse, water temperatures associated with spawning sites varied widely among redhorse species (7.5–21.1°C); however, mean water temperatures associated with spawning sites among species were similar (16.8–18.8 °C; Table 4). All redhorse species, except the golden redhorse, had a propensity to spawn close to the bank. Black redhorse and golden redhorse spawned in shallow depths, while sicklefin redhorse sites were common in moderate depths, and silver redhorse and river redhorse spawning sites occurred at deeper depths. Black redhorse spawning sites occurred in slow current velocities, and sicklefin redhorse sites were common in moderate velocities, while golden redhorse, river redhorse, and silver redhorse spawned in swift velocities. Black redhorse and golden redhorse used spawning substrates ranging from coarse gravel to small cobble, while river redhorse, sicklefin redhorse, and silver redhorse spawning sites were common in small cobble. Sicklefin redhorse was the only species to spawn occasionally over small boulder substrate. Golden redhorse, river redhorse, and silver redhorse spawning sites were typically not associated with cover, while those of black redhorse were commonly near a boulder or overhanging terrestrial vegetation. Those of sicklefin redhorse were typically adjacent to a boulder or embedded coarse woody debris. Mean distance to cover for black redhorse spawning sites was 0.95 m, while those of sicklefin redhorse typically occurred < 0.25 m from cover (Table 4).

Kruskal-Wallis tests revealed significant differences among redhorse species spawning microhabitat for water temperature, distance to bank, depth, bottom velocity, mean column velocity, dominant substrate, and distance to cover (Table 5;  $P < 0.0001$ ), and

contingency table analysis of cover revealed significant differences among species (Table 5;  $P < 0.0001$ ). Additional contingency table analyses revealed that golden redhorse, river redhorse, and silver redhorse were not significantly different in cover type associations (Table 6;  $P = 0.1783$ ), but sicklefin redhorse and black redhorse were significantly different from the other redhorse species, as well as each other (Table 6;  $P < 0.0001$ ).

Redhorse spawning sites were generally significantly different for microhabitat variables due to considerable deviation of one or two species, rather than all redhorses using unique microhabitats. Specifically, Dunn's multiple comparison tests reveal that black redhorse and silver redhorse differ significantly for spawning water temperature (Figure 8). Black redhorse, sicklefin redhorse, and silver redhorse spawning sites were all significantly different from the other for depth (Figure 9). Bottom velocity of silver redhorse spawning sites was significantly faster compared to those of all other redhorses, but those of all other redhorses were not significantly different from one another (Figure 10). Mean column velocity of black redhorse spawning sites was significantly slower from that of the other redhorses species, while all the other redhorse spawning sites were not significantly different from one another (Figure 11). Dominant substrate of black redhorse spawning sites was significantly finer from those of all the other redhorse species, while river redhorse, sicklefin redhorse, and silver redhorse spawning sites were not significantly different (Figure 12). Spawning sites of all redhorse species were not significantly different for distance to bank (Figure 13). Spawning sites of black redhorse and sicklefin redhorse were significantly different from one another for distance to cover, but from those of the other redhorse species

(Figure 14). However, golden redhorse, river redhorse, and silver redhorse spawning sites were rarely associated with cover and therefore had small distance to cover sample sizes (Table 4).

Univariate frequency distributions of microhabitat use and microhabitat availability were significantly different for depth, bottom velocity, mean column velocity, dominant substrate, cover, and occurrence of river weed for all five redhorse species (Figures 15-21;  $P < 0.05$ ), suggesting that these fish select and use spawning microhabitat nonrandomly. Exceptions to non-random spawning microhabitat selection were found in golden redhorse and silver redhorse for distance to bank (Figure 19) and for all redhorses except sicklefin redhorse for distance to cover (Figure 21). Frequency distributions of microhabitat use revealed that all five redhorse species spawn in shallow water, despite deeper water being widely available (Figure 15). Black redhorse tended to select the shallowest spawning sites, while silver redhorse and river redhorse generally selected the deepest (Figure 15). All redhorse species most frequently spawned in relatively slow to moderate bottom velocities, despite considerably greater bottom velocities being available (Figure 16). Black redhorse tended to spawn within the narrowest range of bottom velocities, while golden redhorse and sicklefin redhorse used the broadest range. Conversely, all redhorse species spawned in relatively swift mean column velocities, yet these swifter velocities were substantially less common than slow velocities (Figure 17). Black redhorse and river redhorse spawned in the narrowest range of mean column velocities, while golden redhorse spawned in the widest range. With the exception of black redhorse, the majority of redhorse spawning sites were

associated with small cobble (Figure 18). Black redhorse dominant substrate use was broad, but most frequently associated with medium gravel. River redhorse and silver redhorse dominant substrates were most narrowly selected. All redhorse species tended to select spawning sites near the bank (Figure 19). With the exception of sicklefin redhorse, spawning sites of all species were most commonly not associated with cover, but sicklefin redhorse spawning sites were most frequently associated with boulders and occasionally coarse woody debris (Figure 20). Sicklefin redhorse deviated considerably from the other redhorses in that sicklefin redhorse typically spawned very close to cover (Table 4; Figure 20). Although river weed was available within stream reaches being utilized for spawning, no redhorse spawning site contained river weed.

For all redhorse species, interspecific and intraspecific patterns in spawning microhabitat suitability are similar to corresponding microhabitat use frequency distributions (Figures 15-28). Depth suitability distributions reveal that black redhorse and golden redhorse preferentially select relatively shallow depths for spawning, while river redhorse, sicklefin redhorse, and silver redhorse find moderate and deeper depths optimal (Figure 22). Bottom velocity suitability distributions reveal that black redhorse and river redhorse find slow to moderate velocities optimal, while a narrow range of moderate bottom velocities were optimal for silver redhorse (Figure 23). In addition to the golden redhorse and sicklefin redhorse bottom velocity optimum suitabilities being inclusive of a broad range of velocities, swift bottom velocities are most suitable compared to slow and moderate bottom velocities that were utilized most frequently (Figures 16, 23). Mean column velocity suitabilities

among all species are diverse (Figure 24). Black redhorse, river redhorse, and sicklefin redhorse found intermediate mean column velocities most suitable, while extremely swift mean column velocities were optimum for golden redhorse and silver redhorse spawning (Figure 24). Golden redhorse and silver redhorse primarily spawned in intermediate mean column velocities; however, swift velocities were most suitable because slow and moderate velocities were abundant and swift currents were rare (Figures 17, 24). Similar to interspecific and intraspecific patterns in microhabitat use for substrate, river redhorse, sicklefin redhorse, and silver redhorse selected coarser optimal substrates than did black redhorse and golden redhorse (Figures 18, 25). Black redhorse, golden redhorse, and sicklefin redhorse had a wide range of dominant spawning substrate suitabilities, while the river redhorse and silver redhorse had relatively narrow spawning ranges (Figure 25). With the exception of black redhorse and sicklefin redhorse, interspecific and intraspecific patterns in microhabitat suitability for distance to bank, cover type, and distance to cover for all species are similar to those for corresponding microhabitat use frequency distributions (Figures 19-21, 26-28). Suitability distributions indicate that terrestrial vegetation is optimum spawning cover for black redhorse and that coarse woody debris (embedded logs) is optimal cover for sicklefin redhorse, despite these cover types being less commonly utilized (Figures 20, 27).

Principal components analysis (PCA) revealed multivariate habitat gradients describing marginal slow and silty habitat to mid-channel swift habitat primarily composed of bedrock (component 1) and shallow swift habitat near cover to deep slow habitat with

cover absent (component 2). All redhorses occupied spawning macrohabitats nonrandomly for component 1 and 2 ( $P < 0.05$ ; Table 7). For redhorse spawning microhabitat use comparisons, significant differences ( $P < 0.05$ ) were found for all comparisons for at least one principal component, except for the golden redhorse and silver redhorse comparison (PC 1,  $P = 0.1344$ ; PC 2,  $P = 0.6945$ ; Table 8). Component 1 and component 2 cumulative available microhabitat variance explained is 61.1% (Table 9). Spawning microhabitat use for all five species overlapped to some degree; however, PCA illustrates that each species diverges from the other to some extent (Figure 29). Redhorse spawning sites were not found in slow, deep habitats over fine substrates (high component 2 scores; Figure 29), further confirming that spawning microhabitats were not selected randomly. Spawning sites of silver redhorse and river redhorse were most similar; however, those of the river redhorse were more specific than those of silver redhorse (Figure 29). Sicklefin redhorse and golden redhorse spawning sites were least similar; however, overlap occurred in shallow, swift habitats (low component 2 scores; Figure 29). Black redhorse selected the narrowest range of spawning habitats, while the golden redhorse showed the broadest range (Figure 29). Golden redhorse and sicklefin redhorse deviated most from other redhorse species in spawning habitat. Golden redhorse spawned in extremely swift, shallow riffles near the thalweg (high component 1 scores and low component 2 scores), while sicklefin redhorse spawned in swift, shallow marginal riffles in close proximity to cover (low component 1 scores and low component 2 scores; Figure 29).

### *Spawning Behavior*

Similarities are common in the spawning behavior of all five redhorse species with several notable divergences. Mean quiver duration was similar for all species with golden redhorse generally displaying the briefest quiver duration and sicklefin redhorse typically displaying the longest quiver duration (Table 10). Generally, all species spawned in trios most commonly, but pair spawning was frequently observed for golden redhorse. Black redhorse and sicklefin redhorse frequently engaged in quivering when more than three individuals (i.e., sicklefin redhorse, 4 – 15) were participating in the spawning act (Table 10). Agonistic behavior was only observed in male golden redhorse and river redhorse, but aggressive behavior in close proximity to the spawning site was observed more frequently for golden redhorse than for river redhorse (Table 10). Interspecific quivering was observed, albeit rarely, between black redhorse and sicklefin redhorse in Valley River, Brasstown Creek, and Beaverdam Creek (Table 10). Interbreeding occurred in both directions, such that observations were made when a single black redhorse was observed courting and spawning with a sicklefin redhorse troupe and two sicklefin redhorse were observed courting with a black redhorse troupe over an apparent spawning site. An undescribed behavior for redhorses was exhibited by 8 (25.8%) of 31 spawning female sicklefin redhorse and best characterized as a repetitive and violent body undulation behavior, distinctly different from quivering that occurred immediately upstream of the spawning site while rapidly advancing upstream. Quivering was never observed following this body undulation behavior, but undulation was observed to occur on a repetitious basis immediately following quivering for

two identified spawning sites. These two post-spawning body undulations by female sicklefin redhorse lasted for 1 – 3 s and occurred approximately every 1 – 2 min and continued for up to 2 h. No other redhorse species observed spawning within the upper Hiwassee Basin displayed this apparently unique and undescribed behavior (post-spawning digging) during the 2007 spring spawning season.

### *Post-Spawning Mortality*

During 2006, very few deceased redhorses were observed at either weir; this may have been a function of only sampling twice per week. During 2007, when the weirs were operated four days per week, considerable numbers of moribund and dead adult redhorses were observed impinged on the upstream section of each resistance board weir (Table 11). Dead redhorses impinged at the upstream weir ( $N = 111$ ) were considerably greater than those impinged at the downstream weir ( $N = 27$ ). No dead silver redhorse were observed at either weir during 2006 or 2007 (Table 11). Few dead black redhorse, river redhorse, and sicklefin redhorse were observed at the weirs, but considerable numbers of dead golden redhorse were recovered at the weirs (Table 11). Impinged dead redhorses recovered at weirs were not included in spawning migration counts, because it could not be determined that these fish were in the process of actively migrating downstream prior to mortality. A high percentage of dead redhorses recovered at weirs displayed caudle bruising, anal fin erosion, substantial loss of pigmentation on the outer margin of the fins, and *Flexibacter columnaris* symptoms (Table 11). Golden redhorse males demonstrated higher mortality

despite females constituting 62.4% of the adult migrating population. The majority of the mortalities occurred during May of 2007 (Table 11).

## **Discussion**

Hybridization between sympatric species is infrequent between internal breeders (e.g., birds, mammals, reptiles); however, external breeders (e.g., the majority of fish) commonly and either intentionally or inadvertently, produce hybrid offspring (Mayr 1971). This phenomenon suggests that sympatric fishes are subject to an elevated probability of interspecific hybridization, and thus, may be more susceptible to altered environmental factors that weaken reproductive isolating mechanisms. Natural hybridization is not an uncommon occurrence for the family Catostomidae (Hubbs 1955; Smith 1966; Nelson 1968; Schwartz 1972, 1981); however, naturally occurring interspecific hybrids belonging to the genus *Moxostoma* are virtually nonexistent, prompting Jenkins (1970) to conclude that operational reproductive isolating mechanisms must be functioning effectively where sympatric redhorses occur (Kwak and Skelly 1992). Virtual absence of interspecific hybrids in the genus *Moxostoma*, assuming some level of genetic compatibility, is presumably a function of sympatry and operational, reinforced reproductive isolating mechanisms (Dobzhansky 1937).

Redhorses exhibit several characteristics that promote reproductive isolation and lack of interspecific hybrids. Mayr (1971) suggested that courtship, a behavior demonstrated by most redhorses (Jenkins 1970; Page and Johnston 1990; Jenkins and Burkhead 1993), is an

efficient ethological isolating mechanism. In addition, spawning tributary homing is a prime factor responsible for maintaining reproductive isolation within sympatric species (Hubbs 1961). Radiotelemetry studies indicate that adult spawning sicklefin redhorse that conduct concurrent annual spawning migrations, do so to the same spawning tributary and same general stream reach in the upper Hiwassee Basin (see Chapter 1). Other redhorse species also exhibit considerable spawning site fidelity (Sule and Skelly 1985; Grabowski and Isely 2006). Aquatic species hybridization is less likely to occur if reproduction is limited to specific water temperatures (Mayr 1971), and spawning migration chronology is annually repetitive among sympatric species and correlated with regular external environmental stimuli (e.g., photoperiod, stream discharge, water temperature). Annual sequential spawning migrations, in addition to that in Valley River described here, have been reported for other sympatric redhorse populations in central and eastern North America (Meyer 1962; Curry and Spacie 1984; Grabowski and Isely 2007).

The affect of aberrant temperature regimes was observed in 2007 due to a natural phenomenon (i.e., cold front) that temporarily decreased water temperatures and interrupted the redhorse spawning migration, resulting in minimal migration and spawning during mid-April and simultaneous spawning observations after water temperatures warmed. Hubbs (1961) stated that “disturbances of habitats, either natural or artificial, often lead to crossbreeding” due to reproductive isolating mechanisms becoming compromised. The consequences of this natural phenomenon elucidate the effect that hypolimnetic releases by hydroelectric dams may have on migration chronology of interspecific species. Water

temperature for Hiwassee Basin redhorses appears to be a primary seasonal reproductive isolating mechanism affecting not only timing, but occurrence of spawning migration for each species; stressing the importance of ecological factors (i.e., environmental potential) over genetic mechanisms as the central factor guiding speciation (Hubbs 1961).

Hubbs (1961) stressed that “environmental potential” is the primary factor guiding reproductive isolating mechanisms and subsequent speciation, and therefore is a principal factor establishing the rate of genetic isolation. Inevitably, genetic variation exists among sympatric species and is presumably responsible for current reproductive isolating mechanisms; however, environmental potential ultimately yielded genetic variation through natural selection, and therefore was a focus of this study to promote reproductive isolation understanding and enable management practices that facilitate continued reproductive segregation. Liley (1966) argued that understanding and maintenance of reproductive isolating mechanisms is of considerable importance, “irrespective of the existence of partial or complete barriers due to genetic incompatibility.”

It has been broadly reported that male redhorse precede conspecific females in their spawning migration and arrival to spawning areas, and that males achieve earlier spawning readiness and a greater level of tubercle development than conspecific females (Meyer 1962; Hackney et al. 1967, 1971; Page and Johnston 1990). Unexpectedly, for all redhorses for which spawning was observed and representative individuals were captured with resistance board weirs, there was no temporal spawning migration sexual dichotomy present. However, similar to previous accounts, sexual differences in percent tubercle development and

maturation stage (i.e., spawning readiness) were clear in all species; such that, males exhibited exceedingly advanced tubercle development as well as earlier occurrence and prolonged expression of gametes compared to females. Considering the universal lack of a temporal sexual dichotomy in spawning migration and sexual differences in tubercle development and maturation stage index in all five redhorse species, it is likely that these three mutual attributes do not function as ethological reproductive isolating mechanisms among redhorses in the upper Hiwassee River Basin.

Mayr (1971) stated that ethological barriers are “the largest and most important class of isolating mechanisms in animals” and compatibilities in courtship behavior (e.g., visual, auditory, tactile, chemical) between conspecific individuals promote species recognition through appropriate stimuli between male and female. The fact that interspecific variation in tubercle location (i.e., morphology) occurred among species, may suggest an ethological isolating mechanism. For example, male golden redhorse that exhibited full body tubercle expression would quiver when physically handled during my data collection, suggesting that tubercles may function as self-stimulating morphological features when physically contacted by conspecifics, in addition to serving as locality stabilizing features or female stimulators during the act of quivering (Burr and Morris 1977). In addition, head tubercles, such as those that were observed on the golden redhorse and river redhorse, have been suggested as characteristics beneficial during agonistic behavior or defending territory (Kwak and Skelly 1992; Jenkins and Burkhead 1993). Tinbergen (1954) stated that analysis of ethological

reproductive isolating mechanisms is complicated, because courtship displays (i.e., color, pattern, form) may serve various functions (Mayr 1971).

Hubbs (1961) suggested that unbalanced sex ratios promote interspecific hybridization. Redhorse sex ratios are typically skewed with a greater proportion of males compared to females (Meyer 1962; Grabowski and Isely 2007). However, Grabowski and Isely (2007) suggested that a male dominated sex ratio may be an artifact of biased sampling from spawning grounds to which males display site fidelity not shared by nearby staging female conspecifics not subject to sampling techniques. This sampling bias may also partially explain why it is commonly concluded that males arrive at spawning sites prior to conspecific females. I found for actively migrating black redhorse, golden redhorse, sicklefin redhorse, and silver redhorse captured by weirs, considerably skewed sex ratios favoring females during the spawning migration. Regardless of sex ratios, interspecific spawning was practically absent among observed spawning acts during this study. Therefore, it is reasonable to conclude that moderately unbalanced sex ratios favoring females do not readily result in interspecific breeding within the genus *Moxostoma*.

The greatest temporal discrepancies in migration and spawning occurred between the silver redhorse and river redhorse. Silver redhorse and river redhorse spawning microhabitats are virtually identical, but silver redhorse commence spawning migration relatively early (mid-March), while river redhorse arrive on spawning grounds relatively late (mid-May). In fact, several river redhorse spawning observations were precisely from previously occupied silver redhorse spawning sites within Valley River. Deviations between

the measured spawning microhabitat characteristics for silver redhorse and river redhorse are primarily a result of much lower flows during May relative to those of March. These observations eliminate space as an operating reproductive isolating mechanism and indicate that time may function as an important if not the primary isolating mechanism between these two sympatric redhorses. Morphologically, the silver redhorse and the river redhorse were the only species without significant differences in size distributions; however, the river redhorse displayed agonistic behavior, while the silver redhorse demonstrated no territorial behavior. During this study, silver redhorse generally concluded spawning prior to river redhorse commencing spawning, and it is therefore uncertain if river redhorse agonistic behavior could function as an isolating mechanism if environmental factors resulted in a common spawning season between the species. However, interspecific spawning between silver redhorse and river redhorse have previously been observed in the upper Hiwassee River Basin (B. J. Freeman, University of Georgia, and M. C. Freeman, U.S. Geological Survey, personal communication), suggesting that river redhorse agonistic behavior may not serve as an absolute ethological isolating mechanism between these two sympatric species. Space, morphology, and behavior do not appear to operate as reproductive isolating mechanisms between silver redhorse and river redhorse, while time appears to function in that role.

Black redhorse, golden redhorse, and sicklefin redhorse not only migrated and spawned synchronously, but also occupied the same stream reaches while courting and spawning. Interspecific interbreeding was observed, albeit negligible, and only present

between the black redhorse and sicklefin redhorse. Kwak and Skelly (1992) concluded that time is not an operational isolating mechanism between black redhorse and golden redhorse in a spawning tributary of east-central Illinois, and that spawning microhabitat, morphology, and behavior are responsible for isolating these simultaneous spawning syntopic species. Similarly, I also found that these two species, in addition to sicklefin redhorse, segregate by spawning microhabitat and are not isolated by time. Black redhorse spawning sites in Valley River were typically located in slow to moderate flow of extremely shallow depth and generally located in marginal habitat where gravel substrates were dominant and overhanging terrestrial vegetation was common. Golden redhorse spawning sites, in Valley River, were typically located within exceptionally swift currents (e.g., 0.7–1.1 m/s) near the thalweg and commonly constructed over gravel and cobble absent of cover. Conversely, Kwak and Skelly (1992) reported that black redhorse spawned in shallow, swift and turbulent riffles comprised of gravel and cobble and that golden redhorse used shallow shoals characterized as having low to moderate flows and sand and gravel as the dominant substrates. These disparate spawning microhabitat relationships between conspecifics of different geographic regions has been recognized and documented by Baker et al. (1994), Freeman et al. (1997), and Williams et al. (1999) and emphasizes the importance of managing aquatic systems on an ecosystem basis rather than for any single criterion (e.g., temperature or flow) to enhance microhabitat heterogeneity and diversity. In addition to promoting overall bionetwork health, ecosystem management precludes resource agencies from mistakenly managing for

published population specific suitable spawning microhabitat that may differ from that for the population being managed in a different geographic region.

A plausible explanation for the dissimilarity between black redhorse and golden redhorse spawning microhabitat may be due to spawning substrate suitability and morphology (i.e., body size). Kwak and Skelly (1992) found that staging adult golden redhorse that selected fine spawning substrates and slower currents were generally smaller than adult spawning black redhorse that used swifter currents and larger spawning substrates. Contrarily, adult black redhorse in Valley River, that selected slow currents and gravel substrates, were generally the same length as sympatric golden redhorse; however, adult golden redhorse, that selected extremely swift water velocities and larger substrates, typically weighed more and were more robust. Adult river redhorse, sicklefin redhorse, and silver redhorse, on average, were all larger than black redhorse and golden redhorse in Valley River, and incidentally, all selected even larger substrates (i.e., small cobble – small boulder) for spawning. Witzel and MacCrimmon (1983) found that sympatric brook trout *Salvelinus fontinalis* and brown trout *Salmo trutta* in a southwestern Ontario stream used stream velocities and substrate in a similar fashion, such that the larger adult spawning brown trout selected spawning sites in swifter currents with larger substrate than did the smaller adult brook trout. The correlation between body size and current velocity and substrate size may be a direct spatial reproductive isolating mechanism and indirectly an ethological reproductive isolating mechanism segregating sympatric fishes belonging to the genus *Moxostoma* in the Hiwassee Basin and likely other systems containing sympatric riverine

fishes that deposit gametes on or in substratum during spawning (i.e., lithophils) (Balon 1975). Disparate relationships between Illinois (Kwak and Skelly 1992) and North Carolina black redhorse and golden redhorse suggest that fisheries managers would be prudent to validate microhabitat suitabilities that originated from geographically distinct riverine systems, especially when the species being managed significantly deviates morphologically between systems or composition of sympatric species is dissimilar.

In addition to being the most abundant adult redhorse captured during this study, the sicklefin redhorse migration overlapped, to some degree, with the migration of all the other redhorse species sampled. This scenario suggests that sicklefin redhorse were poised to potentially interbreed with any of the other sympatric redhorses in the absence of other isolating mechanisms. This undescribed and imperiled species that is restricted to only two river basins throughout its range in western North Carolina and northern Georgia was identified as a separate and unique species within *Moxostoma* by R. E. Jenkins in 1992 and has been described to only spawn with conspecifics (Jenkins 1999). My results support Jenkins' (1999, 2005) accounts that this sucker is unique in its reproductive ecology and associated isolating mechanisms. Sicklefin redhorse spawning microhabitat is categorically different than that of any other redhorse species. Specifically, sicklefin redhorse typically spawn close to the bank in shallow water of moderate to swift flow. Suitable or usable spawning substrate ranges from very coarse gravel to small boulder and is closely associated with embedded cover such as a raised boulder, piece of large cobble, or large embedded log. In addition, similar to an account by Jenkins (1999), the sicklefin redhorse demonstrated no

agonistic behavior during my study. As a result of concurrent migration and spawning with the other four sympatric redhorses during my study, time can not be considered an operating isolating mechanism. The unique spawning microhabitat and statistically dissimilar body size distribution of the sicklefin redhorse indicate that habitat and ethological (i.e., courting and morphology) isolating mechanisms serve as operational reproductive barriers.

Courting behavior can prevent interbreeding between sympatric species (Mayr 1971). The courting behavior of the sicklefin redhorse is unique among redhorses (Jenkins 1999). Most redhorse males either nonaggressively hold position near or actively defend spawning sites, while the spawning site of the sicklefin redhorse is typically not attended by males, but by females. In addition, sicklefin redhorse are a roaming and pod-forming group spawner that, similar to other redhorses (Jenkins 1999), spawn at several different spawning sites within a spawning area. My observations of sicklefin redhorse courting behavior during 2007 are in agreement with those of Jenkins (1999). Unique courting behavior by the sicklefin redhorse, among redhorses, presumably acts as an ethological isolating mechanism from other sympatric redhorses spawning within the same spatiotemporal bounds.

It is unclear why interspecific courting and spawning was observed on four occasions in three spawning tributaries between sicklefin redhorse and black redhorse. One occasion of interspecific courting occurred at a black redhorse spawning site in Beaverdam Creek was between two sicklefin redhorse and greater than 10 black redhorse. This occurrence may be related to a lack of conspecifics or suitable spawning microhabitat in that tributary. Sicklefin redhorse typically spawn in trios or troupes (i.e., greater than three individuals) and rarely in

pairs. The two sicklefin redhorse observed in this interspecific courting act may have been seeking a third individual to complete a trio or troupe spawning act. Lower Beaverdam Creek is a high gradient stream largely composed of bedrock and large boulders with suitable redhorse spawning substrate being extremely rare. It is possible that the interspecific courting act there was a result of the absence of a habitat isolating mechanism and a low density of sicklefin redhorse. Mayr (1971) stated that “ethological isolation is the result of an interaction between external stimuli and the totality of internal drives. If no appropriate sex partner is available, internal drives continue to build up until readiness for mating can be induced even by a highly inadequate stimulus.” However, the interbreeding that was observed in Brasstown Creek and Valley River between multiple sicklefin redhorse and a single black redhorse is not readily explained. Substantial suitable spawning microhabitat and fish density of both species were abundant in both tributaries. However, Jenkins (1999) stated that Hiwassee Basin black redhorse and sicklefin redhorse have possibly previously exchanged alleles due to black redhorse exhibiting phenotypes unique to the Blue Ridge population and similar to the sicklefin redhorse. In addition, no post-spawning digging was observed by the female sicklefin redhorse during these interspecific spawning acts.

River weed in the Hiwassee Basin is presumably not important relative to reproductive isolation, because spawning sites of all five redhorse species occurred over clean substrate free of river weed. Nevertheless, this result is significant relative to the reproductive ecology of Hiwassee Basin redhorses. River weed has been correlated with decreased to absent flow near the substratum (Grubaugh and Wallace 1995) and subsequent

increases in sediment deposition (Madsen et al. 2001), possibly rendering subsurface spawning site environmental conditions unsuitable for zygote incubation due to poor water circulation and low dissolved oxygen concentrations.

This study highlights that knowledge of reproductive isolating mechanisms, particularly as they pertain to sympatric fishes, can be beneficial to fisheries management, environmental policy, and justification for strategic planning. Increasing understanding of extrinsic reproductive isolating mechanisms (e.g., space and time) are of more practical application to management, relative to intrinsic genetic barriers (e.g., ethological isolating mechanisms), and their identification would be critical, because extrinsic barriers are most prone to breakdown by anthropomorphic disturbance and thus most susceptible to rehabilitation. Similarly, the sterility barrier (i.e., hybrid sterility) has less management relevance, because this barrier is typically the last reproductive barrier (i.e., hurdle) preventing the interbreeding of populations and therefore a less pressing concern given the primary and secondary isolation mechanisms are functioning properly or rehabilitation management is feasible.

Human induced hydrologic and microhabitat degraded states primarily alter extrinsic isolating mechanisms related to time and space (e.g., water temperature and depth, respectively). Mechanisms based on one of these two categories (time or space) are primarily responsible for governing the spawning migration and selection of spawning microhabitat of many potamodromous fishes (Hubbs 1961). Morphological and behavioral isolating mechanisms (i.e., intrinsic isolating mechanisms) should persist as they did for fish

populations that occurred prior to anthropogenic hydrologic and habitat degradations, as these isolating mechanisms are less susceptible to atypical variation as a result of natural or otherwise degradation. Hubbs (1961) references the fact that many sympatric species will readily hybridize in forced captivity however, seldom, if ever, hybridize in nature. The reproductive isolation of most sympatric fishes is facilitated by a dominant barrier; however, subdominant reproductive barriers must breakdown substantially for interspecific hybridization to be prominent (Mayr 1971).

My study suggests that habitat and seasonal reproductive isolating mechanisms are the primary reproductive barriers among redhorse species in the upper Hiwassee Basin and that several ethological isolating mechanisms (e.g., morphology and behavior) also play segregating roles. Altered and degraded conditions that currently exist in the upper Hiwassee Basin have potential to degrade or remove redhorse reproductive isolating mechanisms. These conditions include sediment loading, riparian zone vegetation loss, and aberrant temperature and flow regimes (Jenkins 1999). This management tool may best be applied below Nottely and Chatuge Dam as both of these hydroelectric dams discharge hypolimnetic waters from upstream reservoirs throughout the spring spawning season resulting in drastically colder water temperatures compared to a pre-impoundment era. In addition, implementing urbanization, grazing, and agriculture best management practices to reduce sediment loading and promote spawning habitat diversity and heterogeneity would potentially foster black redhorse, golden redhorse, and sicklefin redhorse habitat isolating mechanisms. Also, reproductive isolating mechanisms exhibited by the smallmouth redhorse

need to be addressed within the upper Hiwassee Basin, as this species appears constricted to the lower reaches of its utilized spawning tributaries and could possibly be the rarest redhorse within the hydrologic system.

Most research on sympatric species and isolating mechanisms has been undertaken to either advance speciation or hybridization understanding as it pertains to speciation.

Although thorough understanding of speciation and natural sympatric hybridization is important, habitat degradation is the principle threat to suckers in North America (Cooke et al. 2005) and is applicable to management planning and implementation. Habitat degradation has been suggested to be most frequently associated with sympatric hybridization following the breakdown of habitat isolating mechanisms (Hubbs 1961; Mayr 1971). Practically, identification of reproductive isolating mechanisms is crucial to conceiving and implementing successful management plans and conservation strategies to maintain separate and distinct reproductive niches among sympatric species. Cooke et al. (2005) implied that this might best be achieved through ecosystem management. Habitat conservation and restoration at an ecosystem scale would ensure maintenance of primary isolating mechanisms dependant on habitat diversity and heterogeneity and an unaltered water temperature and flow regime among the sympatric redhorse community within the Hiwassee Basin.

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Table 1.—Particle size categories and associated continuous variables used to visually estimate dominant and subdominant surface substrate size for all observed spawning and courting acts as well as associated habitat availability survey points based on a modified Wentworth scale (Bovee 1986).

| Particle category  | Particle size (mm) | Continuous variable |
|--------------------|--------------------|---------------------|
| Bedrock            |                    | 13                  |
| Large boulder      | >1024              | 12                  |
| Medium boulder     | 508-1024           | 11                  |
| Small boulder      | 256-508            | 10                  |
| Large cobble       | 128-256            | 9                   |
| Small cobble       | 64-128             | 8                   |
| Very coarse gravel | 32-64              | 7                   |
| Coarse gravel      | 16-32              | 6                   |
| Medium gravel      | 8-16               | 5                   |
| Fine gravel        | 2-8                | 4                   |
| Sand               | 0.062-2.0          | 3                   |
| Silt               | 0.004-0.062        | 2                   |
| Clay               | <0.004             | 1                   |

Table 2.—Length (L) and weight (W) statistics for adult migratory black redhorse, golden redhorse, river redhorse, sicklefin redhorse, and silver redhorse sampled from Valley River, North Carolina, with a two-way resistance board weir during 2006 and 2007.

| Species/sex                                 | <i>N</i> | L, mean ± S.E. | Lmin – Lmax | W, mean ± S.E.   | Wmin – Wmax   |
|---|----------|----------------|-------------|------------------|---------------|
| black redhorse <i>Moxostoma duquesnei</i>   |          |                |             |                  |               |
| Female                                      | 186      | 459.3 ± 2.13   | 364 – 554   | 974.8 ± 14.75    | 458 – 1,610   |
| Male  | 56       | 430.6 ± 4.32   | 353 – 510   | 753.2 ± 28.07    | 438 – 1,460   |
| Combined                                    | 242      | 452.3 ± 2.09   | 353 – 554   | 921.2 ± 14.48    | 370 – 1,610   |
| golden redhorse <i>Moxostoma erythrurum</i> |          |                |             |                  |               |
| Female                                      | 156      | 452.2 ± 2.07   | 393 – 513   | 1,083.4 ± 15.54  | 702 – 1,778   |
| Male  | 94       | 432.2 ± 2.99   | 366 – 550   | 883.8 ± 15.74    | 515 – 1,344   |
| Combined                                    | 250      | 444.7 ± 1.82   | 366 – 550   | 1,008.9 ± 12.89  | 515 – 1,778   |
| river redhorse <i>Moxostoma carinatum</i>   |          |                |             |                  |               |
| Female                                      | 11       | 557.3 ± 16.15  | 430 – 647   | 1,656.5 ± 121.92 | 680 – 2,423   |
| Male  | 14       | 547.5 ± 9.95   | 462 – 610   | 1,560.7 ± 76.51  | 870 – 2,080   |
| Combined                                    | 25       | 552.0 ± 8.76   | 430 – 647   | 1,604.6 ± 66.86  | 680 – 2,423   |
| sicklefin redhorse <i>Moxostoma</i> sp.     |          |                |             |                  |               |
| Female                                      | 186      | 523.2 ± 1.78   | 462 – 604   | 1,338.6 ± 14.10  | 949 – 2,122   |
| Male  | 81       | 495.0 ± 2.89   | 421 – 562   | 1,063.9 ± 17.80  | 722 – 1,649   |
| Combined                                    | 267      | 514.6 ± 1.71   | 421 – 604   | 1,255.3 ± 13.58  | 722 – 2,122   |
| silver redhorse <i>Moxostoma anisurum</i>   |          |                |             |                  |               |
| Female                                      | 103      | 575.7 ± 3.87   | 371 – 634   | 1,813.6 ± 31.52  | 602 – 2,718   |
| Male  | 90       | 552.3 ± 3.10   | 408 – 604   | 1,570.7 ± 21.74  | 1,058 – 2,051 |
| Combined                                    | 193      | 564.8 ± 2.65   | 371 – 634   | 1,700.3 ± 21.46  | 602 – 2,718   |

Table 3.—Sample size and results from a comparison of pit and flat spawning microhabitat data for black redhorse, golden redhorse, river redhorse, and sicklefin redhorse from the upper Hiwassee Basin, North Carolina. Flat microhabitat data was not collected for the silver redhorse. Statistical analyses were performed using the parametric Student's *t*-test.

| Species and variable       | <i>N</i> | Student's <i>t</i> | <i>P</i> |
|----------------------------|----------|--------------------|----------|
| Black redhorse             |          |                    |          |
| Depth (m)                  | 38       | -2.080             | 0.0406   |
| Bottom velocity (m/s)      | 38       | 5.220              | <0.0001  |
| Mean column velocity (m/s) | 38       | 1.436              | 0.1550   |
| Dominant substrate         | 38       | -2.571             | 0.0124   |
| Golden redhorse            |          |                    |          |
| Depth (m)                  | 35       | -2.256             | 0.0273   |
| Bottom velocity (m/s)      | 35       | 4.447              | <0.0001  |
| Mean column velocity (m/s) | 35       | 1.622              | 0.1095   |
| Dominant substrate         | 35       | -1.831             | 0.0720   |
| River redhorse             |          |                    |          |
| Depth (m)                  | 24       | -4.563             | <0.0001  |
| Bottom velocity (m/s)      | 24       | 4.881              | <0.0001  |
| Mean column velocity (m/s) | 24       | 3.226              | 0.0023   |
| Dominant substrate         | 24       | -4.112             | 0.0003   |
| Sicklefin redhorse         |          |                    |          |
| Depth (m)                  | 43       | -1.587             | 0.1163   |
| Bottom velocity (m/s)      | 43       | 1.863              | 0.0662   |
| Mean column velocity (m/s) | 43       | -0.062             | 0.9510   |
| Dominant substrate         | 43       | 0.531              | 0.5970   |

Table 4.—Pit and flat spawning microhabitat statistics for black redhorse, golden redhorse, river redhorse, and sicklefin redhorse from the upper Hiwassee Basin, North Carolina. Flat microhabitat data were not collected for the silver redhorse.

| Parameter and statistic | Black redhorse |      | Golden redhorse |      | River redhorse |      | Sicklefin redhorse |      | Silver redhorse |      |
|-------------------------|----------------|------|-----------------|------|----------------|------|--------------------|------|-----------------|------|
|                         | Flat           | Pit  | Flat            | Pit  | Flat           | Pit  | Flat               | Pit  | Flat            | Pit  |
| Temperature (C°)        |                |      |                 |      |                |      |                    |      |                 |      |
| N                       | 38             | 38   | 35              | 35   | 24             | 24   | 43                 | 43   | 0               | 16   |
| Mean                    | 16.8           | 16.8 | 17.8            | 17.8 | 17.7           | 17.7 | 17.4               | 17.4 |                 | 18.8 |
| SE                      | 0.52           | 0.52 | 0.24            | 0.24 | 0.22           | 0.22 | 0.41               | 0.41 |                 | 0.09 |
| Min                     | 7.5            | 7.5  | 16.6            | 16.6 | 15.3           | 15.3 | 11.0               | 11.0 |                 | 18.4 |
| Max                     | 20.8           | 20.8 | 21.1            | 21.1 | 19.0           | 19.0 | 21.1               | 21.1 |                 | 19.2 |
| Distance to bank (m)    |                |      |                 |      |                |      |                    |      |                 |      |
| N                       | 38             | 38   | 35              | 35   | 24             | 24   | 43                 | 43   | 0               | 16   |
| Mean                    | 2.8            | 2.8  | 3.0             | 3.0  | 3.5            | 3.5  | 2.9                | 2.9  |                 | 4.1  |
| SE                      | 0.25           | 0.25 | 0.29            | 0.29 | 0.27           | 0.27 | 0.25               | 0.24 |                 | 0.40 |
| Min                     | 0.5            | 0.5  | 0.3             | 0.3  | 1.0            | 1.0  | 0.8                | 0.8  |                 | 2.0  |
| Max                     | 5.0            | 5.0  | 8.0             | 8.0  | 7.0            | 7.0  | 8.0                | 8.0  |                 | 7.0  |
| Depth (m)               |                |      |                 |      |                |      |                    |      |                 |      |
| N                       | 38             | 38   | 35              | 35   | 24             | 24   | 43                 | 43   | 0               | 16   |
| Mean                    | 0.18           | 0.20 | 0.19            | 0.23 | 0.29           | 0.36 | 0.25               | 0.28 |                 | 0.36 |
| SE                      | 0.01           | 0.01 | 0.01            | 0.01 | 0.01           | 0.01 | 0.01               | 0.01 |                 | 0.02 |
| Min                     | 0.10           | 0.12 | 0.06            | 0.08 | 0.22           | 0.28 | 0.12               | 0.15 |                 | 0.26 |
| Max                     | 0.34           | 0.36 | 0.37            | 0.37 | 0.40           | 0.50 | 0.46               | 0.46 |                 | 0.50 |
| Bottom velocity (m/s)   |                |      |                 |      |                |      |                    |      |                 |      |
| N                       | 38             | 38   | 35              | 35   | 24             | 24   | 43                 | 43   | 0               | 16   |
| Mean                    | 0.25           | 0.15 | 0.41            | 0.22 | 0.34           | 0.17 | 0.26               | 0.20 |                 | 0.28 |
| SE                      | 0.01           | 0.01 | 0.03            | 0.03 | 0.02           | 0.03 | 0.03               | 0.02 |                 | 0.02 |
| Min                     | 0.09           | 0.00 | 0.09            | 0.01 | 0.10           | 0.00 | 0.00               | 0.02 |                 | 0.15 |
| Max                     | 0.41           | 0.29 | 0.85            | 0.72 | 0.51           | 0.33 | 0.53               | 0.55 |                 | 0.40 |
| Mean velocity (m/s)     |                |      |                 |      |                |      |                    |      |                 |      |
| N                       | 38             | 38   | 35              | 35   | 24             | 24   | 43                 | 43   | 0               | 16   |
| Mean                    | 0.41           | 0.37 | 0.68            | 0.59 | 0.66           | 0.55 | 0.50               | 0.51 |                 | 0.66 |
| SE                      | 0.02           | 0.02 | 0.04            | 0.04 | 0.02           | 0.02 | 0.02               | 0.02 |                 | 0.04 |
| Min                     | 0.12           | 0.10 | 0.25            | 0.20 | 0.44           | 0.31 | 0.12               | 0.16 |                 | 0.42 |
| Max                     | 0.65           | 0.63 | 1.10            | 1.05 | 0.87           | 0.71 | 1.01               | 0.90 |                 | 0.93 |
| Dominant substrate      |                |      |                 |      |                |      |                    |      |                 |      |
| N                       | 38             | 38   | 35              | 35   | 24             | 24   | 43                 | 43   | 0               | 16   |
| Mode                    | CG             | CG   | VCG             | SC   | SC             | SC   | SC                 | SC   |                 | SC   |
| SE                      | 0.25           | 0.18 | 0.22            | 0.15 | 0.18           | 0.07 | 0.12               | 0.13 |                 | 0.14 |
| Min                     | SA             | FG   | SA              | MG   | MG             | VCG  | MG                 | MG   |                 | VCG  |
| Max                     | LC             | SC   | SC              | SC   | SC             | SC   | SB                 | SB   |                 | LC   |
| Subdominant substrate   |                |      |                 |      |                |      |                    |      |                 |      |
| N                       | 38             | 38   | 35              | 35   | 24             | 24   | 43                 | 43   | 0               | 16   |
| Mode                    | CG             | VCG  | VCG             | VCG  | VCG            | VCG  | VCG                | VCG  |                 | LC   |
| SE                      | 0.28           | 0.14 | 0.26            | 0.15 | 0.17           | 0.18 | 0.19               | 0.15 |                 | 0.21 |
| Min                     | SA             | FG   | SA              | FG   | CG             | VCG  | SA                 | CG   |                 | VCG  |
| Max                     | LC             | SC   | LC              | LC   | LC             | LC   | LC                 | SB   |                 | LC   |
| Cover                   |                |      |                 |      |                |      |                    |      |                 |      |
| N                       | 38             | 38   | 35              | 35   | 24             | 24   | 43                 | 43   | 0               | 16   |
| Mode                    | B              | B    | NC              | NC   | NC             | NC   | B                  | B    |                 | NC   |

Table 4.—(Continued).

| Parameter and statistic | Black redhorse |      | Golden redhorse |      | River redhorse |      | Sicklefin redhorse |      | Silver redhorse |      |
|-------------------------|----------------|------|-----------------|------|----------------|------|--------------------|------|-----------------|------|
|                         | Flat           | Pit  | Flat            | Pit  | Flat           | Pit  | Flat               | Pit  | Flat            | Pit  |
| Distance to cover (m)   |                |      |                 |      |                |      |                    |      |                 |      |
| N                       | 26             | 26   | 10              | 10   | 4              | 4    | 40                 | 40   | 0               | 1    |
| Mean                    | 0.98           | 0.95 | 0.76            | 0.75 | 0.40           | 0.40 | 0.39               | 0.36 |                 | 1.00 |
| E                       | 0.12           | 0.11 | 0.22            | 0.22 | 0.10           | 0.10 | 0.07               | 0.07 |                 |      |
| Min                     | 0.10           | 0.10 | 0.10            | 0.10 | 0.10           | 0.10 | 0.10               | 0.10 |                 | 1.00 |
| Max                     | 2.00           | 2.00 | 2.00            | 2.00 | 0.50           | 0.50 | 2.00               | 2.00 |                 | 1.00 |

Table 5.—Statistics and associated *P*-values from Kruskal-Wallis tests comparing continuous spawning microhabitat variables and contingency table analysis comparing categorical spawning microhabitat variables (i.e., cover) among redhorses from Hiwassee Basin, North Carolina and Georgia.

| Microhabitat variable | $X^2$ Statistic | <i>P</i> |
|-----------------------|-----------------|----------|
| Temperature (°C)      | 10.793          | 0.029    |
| Distance to bank (m)  | 11.33           | 0.023    |
| Depth (m)             | 66.967          | <0.0001  |
| Bottom velocity (m/s) | 17.819          | 0.0013   |
| Mean velocity (m/s)   | 39.018          | <0.0001  |
| Dominant substrate    | 70.794          | <0.0001  |
| Cover                 | 106.327         | <0.0001  |
| Distance to cover (m) | 28.318          | <0.0001  |

Table 6.—Contingency tables and subset contingency tables displaying row percentages for each species and cover type. Average expected frequency ( $n/rc$ ) is given along with the Pearson chi-square value and  $p$ -value for each contingency table.

| Contingency table and species | NC    | CWD   | FWD  | VT    | B     | $n/rc$ | $\chi^2$ | $P$     |
|-------------------------------|-------|-------|------|-------|-------|--------|----------|---------|
| Table 1                       |       |       |      |       |       |        |          |         |
| Black redhorse                | 31.58 | 0.00  | 7.89 | 21.05 | 39.47 | 6.2    | 106.33   | <0.0001 |
| Golden redhorse               | 71.43 | 14.29 | 0.00 | 0.00  | 14.29 |        |          |         |
| River redhorse                | 83.33 | 0.00  | 0.00 | 0.00  | 16.67 |        |          |         |
| Sicklefin redhorse            | 7.14  | 14.29 | 0.00 | 0.00  | 78.57 |        |          |         |
| Silver redhorse               | 87.50 | 0.00  | 0.00 | 0.00  | 12.50 |        |          |         |
| Subdivided tables and species |       |       |      |       |       |        |          |         |
| Table 2                       |       |       |      |       |       |        |          |         |
| Golden redhorse               | 71.43 | 14.29 | 0.00 | 0.00  | 14.29 | 9.7    | 61.67    | <0.0001 |
| River redhorse                | 83.33 | 0.00  | 0.00 | 0.00  | 16.67 |        |          |         |
| Sicklefin redhorse            | 7.14  | 14.29 | 0.00 | 0.00  | 78.57 |        |          |         |
| Silver redhorse               | 87.50 | 0.00  | 0.00 | 0.00  | 12.50 |        |          |         |
| Table 3                       |       |       |      |       |       |        |          |         |
| Black redhorse                | 31.58 | 0.00  | 7.89 | 21.05 | 39.47 | 5.7    | 49.15    | <0.0001 |
| Golden redhorse               | 71.43 | 14.29 | 0.00 | 0.00  | 14.29 |        |          |         |
| River redhorse                | 83.33 | 0.00  | 0.00 | 0.00  | 16.67 |        |          |         |
| Silver redhorse               | 87.50 | 0.00  | 0.00 | 0.00  | 12.50 |        |          |         |
| Table 4                       |       |       |      |       |       |        |          |         |
| Black redhorse                | 31.58 | 0.00  | 7.89 | 21.05 | 39.47 | 8.0    | 29.02    | <0.0001 |
| Sicklefin redhorse            | 7.14  | 14.29 | 0.00 | 0.00  | 78.57 |        |          |         |
| Table 5                       |       |       |      |       |       |        |          |         |
| Golden redhorse               | 71.43 | 14.29 | 0.00 | 0.00  | 14.29 | 8.3    | 6.29     | 0.1783  |
| River redhorse                | 83.33 | 0.00  | 0.00 | 0.00  | 16.67 |        |          |         |
| Silver redhorse               | 87.50 | 0.00  | 0.00 | 0.00  | 12.50 |        |          |         |

Table 7.—Redhorse spawning microhabitat statistics and significance values from comparisons of microhabitat use and availability principal component1 and component 2 scores to test for nonrandom microhabitat use. The Komogorov-Smirnov two-sample test was used to compare component scores.

| Species and principal component | <i>D</i> statistic | <i>P</i> -value |
|---------------------------------|--------------------|-----------------|
| Black redhorse                  |                    |                 |
| PC 1                            | 0.3768             | 0.0419          |
| PC 2                            | 0.5822             | 0.0002          |
| Golden redhorse                 |                    |                 |
| PC 1                            | 0.5718             | <0.0001         |
| PC 2                            | 0.5071             | <0.0001         |
| River redhorse                  |                    |                 |
| PC 1                            | 0.4370             | 0.0003          |
| PC 2                            | 0.4083             | 0.0010          |
| Sicklefin redhorse              |                    |                 |
| PC 1                            | 0.3191             | 0.0022          |
| PC 2                            | 0.8193             | <0.0001         |
| Silver redhorse                 |                    |                 |
| PC 1                            | 0.6738             | <0.0001         |
| PC 2                            | 0.6166             | <0.0001         |

Table 8.—Redhorse spawning microhabitat comparison statistics and significance values from comparisons of microhabitat use principal component 1 and component 2 scores. The Komogorov-Smirnov two-sample test was used to compare component scores.

| Season and principal component            | <i>D</i> statistic | <i>P</i> -value |
|---|--------------------|-----------------|
| Black redhorse versus golden redhorse     |                    |                 |
| PC 1                                      | 0.7738             | <0.0001         |
| PC 2                                      | 0.2679             | 0.5499          |
| Black redhorse versus river redhorse      |                    |                 |
| PC 1                                      | 0.5238             | 0.0156          |
| PC 2                                      | 0.2083             | 0.8375          |
| Black redhorse versus sicklefin redhorse  |                    |                 |
| PC 1                                      | 0.3849             | 0.1009          |
| PC 2                                      | 0.7023             | <0.0001         |
| Black redhorse versus silver redhorse     |                    |                 |
| PC 1                                      | 0.8750             | <0.0001         |
| PC 2                                      | 0.3750             | 0.2445          |
| Golden redhorse versus river redhorse     |                    |                 |
| PC 1                                      | 0.3333             | 0.1389          |
| PC 2                                      | 0.4167             | 0.0310          |
| Golden redhorse versus sicklefin redhorse |                    |                 |
| PC 1                                      | 0.5556             | 0.0003          |
| PC 2                                      | 0.5139             | 0.0010          |
| Golden redhorse versus silver redhorse    |                    |                 |
| PC 1                                      | 0.3750             | 0.1344          |
| PC 2                                      | 0.2292             | 0.6945          |
| River redhorse versus sicklefin redhorse  |                    |                 |
| PC 1                                      | 0.2917             | 0.1725          |
| PC 2                                      | 0.8750             | <0.0001         |
| River redhorse versus silver redhorse     |                    |                 |
| PC 1                                      | 0.5208             | 0.0109          |
| PC 2                                      | 0.3542             | 0.1798          |
| Sicklefin redhorse versus silver redhorse |                    |                 |
| PC 1                                      | 0.6875             | <0.0001         |
| PC 2                                      | 0.6667             | 0.0001          |

Table 9.—Spawning principle component eigenvector values (i.e., loadings), eigenvalues, and cumulative variance explained for microhabitat use and availability for utilized redhorse streams and rivers in the Hiwassee Basin, North Carolina and Georgia.

| Variable and statistic            | PCA axis |       |
|-----------------------------------|----------|-------|
|                                   | 1        | 2     |
| Distance to bank (m)              | 0.47     | 0.29  |
| Depth (m)                         | 0.30     | 0.64  |
| Bottom velocity (m/s)             | 0.48     | -0.47 |
| Mean velocity (m/s)               | 0.54     | -0.33 |
| Dominate substrate                | 0.34     | -0.03 |
| Distance to cover (m)             | 0.21     | 0.43  |
| Eigenvalue                        | 2.42     | 1.24  |
| Cumulative variance explained (%) | 40.3     | 61.1  |

Table 10.—Quantitative spawning behavior for the black redhorse, golden redhorse, river redhorse, sicklefin redhorse, and silver redhorse from the upper Hiwassee Basin, North Carolina.

| Spawning act attribute      | Black redhorse     |          | Golden redhorse |          | River redhorse |          | Sicklefin redhorse |                | Silver redhorse |          |
|-----------------------------|--------------------|----------|-----------------|----------|----------------|----------|--------------------|----------------|-----------------|----------|
|                             | Courting           | Spawning | Courting        | Spawning | Courting       | Spawning | Courting           | Spawning       | Courting        | Spawning |
| Number of individuals       |                    |          |                 |          |                |          |                    |                |                 |          |
| Number of obs.              | 1                  | 22       | 1               | 25       |                | 18       | 8                  | 31             |                 | 16       |
| Mean                        | 12.0               | 3.9      | 5.0             | 2.5      |                | 3.2      | 11.8               | 3.8            |                 | 3.1      |
| SE                          |                    | 0.32     |                 | 0.10     |                | 0.13     | 2.43               | 0.43           |                 | 0.06     |
| Min - Max                   | 12 - 12            | 2 - 8    | 5 - 5           | 2 - 3    |                | 2 - 4    | 2 - 20             | 2 - 15         |                 | 3 - 4    |
| Quiver duration (s)         |                    |          |                 |          |                |          |                    |                |                 |          |
| Number of obs.              |                    | 22       |                 | 25       |                | 17       |                    | 27             |                 | 16       |
| Mean                        |                    | 5.2      |                 | 3.8      |                | 4.8      |                    | 6.4            |                 | 4.9      |
| SE                          |                    | 0.41     |                 | 0.21     |                | 0.56     |                    | 0.66           |                 | 0.54     |
| Min -Max                    |                    | 3 - 10   |                 | 2 - 5    |                | 2 - 10   |                    | 3 - 15         |                 | 2 - 9    |
| Agonistic behavior observed |                    |          |                 |          |                |          |                    |                |                 |          |
| Occurrence (% of obs.)      | 0                  | 0        | 0               | 51.4     |                | 16.7     | 0                  | 0              |                 | 0        |
| Additional species in act   | sicklefin redhorse |          |                 |          |                |          | black redhorse     | black redhorse |                 |          |
| Occurrence (% of obs.)      | 100.0              | 0        | 0               | 0        |                | 0        | 12.5               | 9.7            |                 | 0        |

Table 11.—Deceased redhorses by sex recovered at the downstream and upstream resistance board weirs during 2007 in Valley River, North Carolina. Characteristics noted included caudle bruising, loss of marginal fin pigmentation, and *Flexibacter columnaris* symptoms.

| Species            | Sex    | N  | April | May | June | Caudle bruising (%) | Loss of marginal fin pigmentation (%) | <i>Flexibacter columnaris</i> (%) |
|--------------------|--------|----|-------|-----|------|---------------------|---------------------------------------|-----------------------------------|
| Black redhorse     | Male   | 2  | 0     | 2   | 0    | 100                 | 0.0                                   | 100                               |
|                    | Female | 1  | 0     | 1   | 0    | 100                 | 0.0                                   | 100                               |
| Golden redhorse    | Male   | 81 | 0     | 81  | 0    | 84.0                | 43.2                                  | 100                               |
|                    | Female | 41 | 0     | 41  | 0    | 85.4                | 68.3                                  | 100                               |
| River redhorse     | Male   | 4  | 0     | 4   | 0    | 100                 | 75.0                                  | 100                               |
|                    | Female | 2  | 0     | 2   | 0    | 100                 | 0.0                                   | 100                               |
| Sicklefin redhorse | Male   | 3  | 1     | 0   | 2    | 100                 | 100                                   | 66.7                              |
|                    | Female | 4  | 0     | 2   | 2    | 100                 | 100                                   | 100                               |

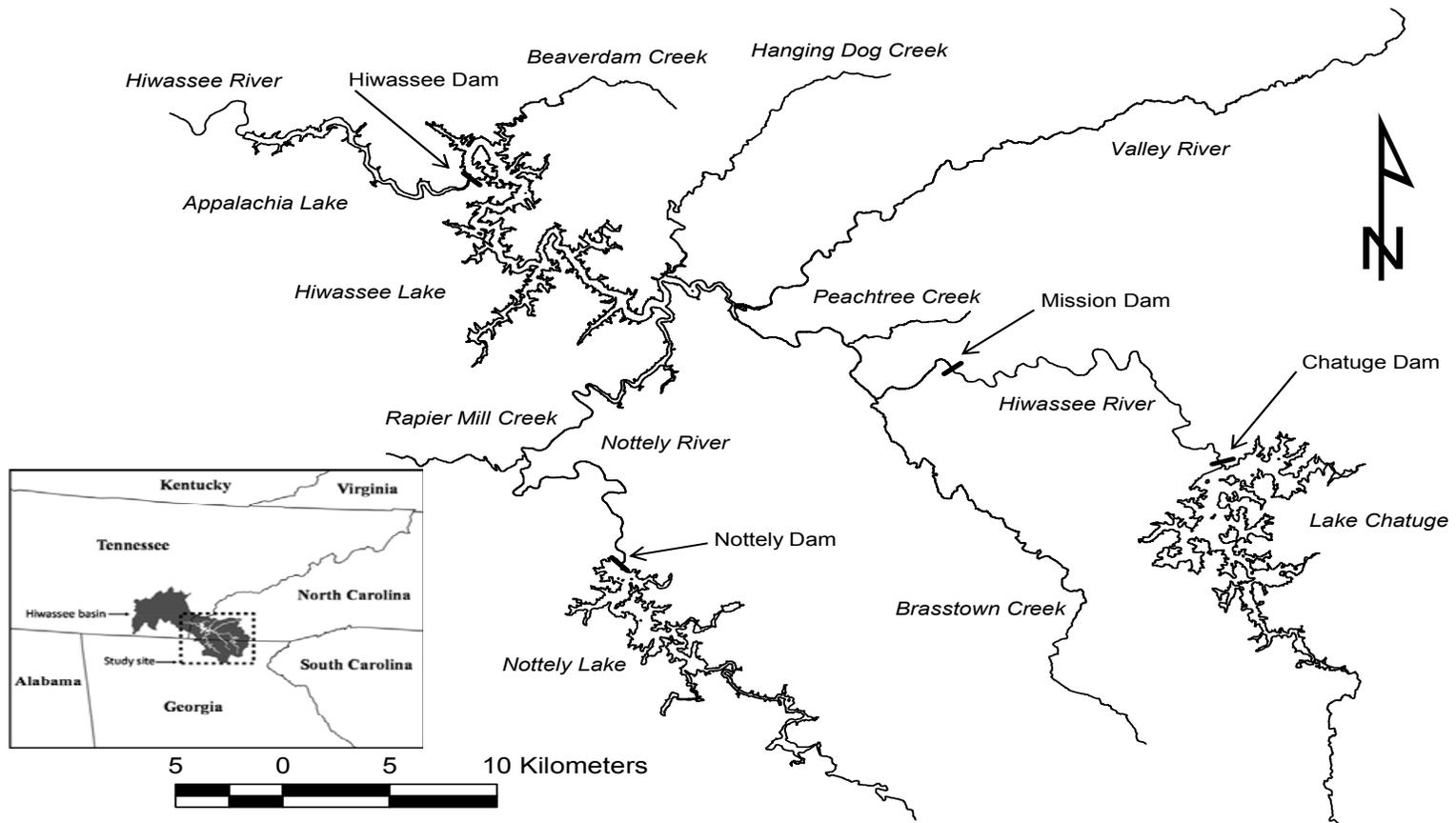


Figure 1.—Map of upper Hiwassee Basin with the study site bounded downstream by Hiwassee Dam and upstream by Mission and Nottely dams.

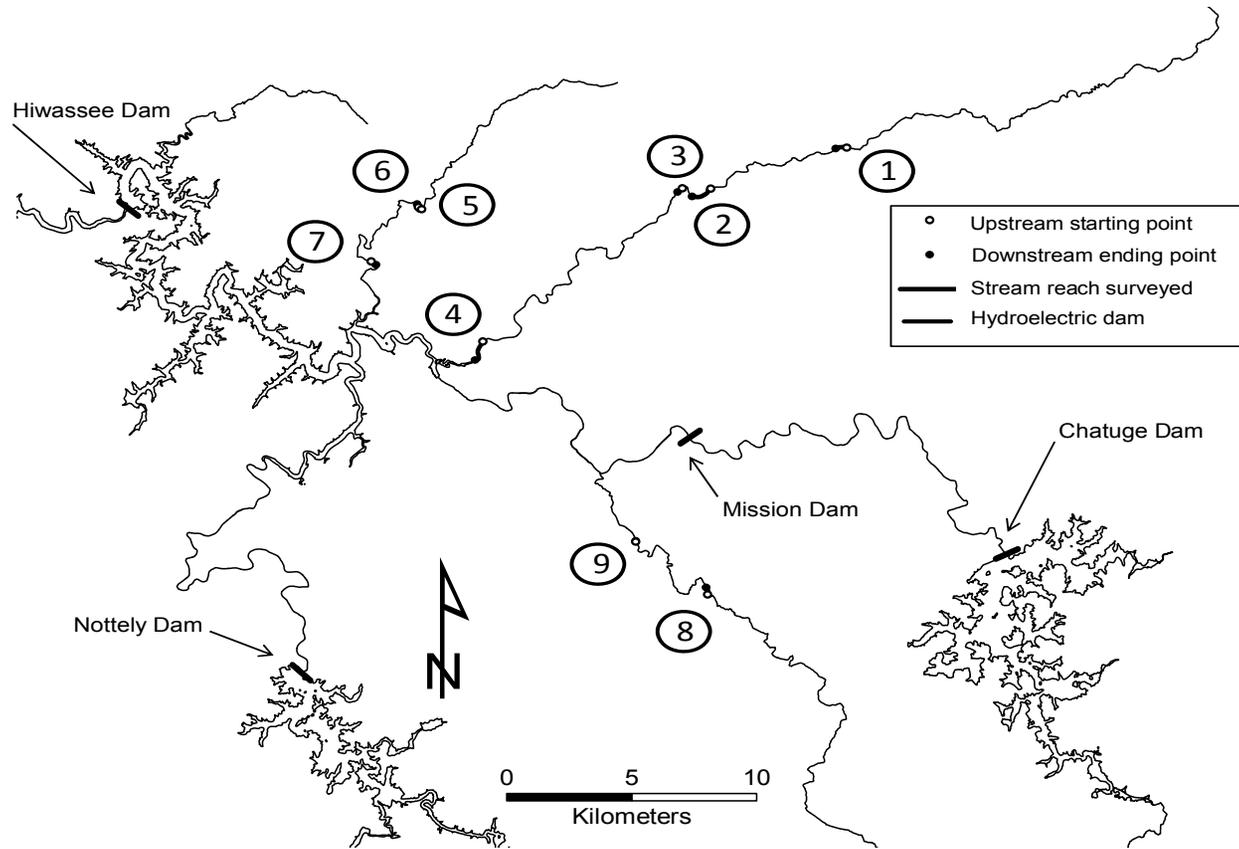


Figure 2.—Map of upper Hiwassee Basin and numbered stream reaches surveyed to quantify habitat availability. Characteristics reported (here) for surveyed stream reaches shown above are stream length (km), number of transects, and number of survey points. Characteristics for surveyed stream reaches numbered above are (1) 0.52, 15, 179; (2) 1.04, 15, 177; (3) 0.30, 10, 137; (4) 0.91, 17, 196; (5) 70, 5, 62; (6) 0.16, 10, 163; (7) 0.28, 10, 103; (8) 0.33, 15, 221; and (9) 0.06, 3, 42.

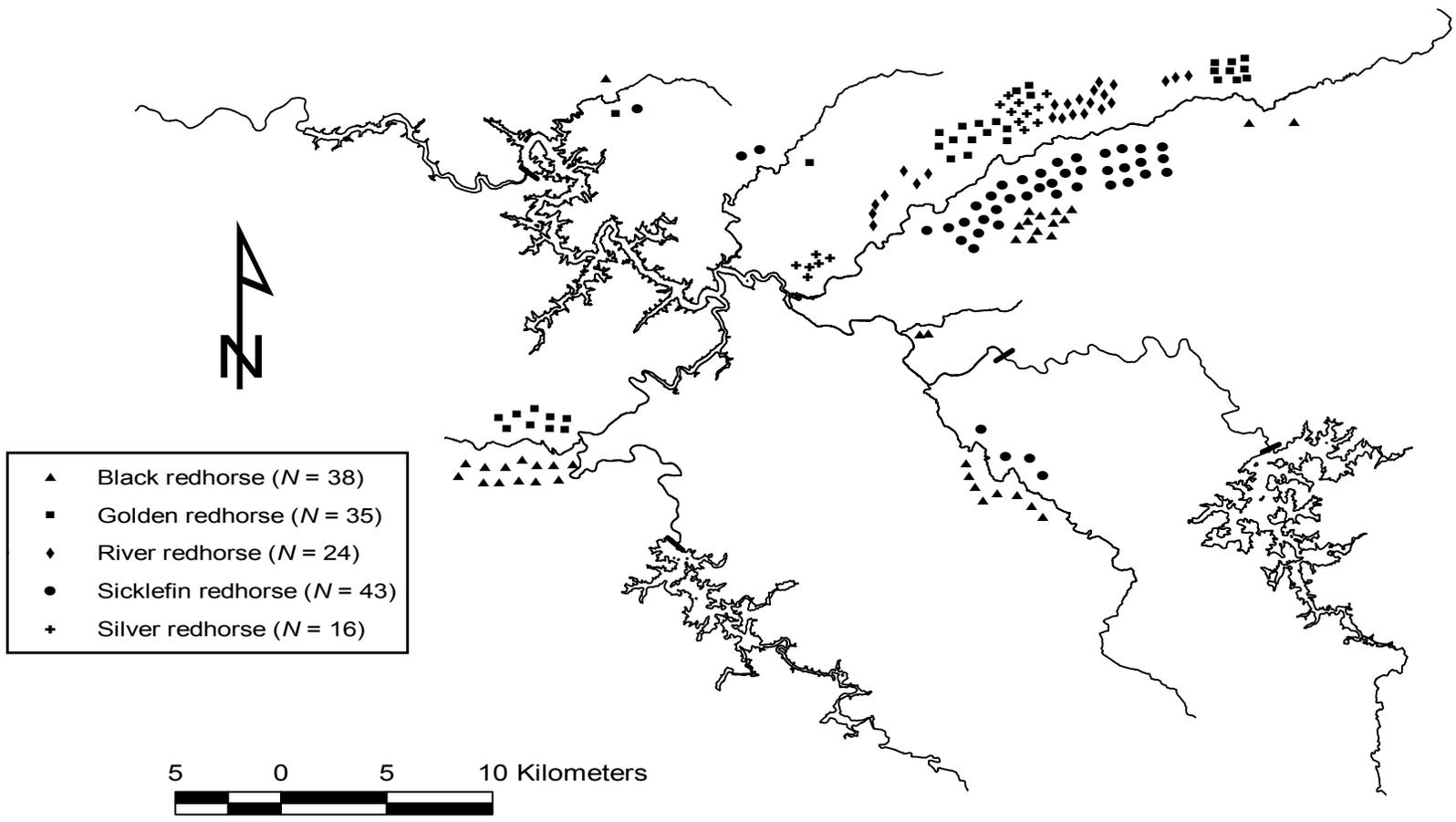


Figure 3.—Redhorse observed spawning and courting sites in upper Hiwassee Basin during the 2007 spring spawning season.

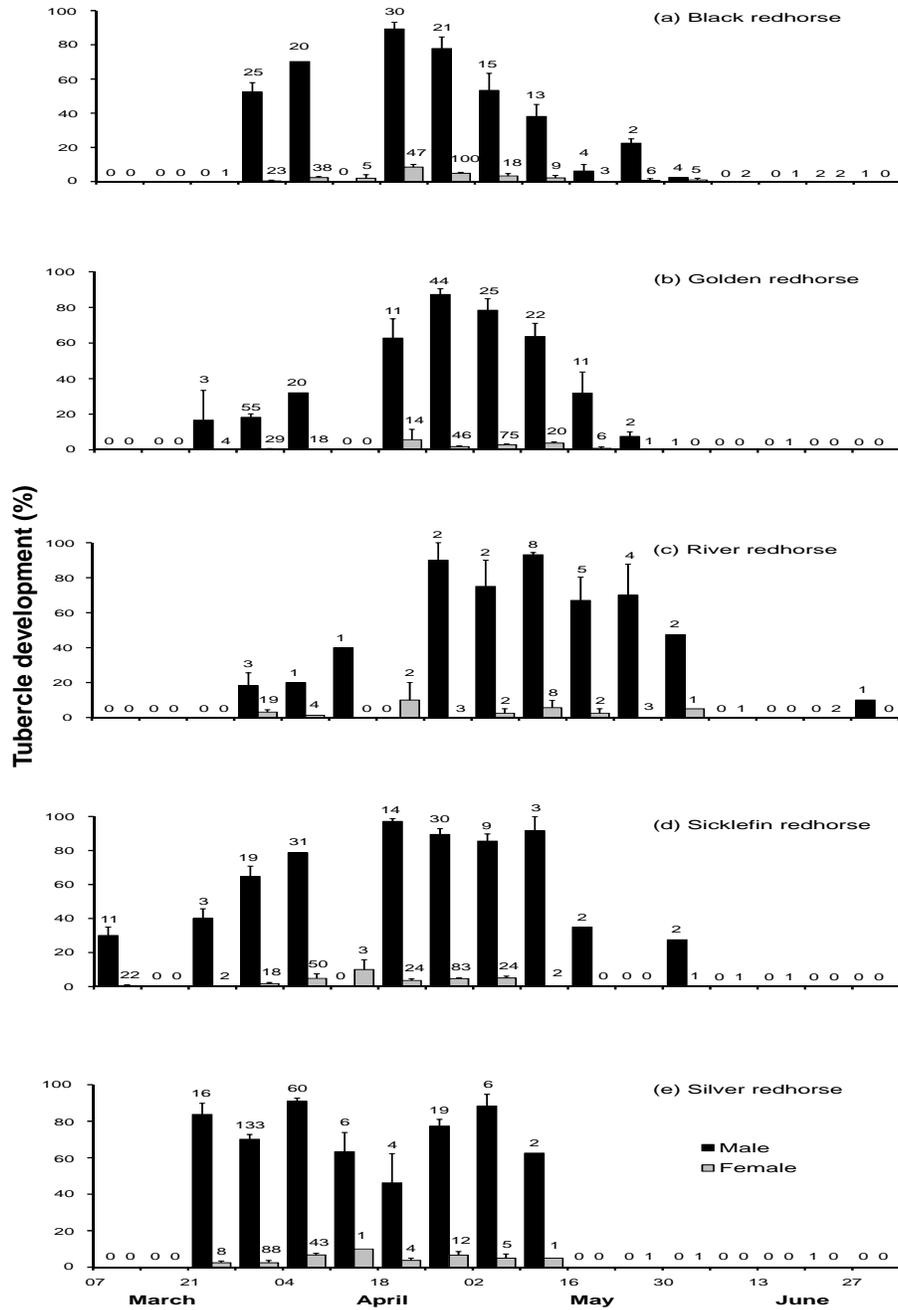


Figure 4.—Mean tubercle development for adult migratory redhorses from the upper Hiwassee Basin, North Carolina. Fish collected with two-way resistance board weirs, PAEs, and boat electrofishing were included to determine if a sexual dichotomy was present relative to tubercle development. Total number of individuals, between 2006 and 2007, that were collected is shown above the weekly mean tubercle percentages.

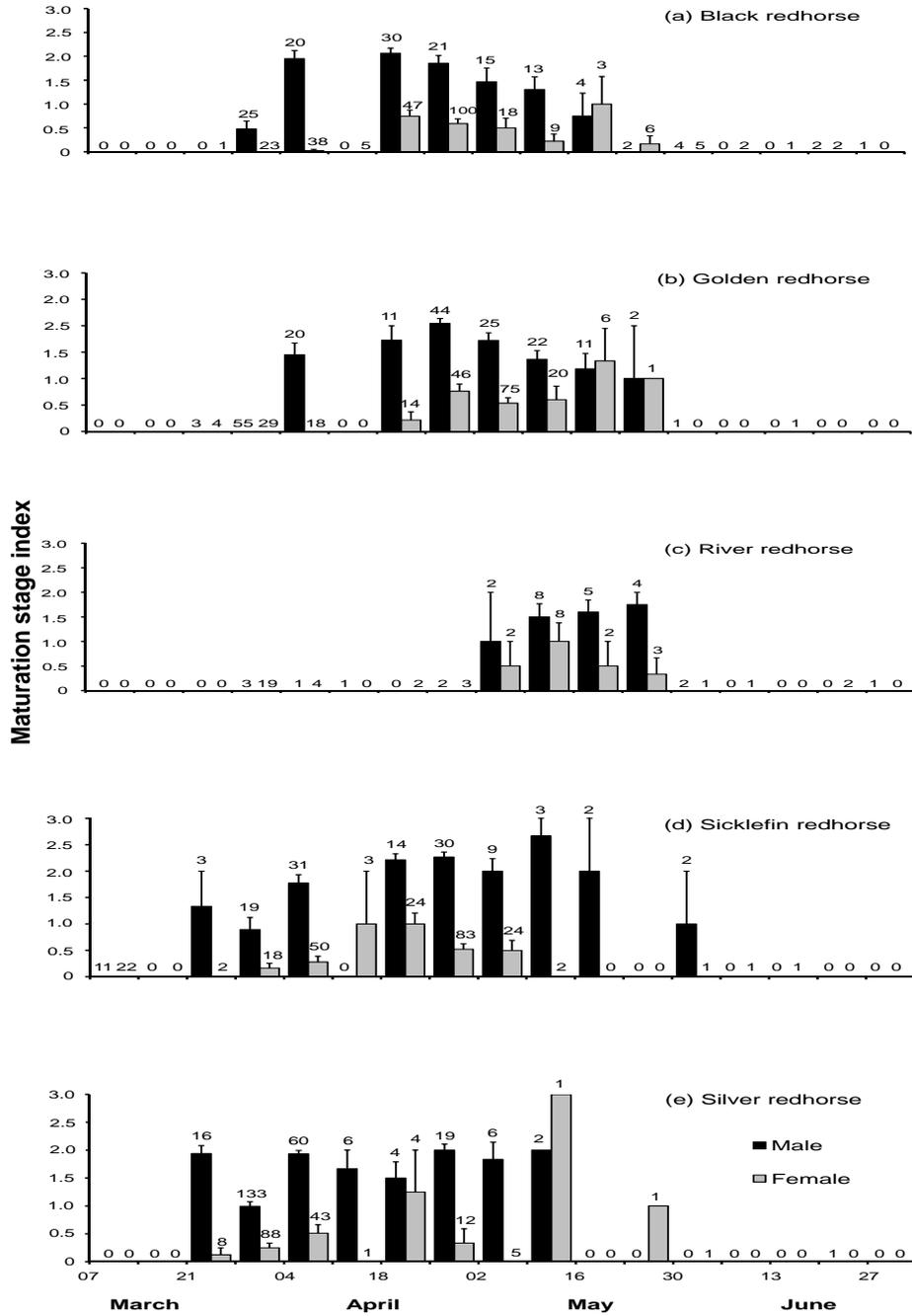


Figure 5.—Mean maturation stage for adult migratory redhorses from the upper Hiwassee Basin, North Carolina. Fish collected with two-way resistance board weirs, PAEs, and boat electrofishing were included to determine if a sexual dichotomy was present relative to maturation stage. Total number of individuals, between 2006 and 2007, that were collected is shown above the weekly mean tubercle percentages.

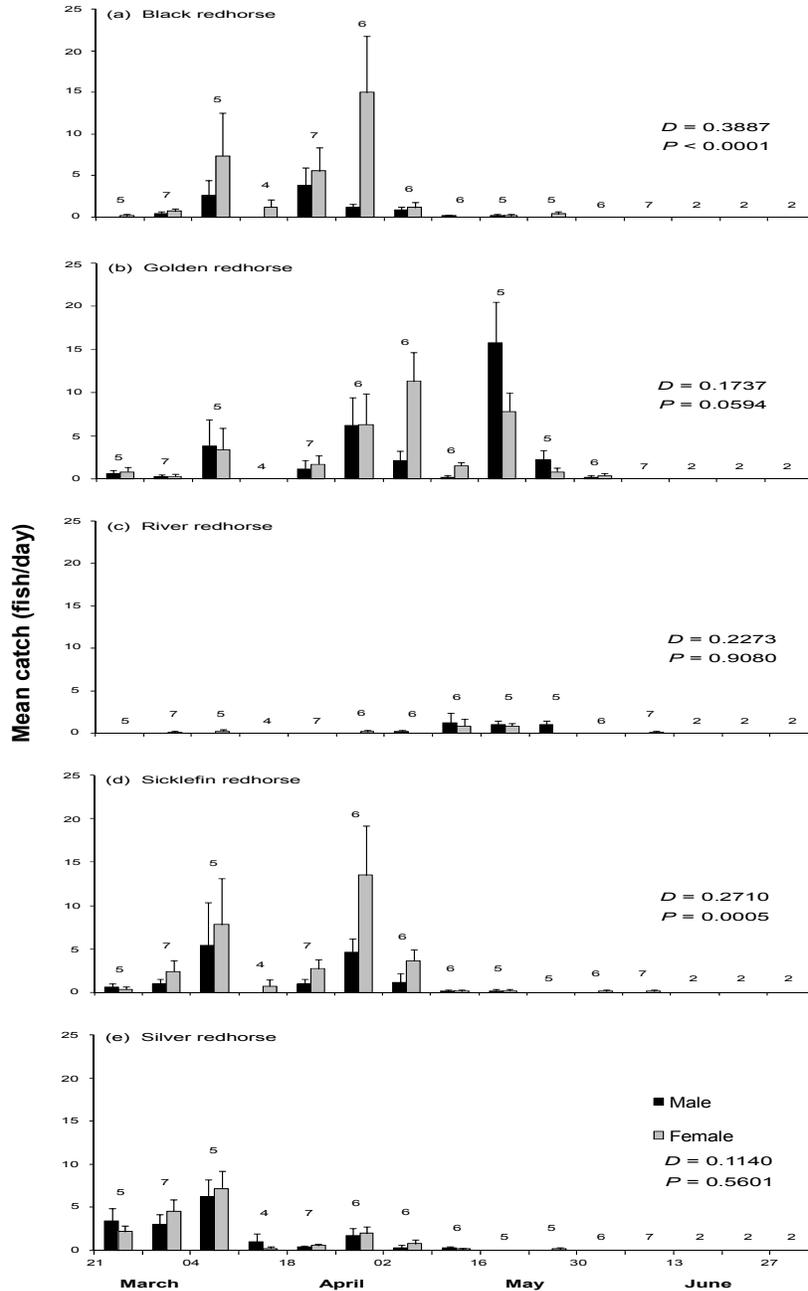


Figure 6.—Mean catch of adult migratory redhorses from Valley River, North Carolina. Only confirmed migratory fish collected with the two-way resistance board weirs were included. A Komogorov-Smirnov two-sample test was performed on male and female catches for black redhorse, golden redhorse, river redhorse, sicklefin redhorse, and silver redhorse to ascertain if a sexual dichotomy was present in relation to the spawning migration. Total number of days, between 2006 and 2007, that were sampled is shown above the daily mean catches reported on a weekly basis.

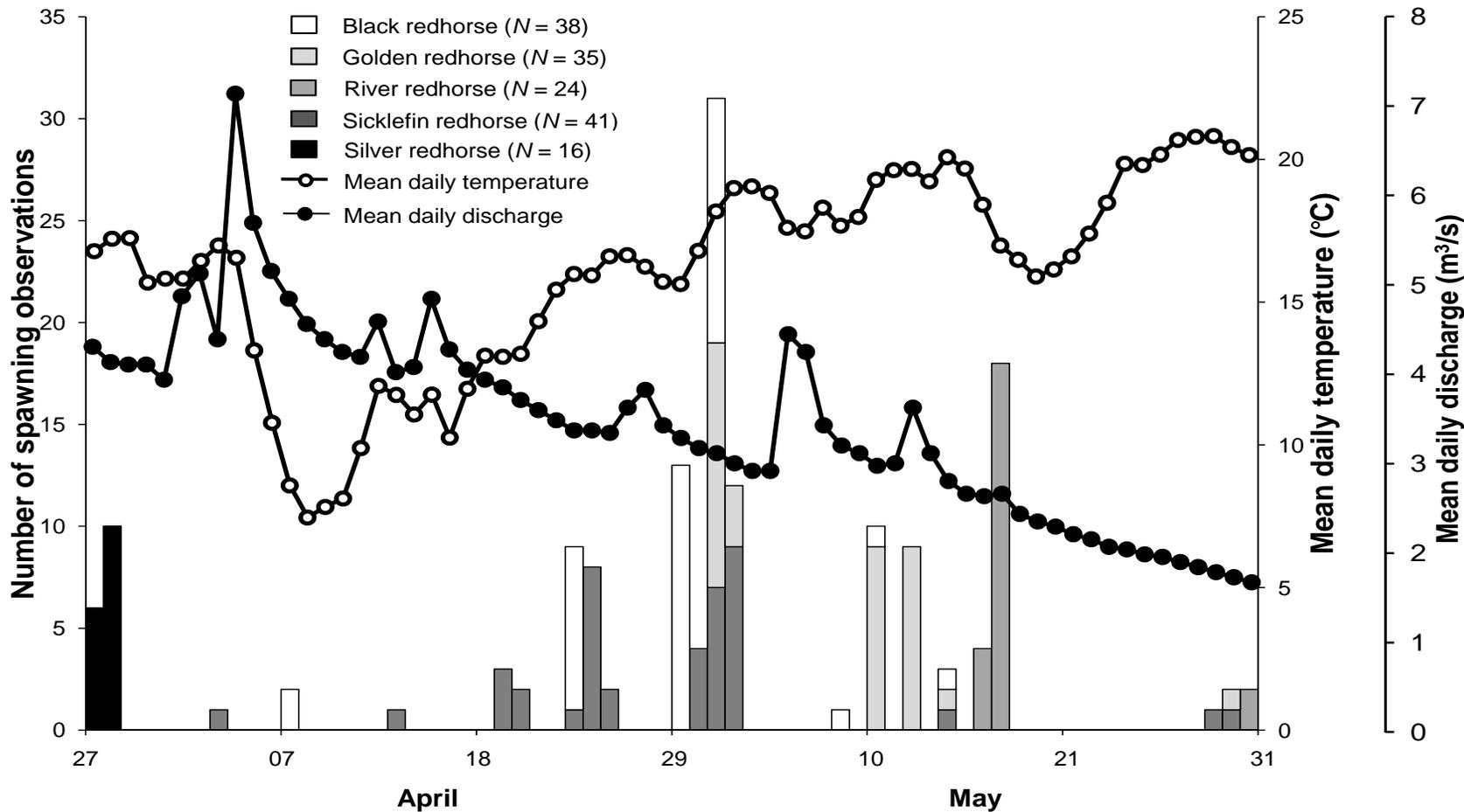


Figure 7.—Number of identified spawning/courting spawning sites for black redhorse, golden redhorse, river redhorse, sicklefin redhorse, and silver redhorse in the Hiwassee Basin, North Carolina, and corresponding mean daily water temperature and mean daily discharge for Valley River, North Carolina, during 2007.

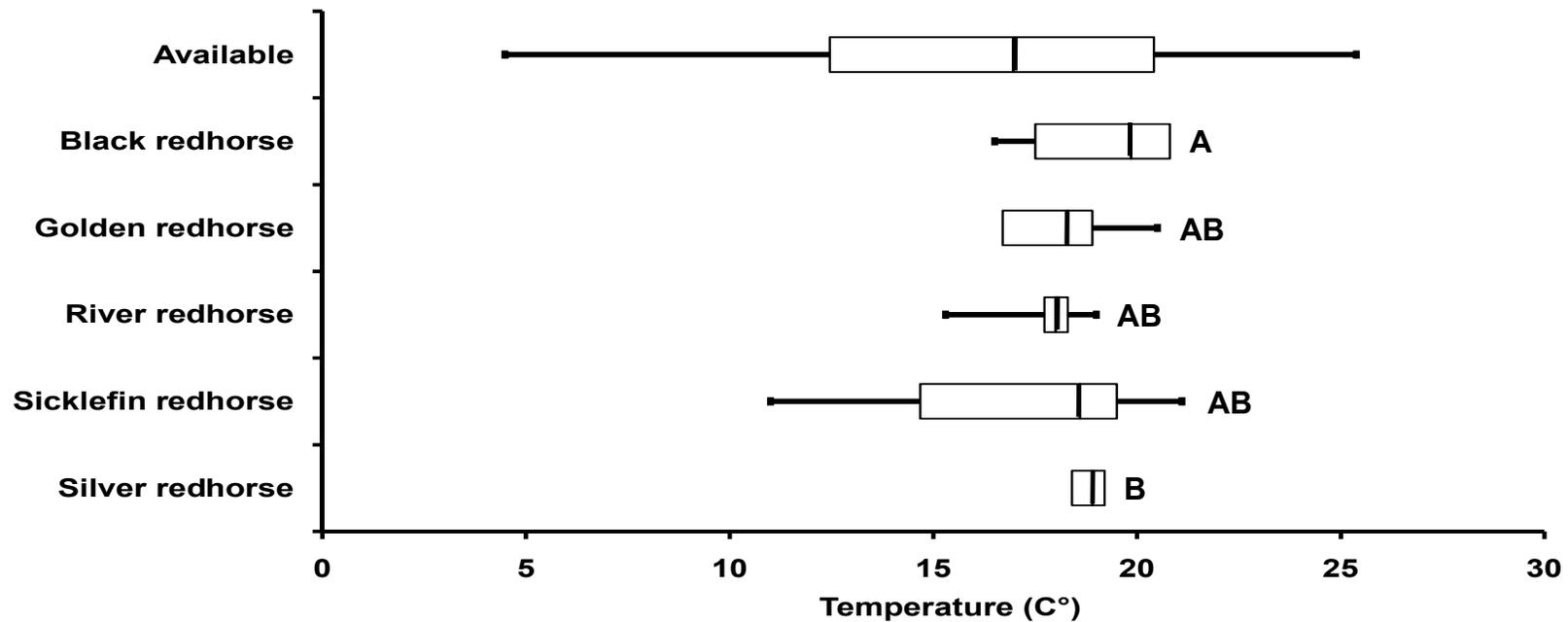


Figure 8.—A box-and-whisker plot depicting the median, minimum, maximum, first quartile, and third quartile for spawning pit instantaneous water temperatures and annual continuous available water temperatures from Valley River, North Carolina, during 2007. Statistical analyses were performed using a non-parametric Kruskal-Wallis test. Dunn multiple comparison tests were performed if a significant difference was detected.

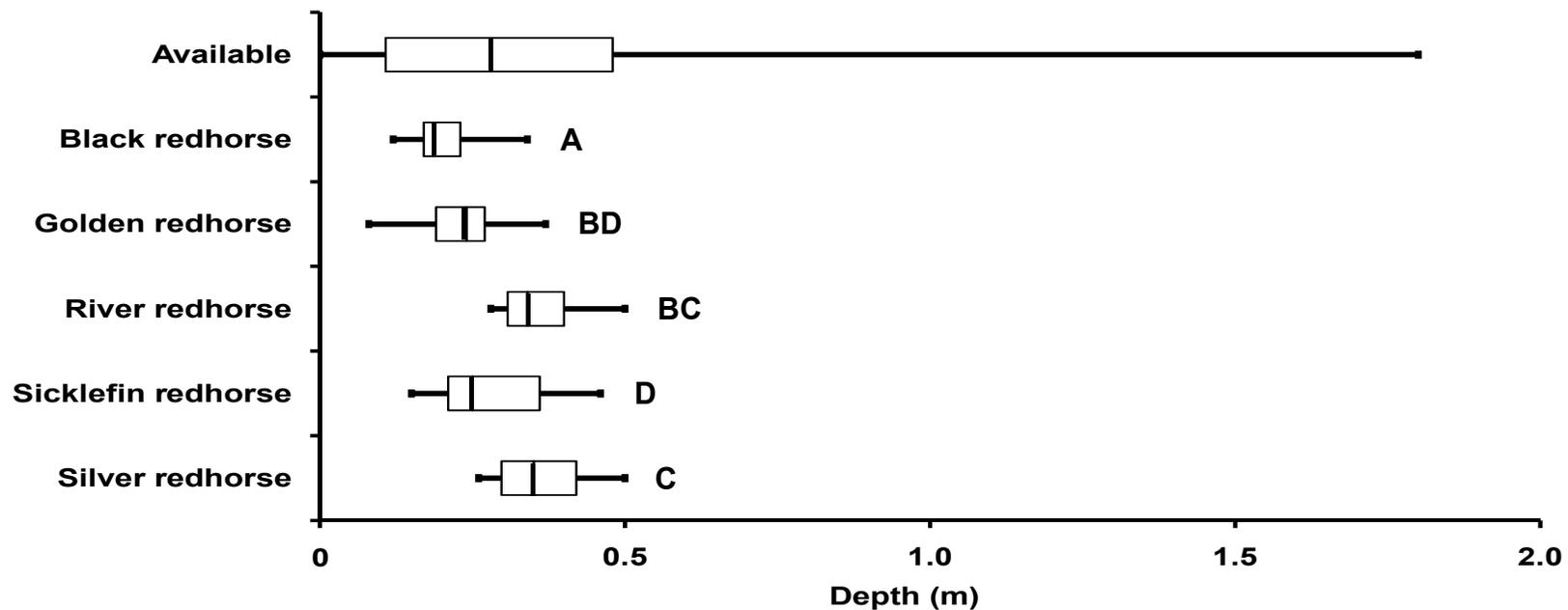


Figure 9.—A box-and-whisker plot depicting the median, minimum, maximum, first quartile, and third quartile for utilized depths associated with spawning and courting pits as well as depth availability in the upper Hiwassee Basin, North Carolina, during 2007. Statistical analyses were performed using a non-parametric Kruskal-Wallis test. Dunn multiple comparison tests were performed if the Kruskal-Wallis test null hypothesis was rejected and a significant difference was detected.

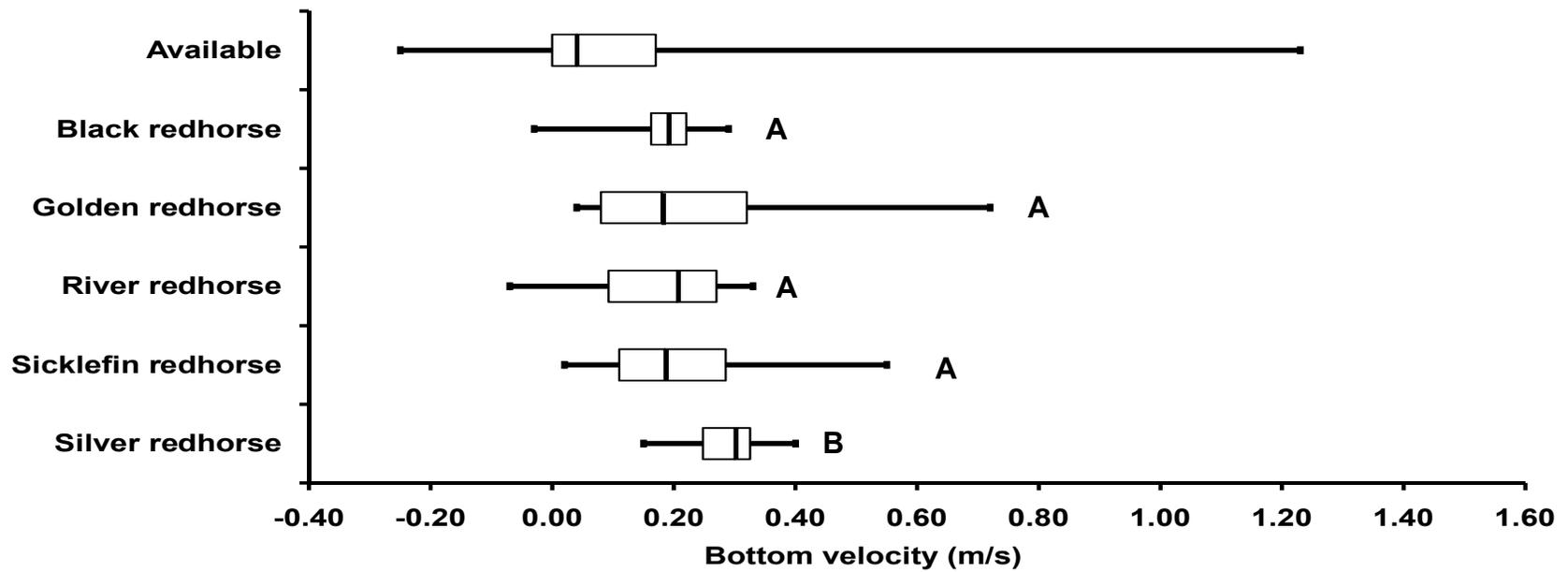


Figure 10.—A box-and-whisker plot depicting the median, minimum, maximum, first quartile, and third quartile for utilized bottom velocities associated with spawning and courting pits as well as bottom velocity availability in the upper Hiwassee Basin, North Carolina, during 2007. Statistical analyses were performed using a non-parametric Kruskal-Wallis test. Dunn multiple comparison tests were performed if a significant difference was detected.

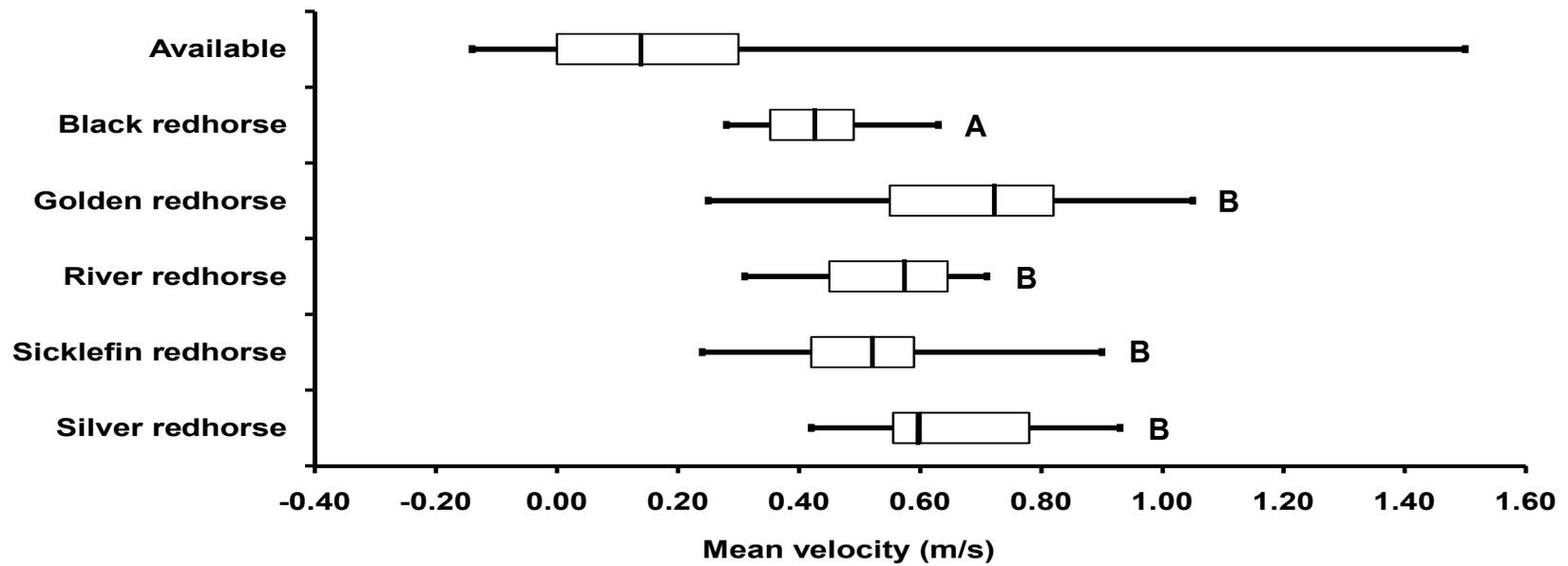


Figure 11.—A box-and-whisker plot depicting the median, minimum, maximum, first quartile, and third quartile for utilized mean column velocities associated with spawning and courting pits as well as mean column velocity availability in the upper Hiwassee Basin, North Carolina, during 2007. Statistical analyses were performed using a non-parametric Kruskal-Wallis test. Dunn multiple comparison tests were performed if a significant difference was detected.

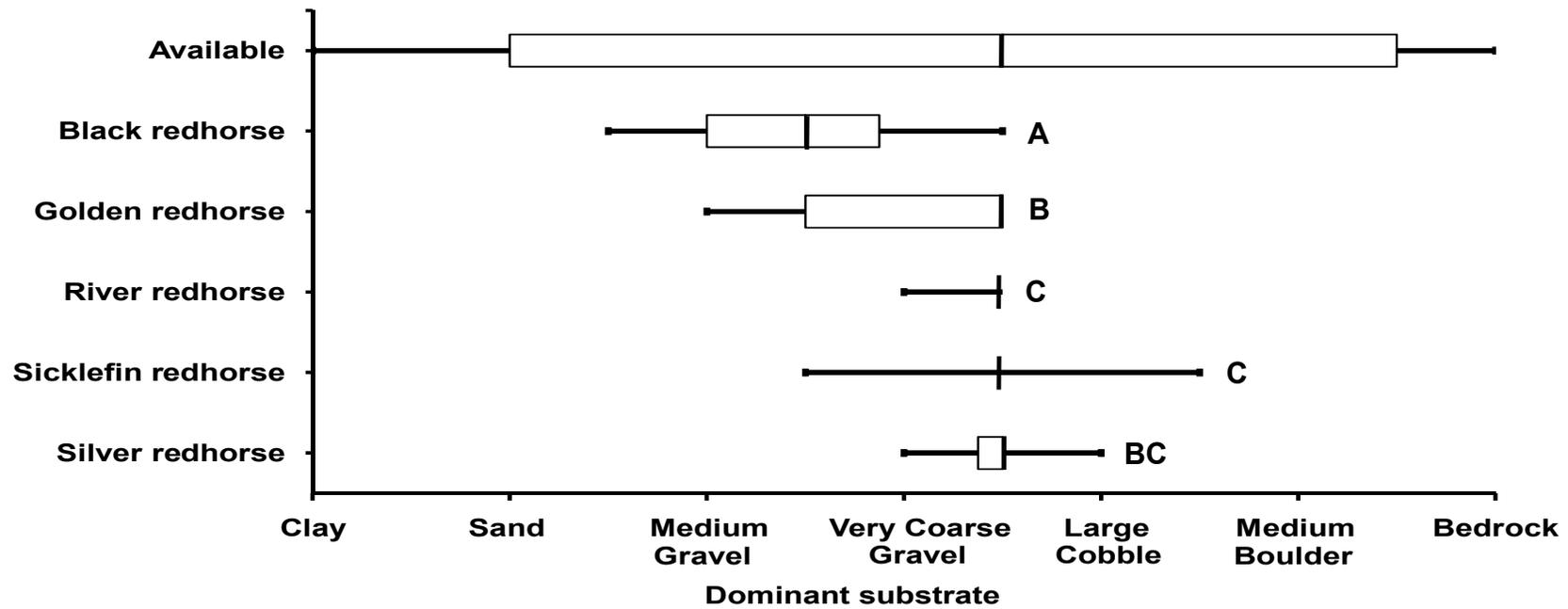


Figure 12.—A box-and-whisker plot depicting the median, minimum, maximum, first quartile, and third quartile for utilized dominant substrate associated with spawning and courting pits as well as dominant substrate availability in the upper Hiwassee Basin, North Carolina, during 2007. Statistical analyses were performed using a non-parametric Kruskal-Wallis test. Dunn multiple comparison tests were performed if a significant difference was detected.

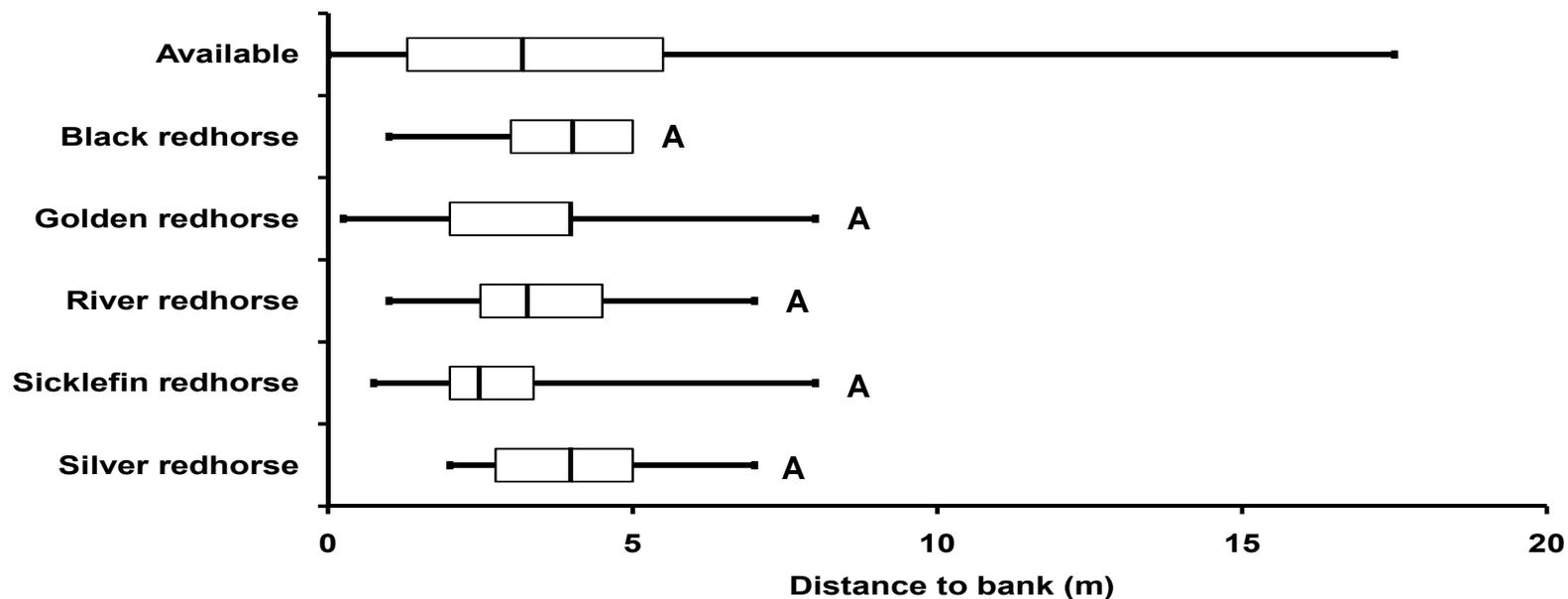


Figure 13.—A box-and-whisker plot depicting the median, minimum, maximum, first quartile, and third quartile for utilized distance to bank associated with spawning and courting pits as well as distance to bank availability in the upper Hiwassee Basin, North Carolina, during 2007. Statistical analyses were performed using a non-parametric Kruskal-Wallis test. Dunn multiple comparison tests were performed if a significant difference was detected.

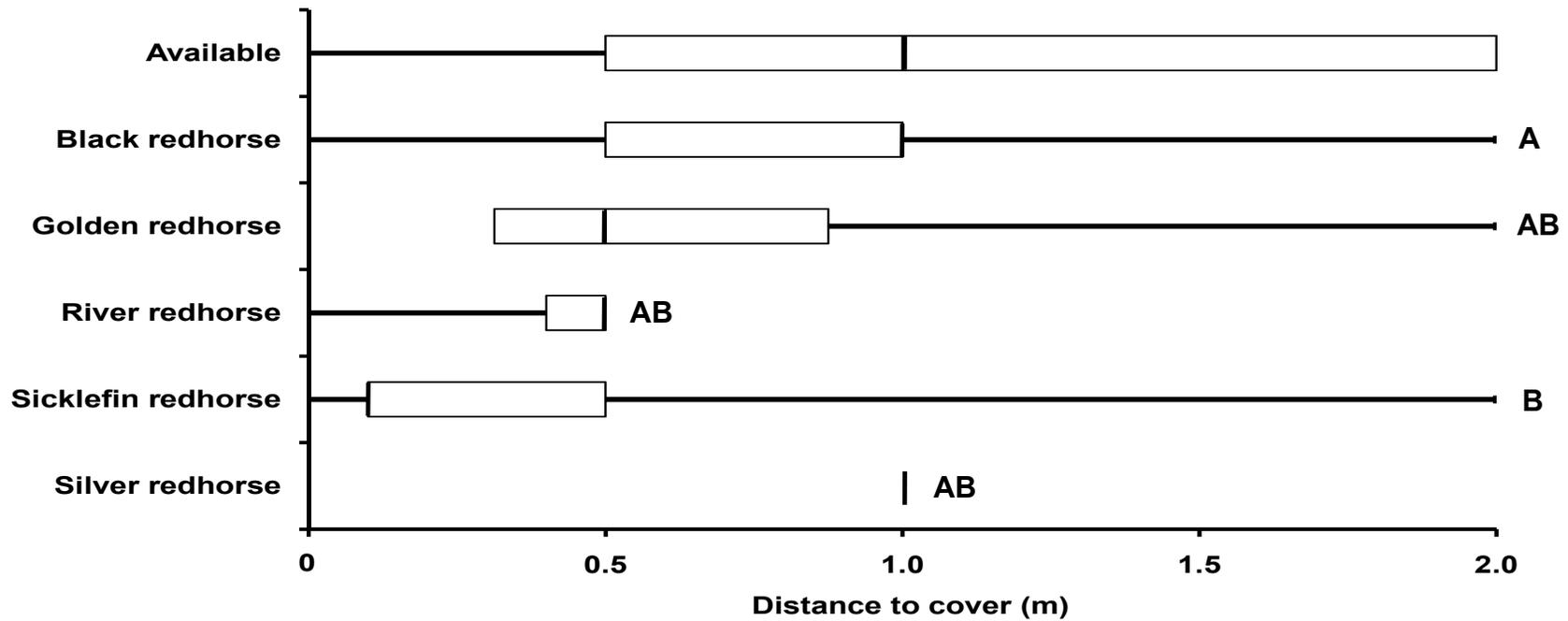


Figure 14.—A box-and-whisker plot depicting the median, minimum, maximum, first quartile, and third quartile for utilized distance to cover associated with spawning and courting pits as well as distance to cover availability in the upper Hiwassee Basin, North Carolina, during 2007. Statistical analyses were performed using a non-parametric Kruskal-Wallis test. Dunn multiple comparison tests were performed if a significant difference was detected.

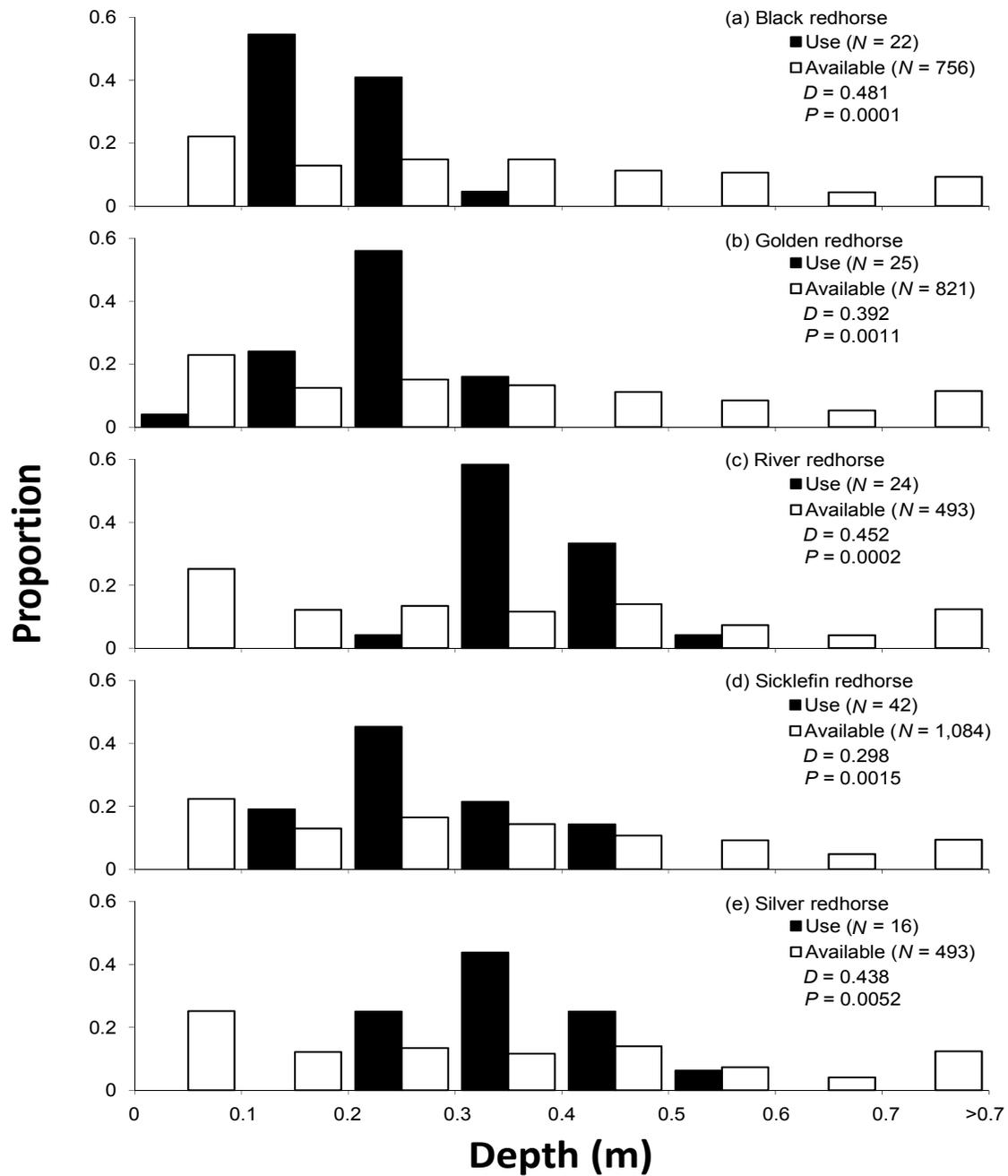


Figure 15.—Redhorse spawning microhabitat use and availability frequency distributions and associated statistics for depth in Hiwassee Basin, North Carolina and Georgia. Use and availability were compared using a Kolmogorov-Smirnov two-sample test.

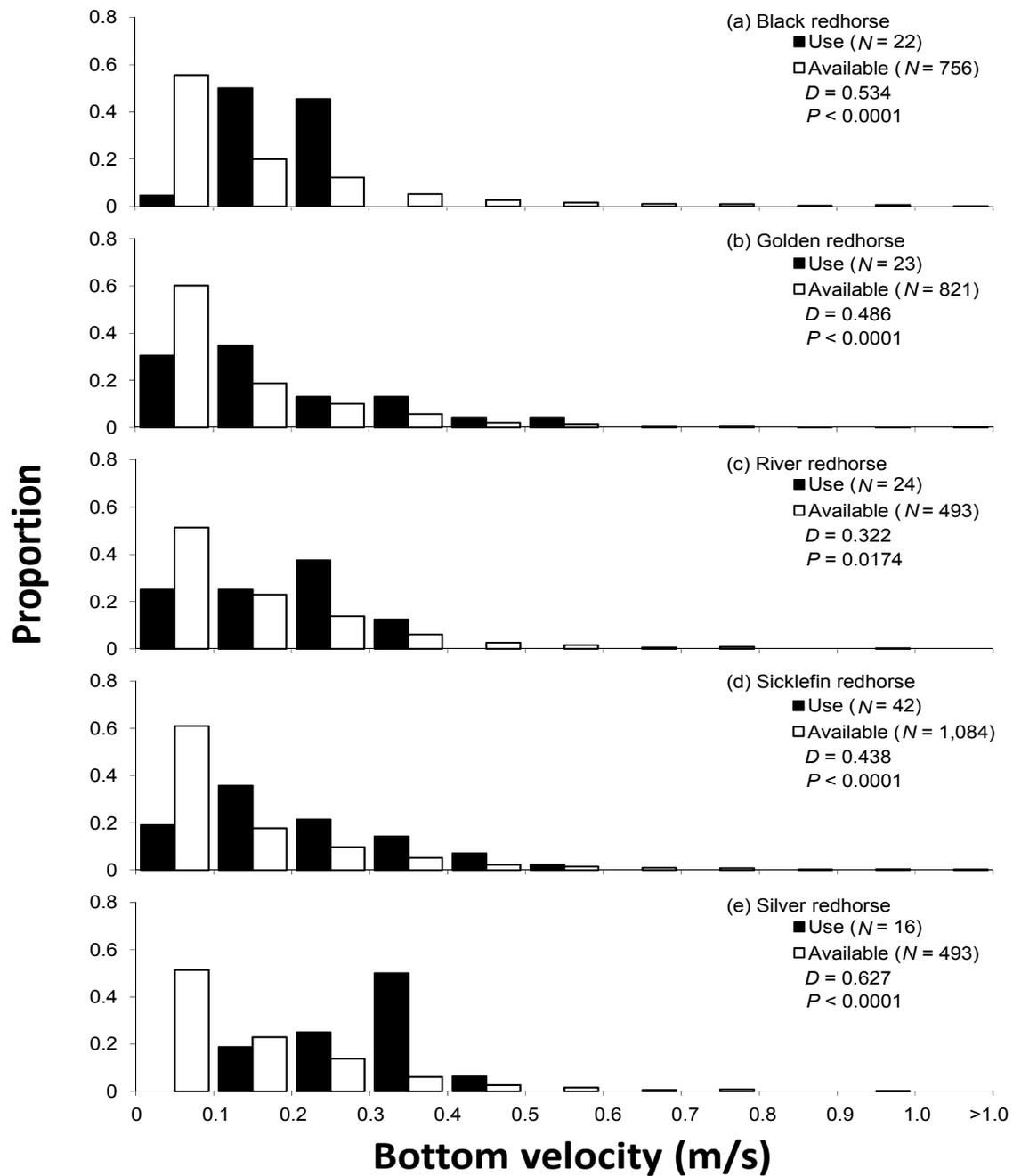


Figure 16.—Redhorse spawning microhabitat use and availability frequency distributions and associated statistics for bottom velocity in Hiwassee Basin, North Carolina and Georgia. Use and availability were compared using a Kolmogorov-Smirnov two-sample test.

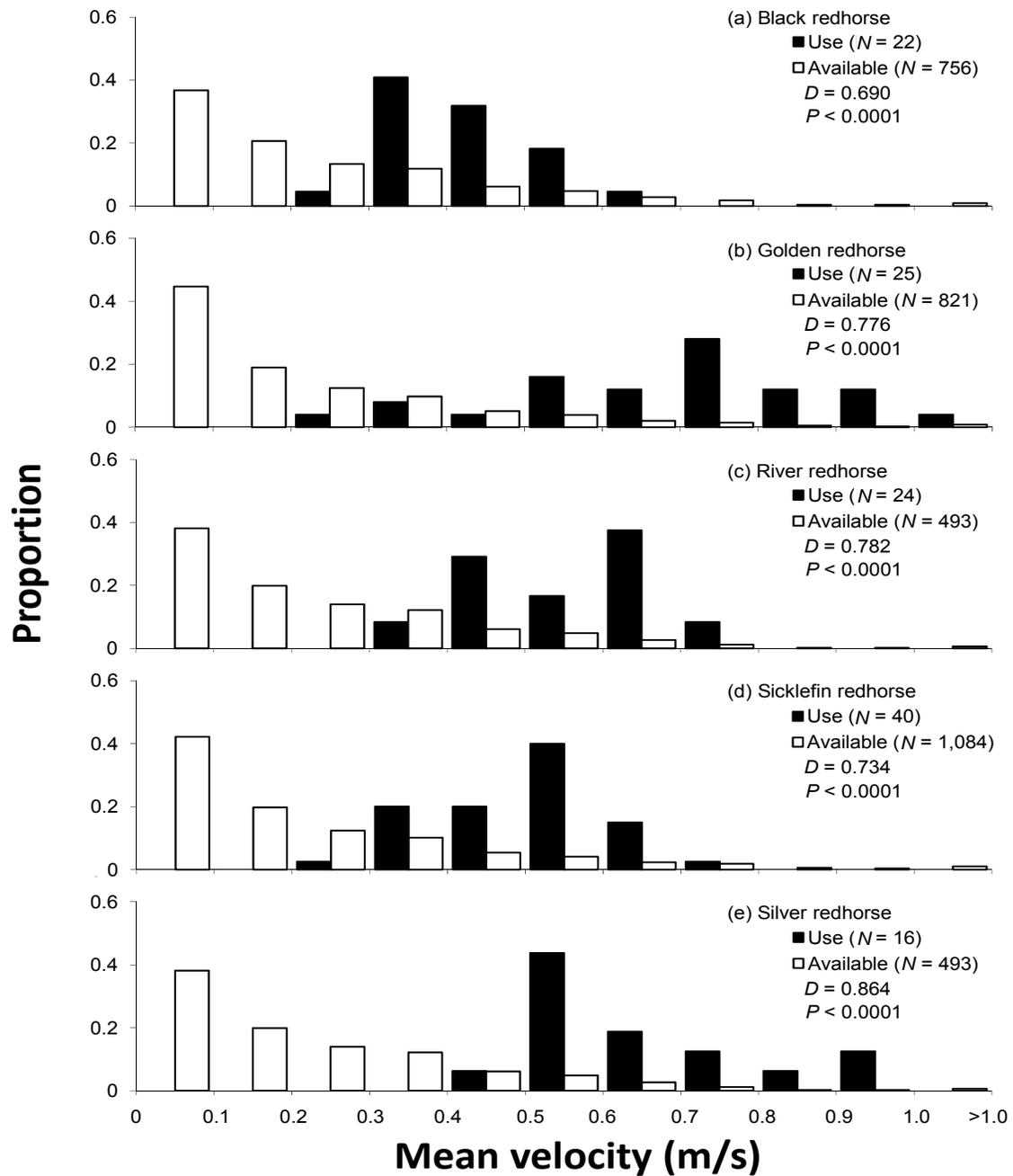


Figure 17.—Redhorse spawning microhabitat use and availability frequency distributions and associated statistics for mean column velocity in Hiwassee Basin, North Carolina and Georgia. Use and availability were compared using a Kolmogorov-Smirnov two-sample test.

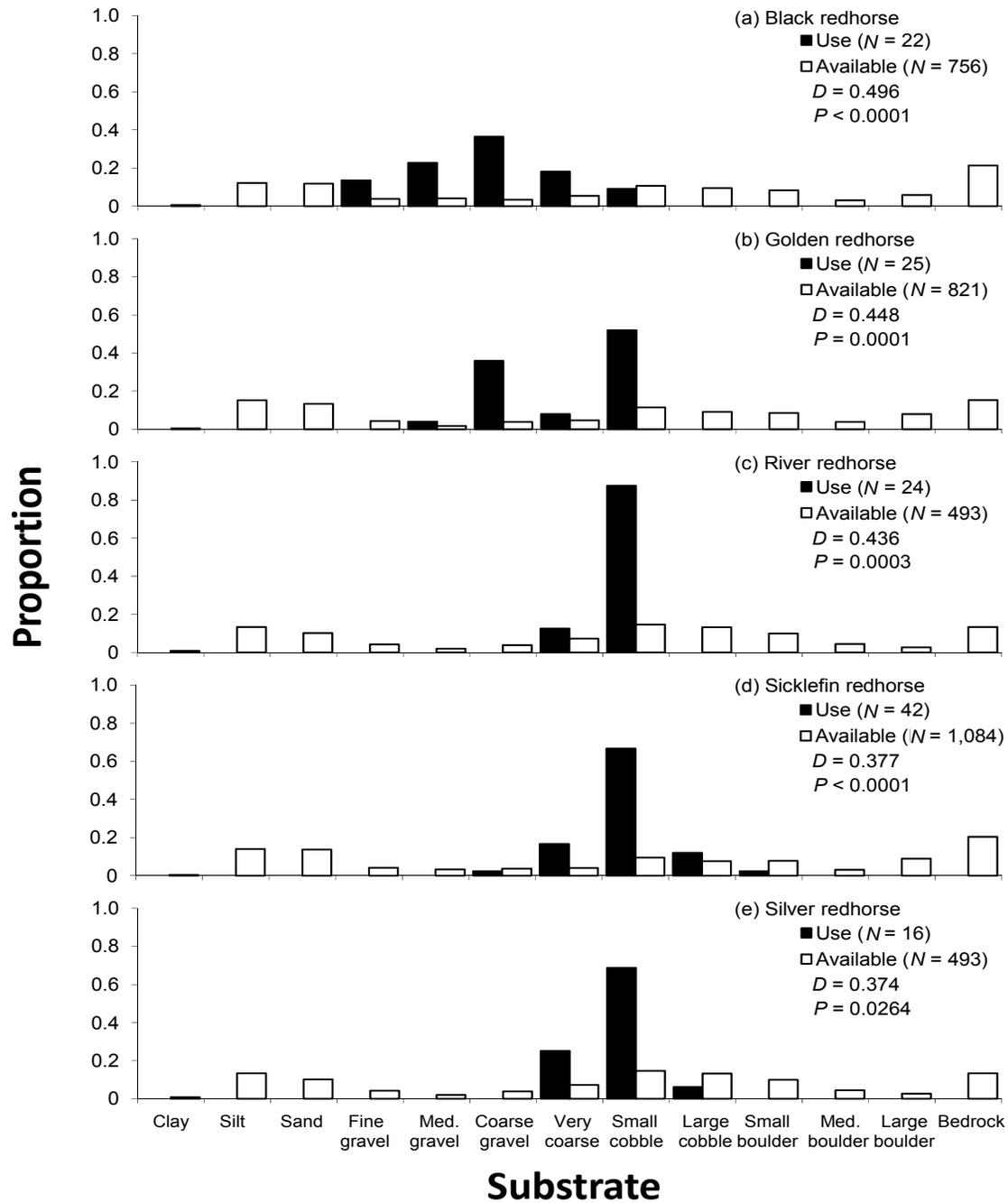


Figure 18.—Redhorse spawning microhabitat use and availability frequency distributions and associated statistics for dominant substrate in Hiwassee Basin, North Carolina and Georgia. Use and availability were compared using a Kolmogorov-Smirnov two-sample test.

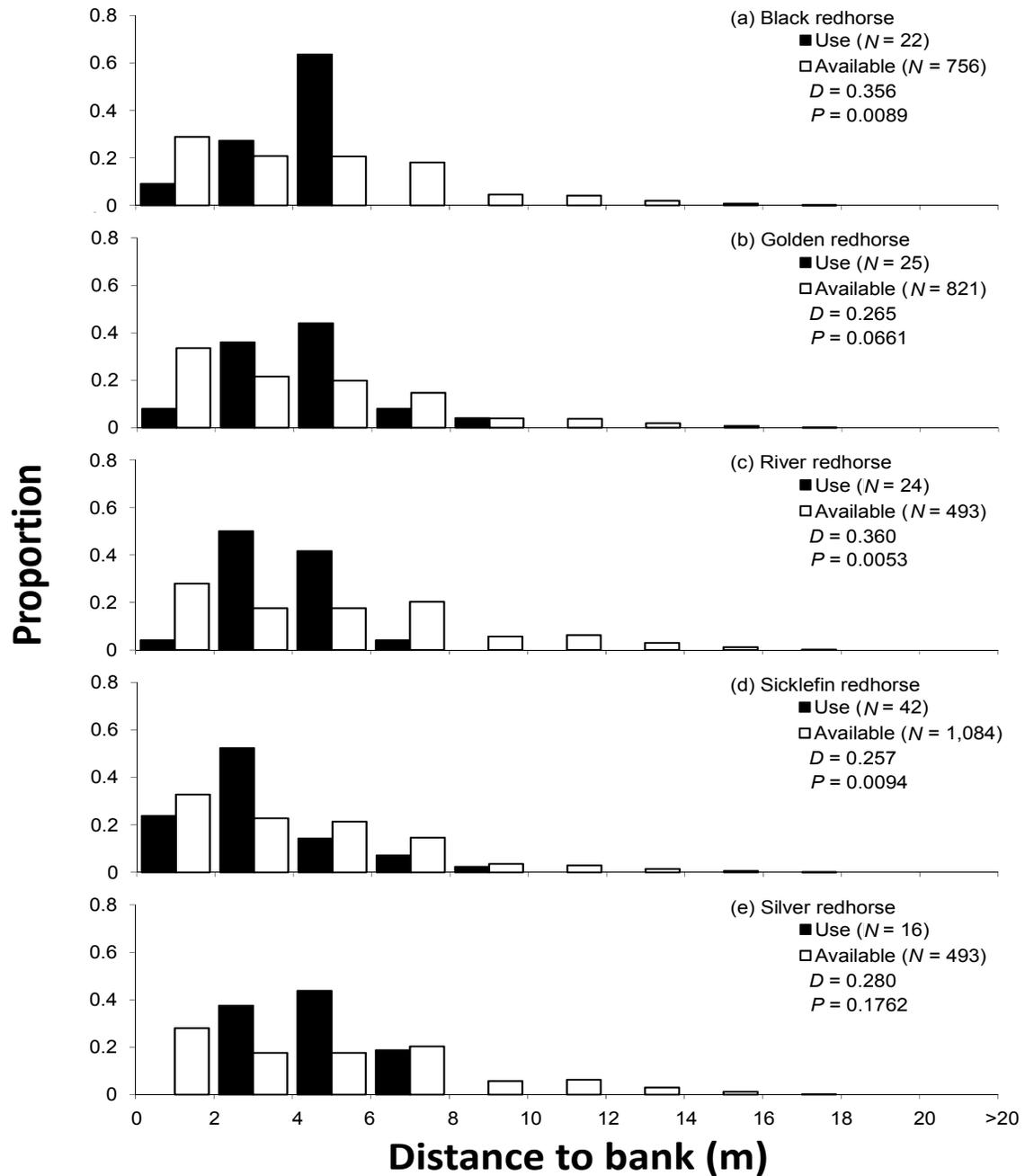


Figure 19.—Redhorse spawning microhabitat use and availability frequency distributions and associated statistics for distance to bank in Hiwassee Basin, North Carolina and Georgia. Use and availability were compared using a Kolmogorov-Smirnov two-sample test.

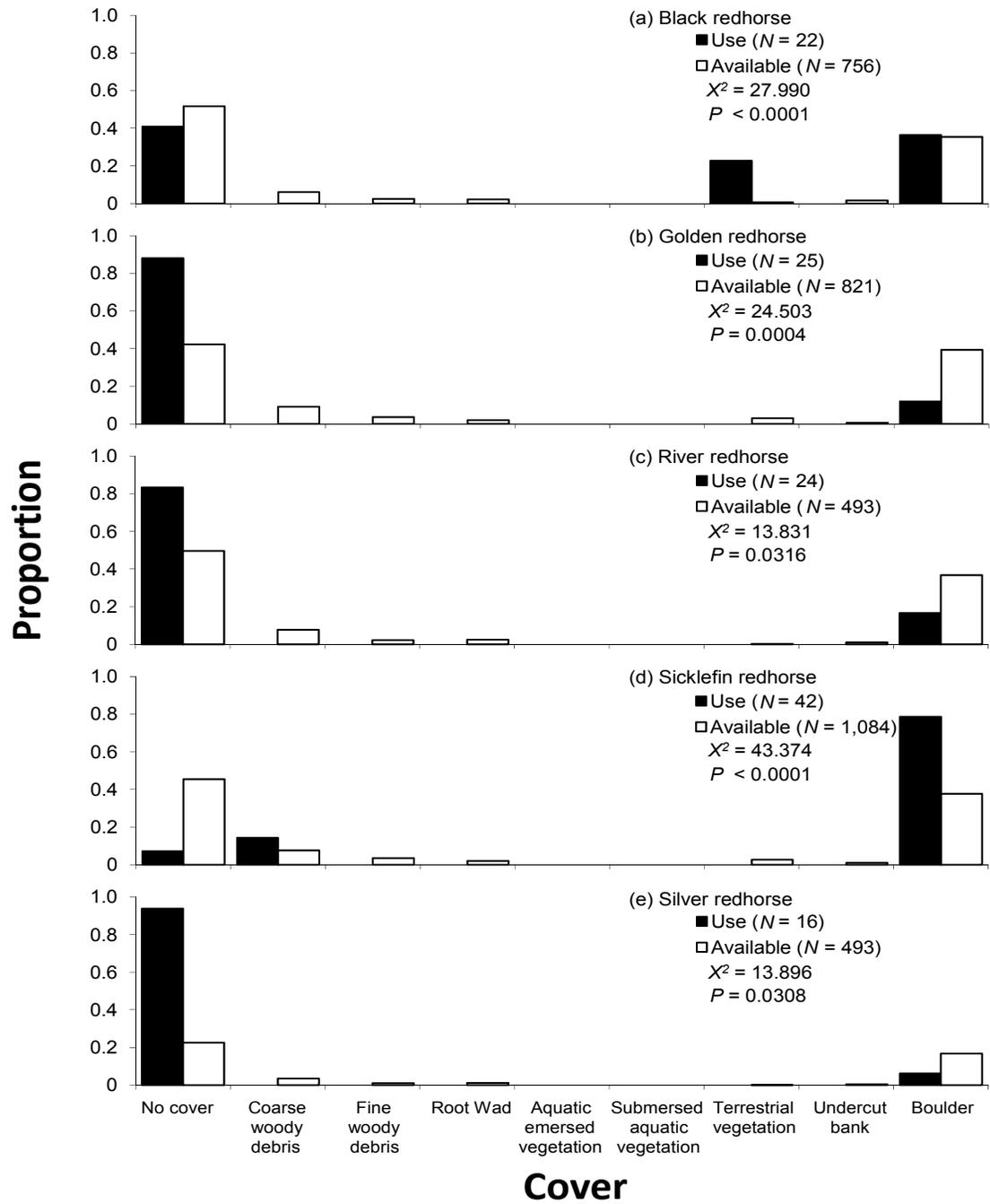


Figure 20.—Redhorse spawning microhabitat use and availability frequency distributions and associated statistics for cover type in Hiwassee Basin, North Carolina and Georgia. Use and availability were compared using a likelihood-ratio chi-square test.

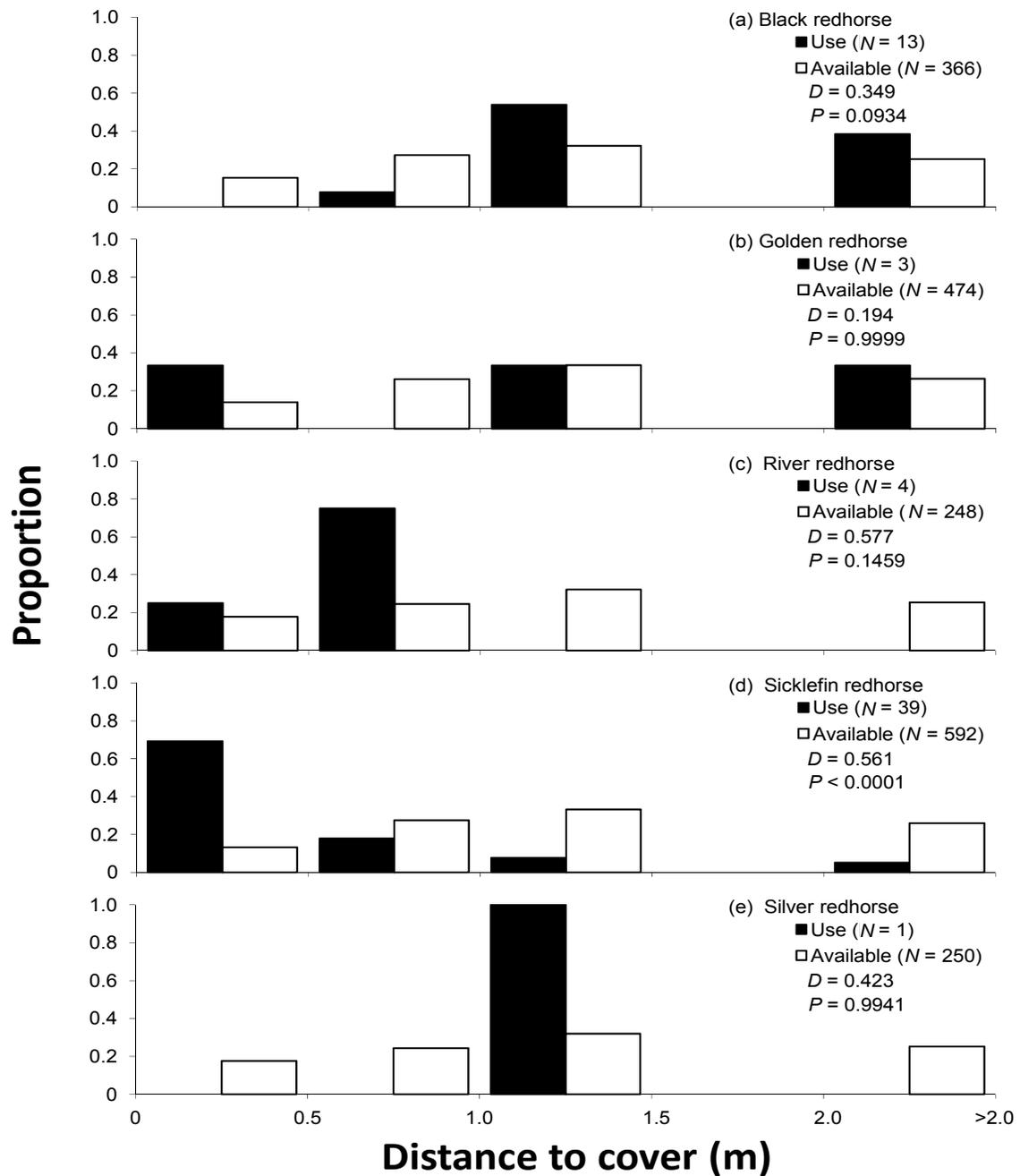


Figure 21.—Redhorse spawning microhabitat use and availability frequency distributions and associated statistics for distance to cover in Hiwassee Basin, North Carolina and Georgia. Use and availability were compared using a Kolmogorov-Smirnov two-sample test.

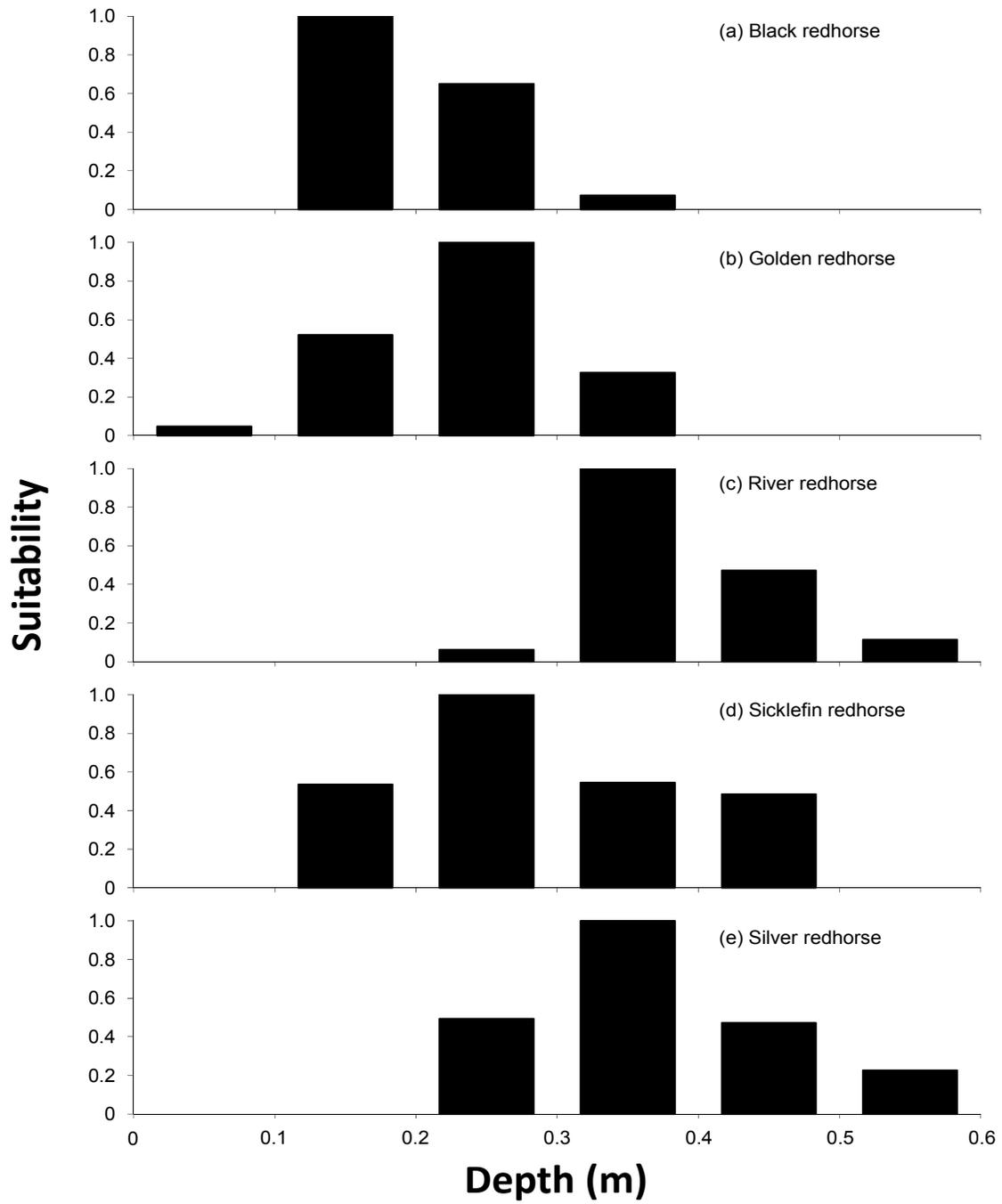


Figure 22.—Redhorse spawning microhabitat suitability distributions for depth in the Hiwassee Basin, North Carolina.

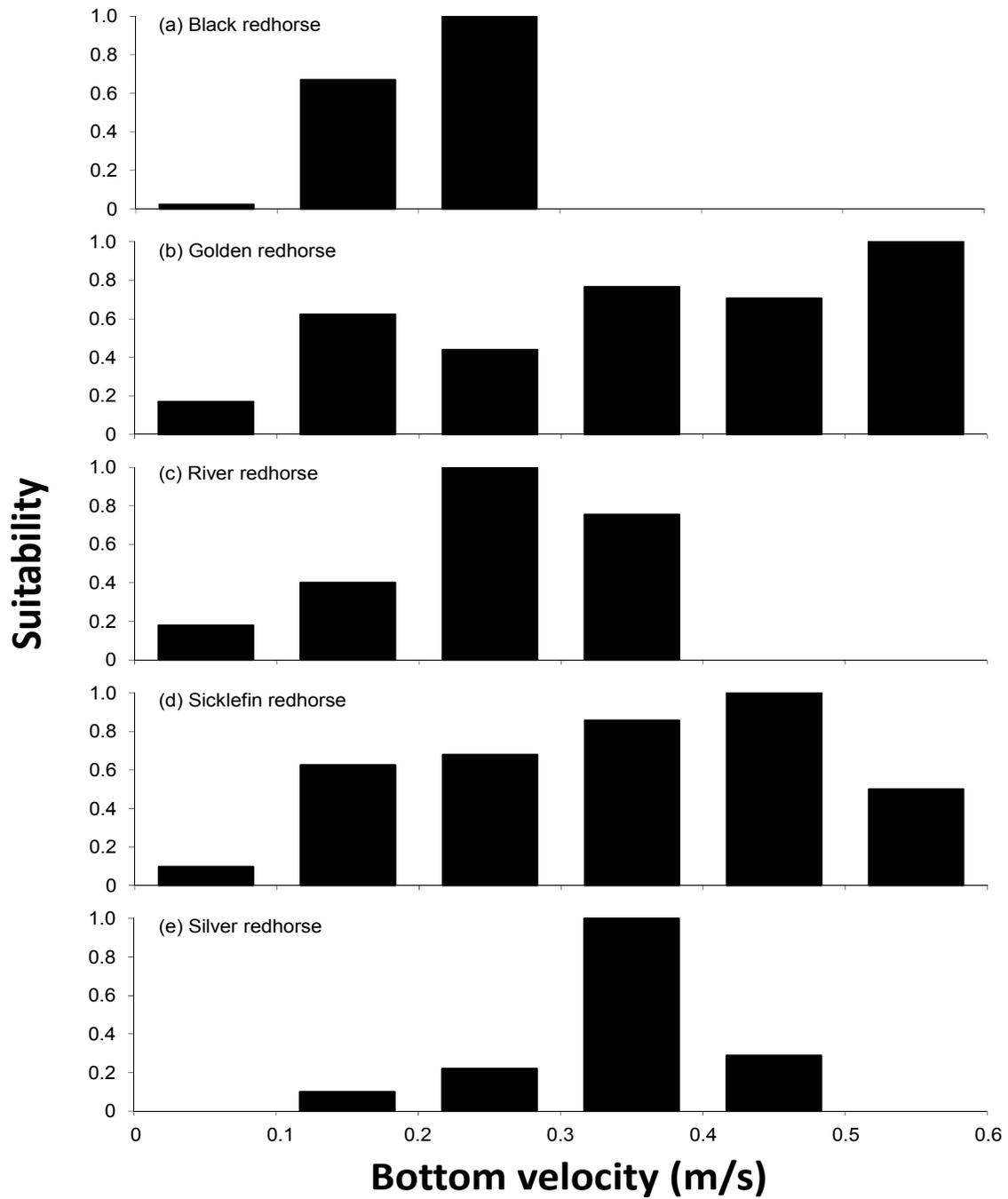


Figure 23.—Redhorse spawning microhabitat suitability distributions for bottom velocity in the Hiwassee Basin, North Carolina.

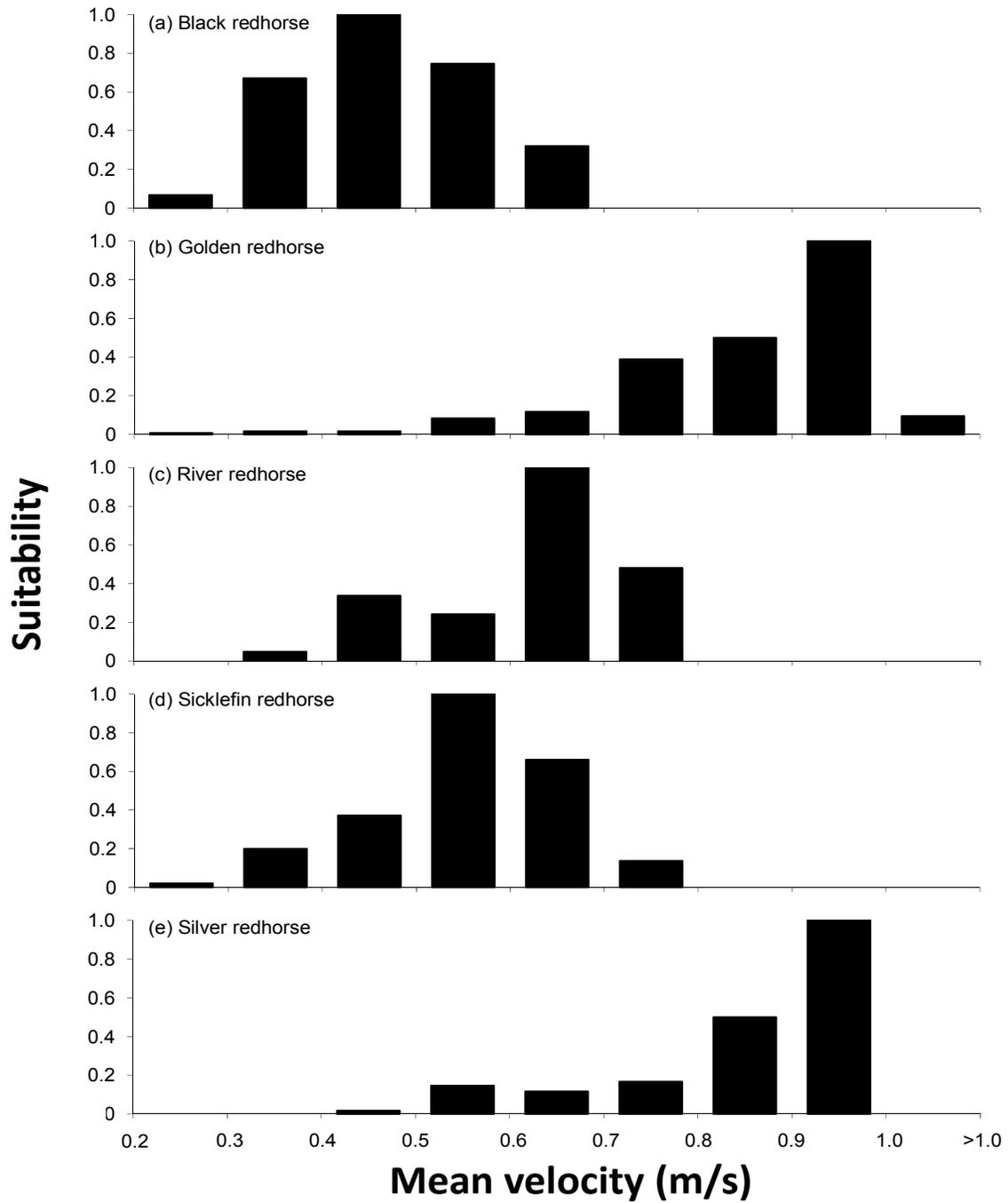


Figure 24.—Redhorse spawning microhabitat suitability distributions for mean column velocity in the Hiwassee Basin, North Carolina.

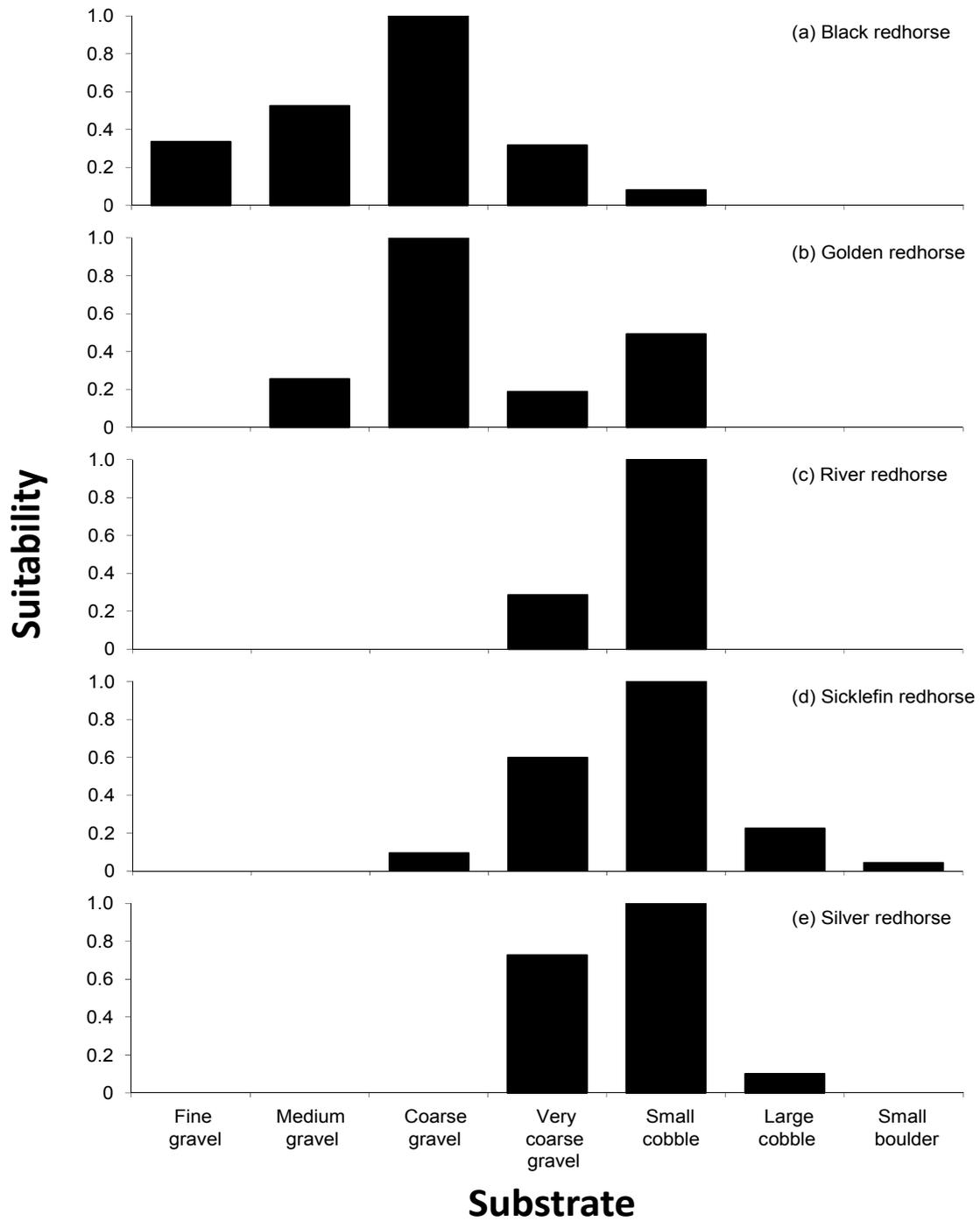


Figure 25.—Redhorse spawning microhabitat suitability distributions for dominant substrate in the Hiwassee Basin, North Carolina.

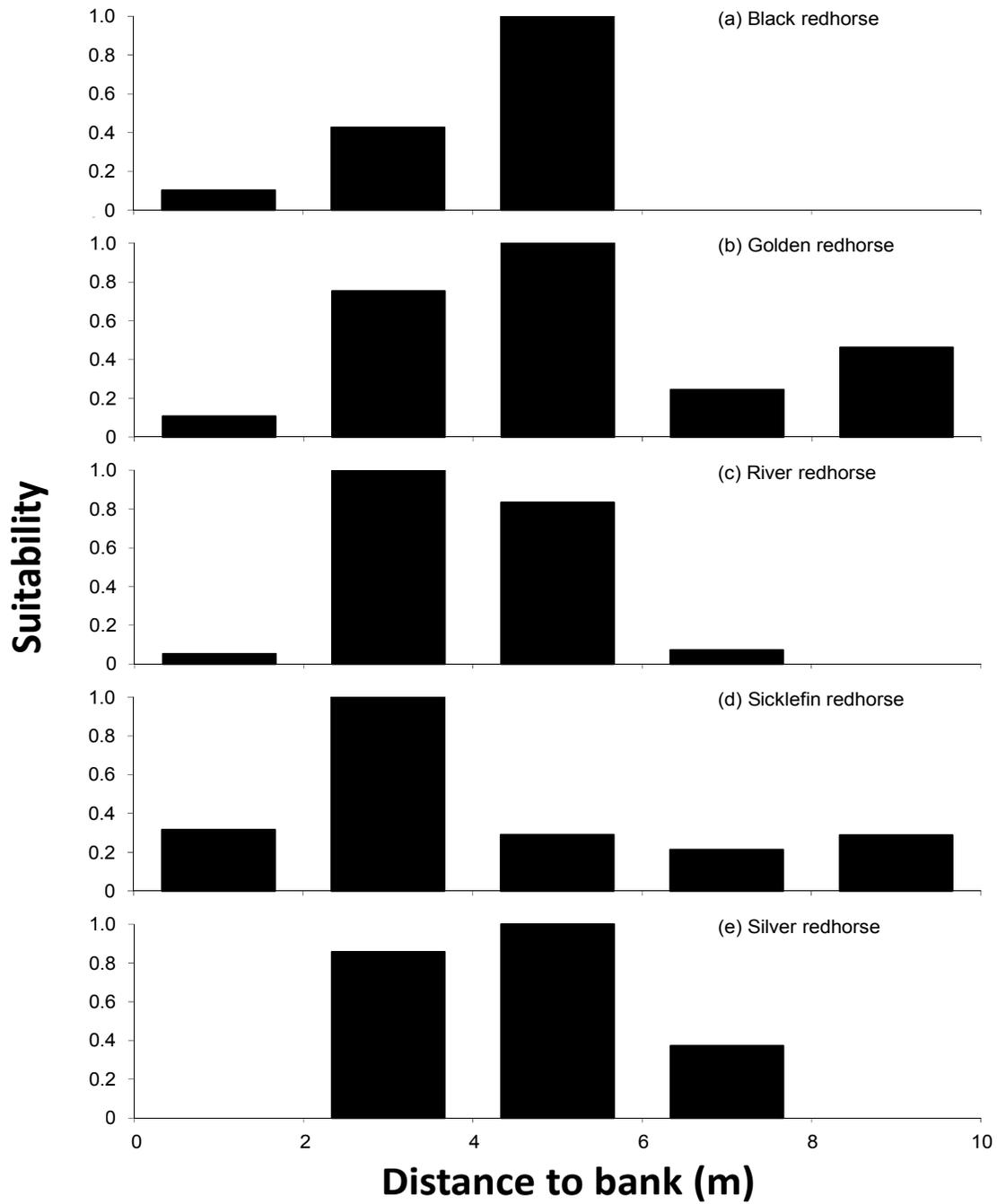


Figure 26.—Redhorse spawning microhabitat suitability distributions for distance to bank in the Hiwassee Basin, North Carolina.

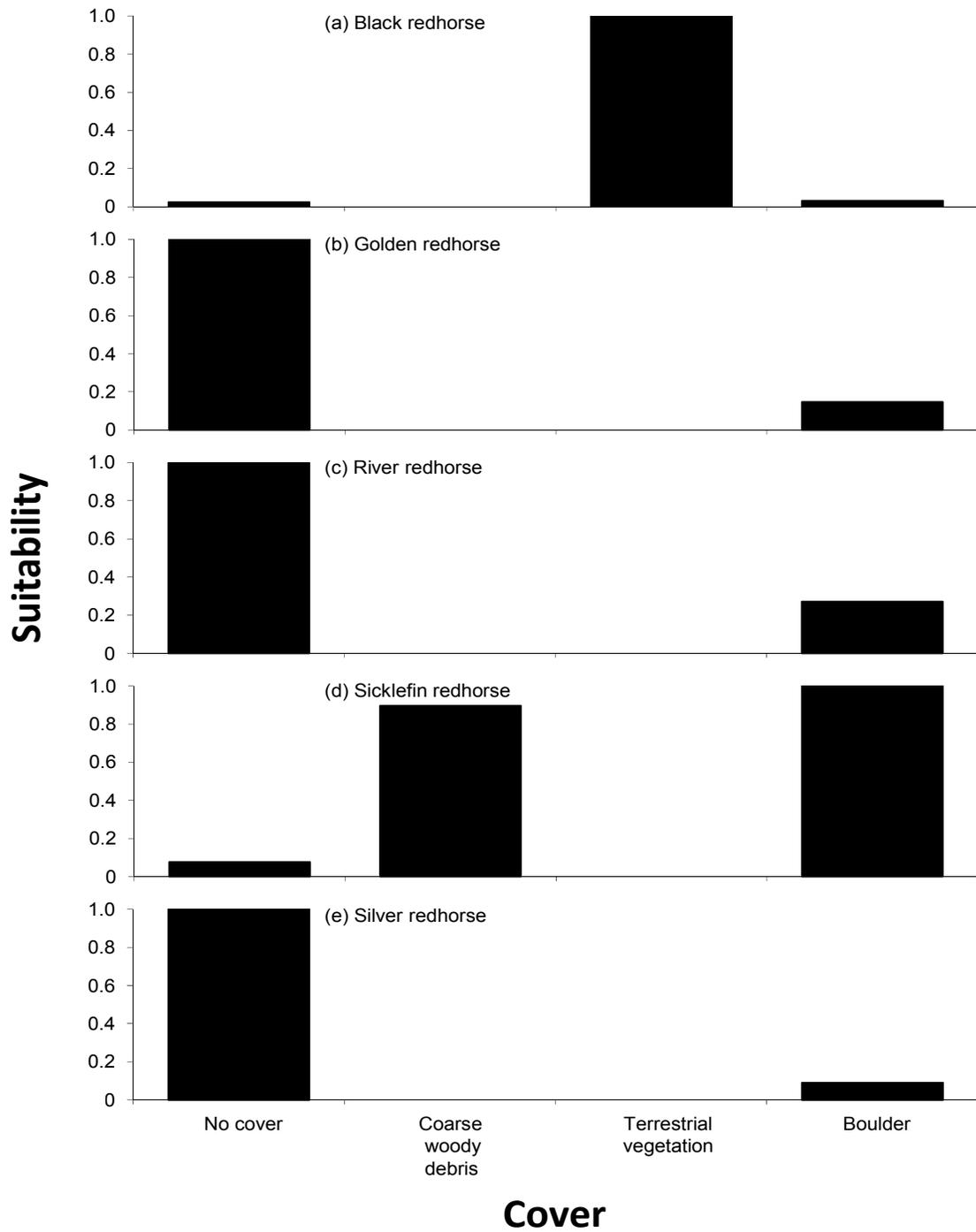


Figure 27.—Redhorse spawning microhabitat suitability distributions for cover type in the Hiwassee Basin, North Carolina.

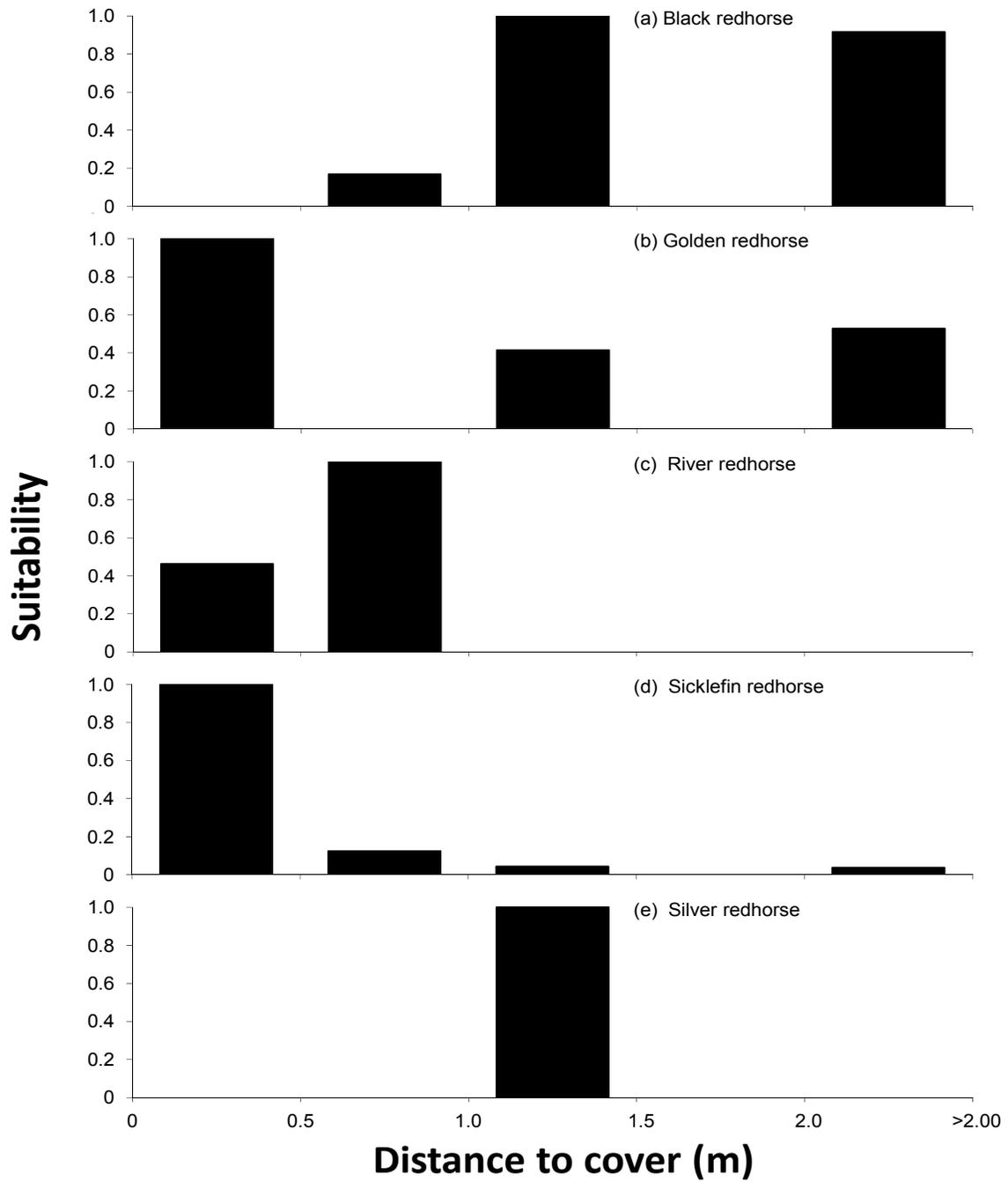


Figure 28.—Redhorse spawning microhabitat suitability distributions for distance to cover in the Hiwassee Basin, North Carolina.

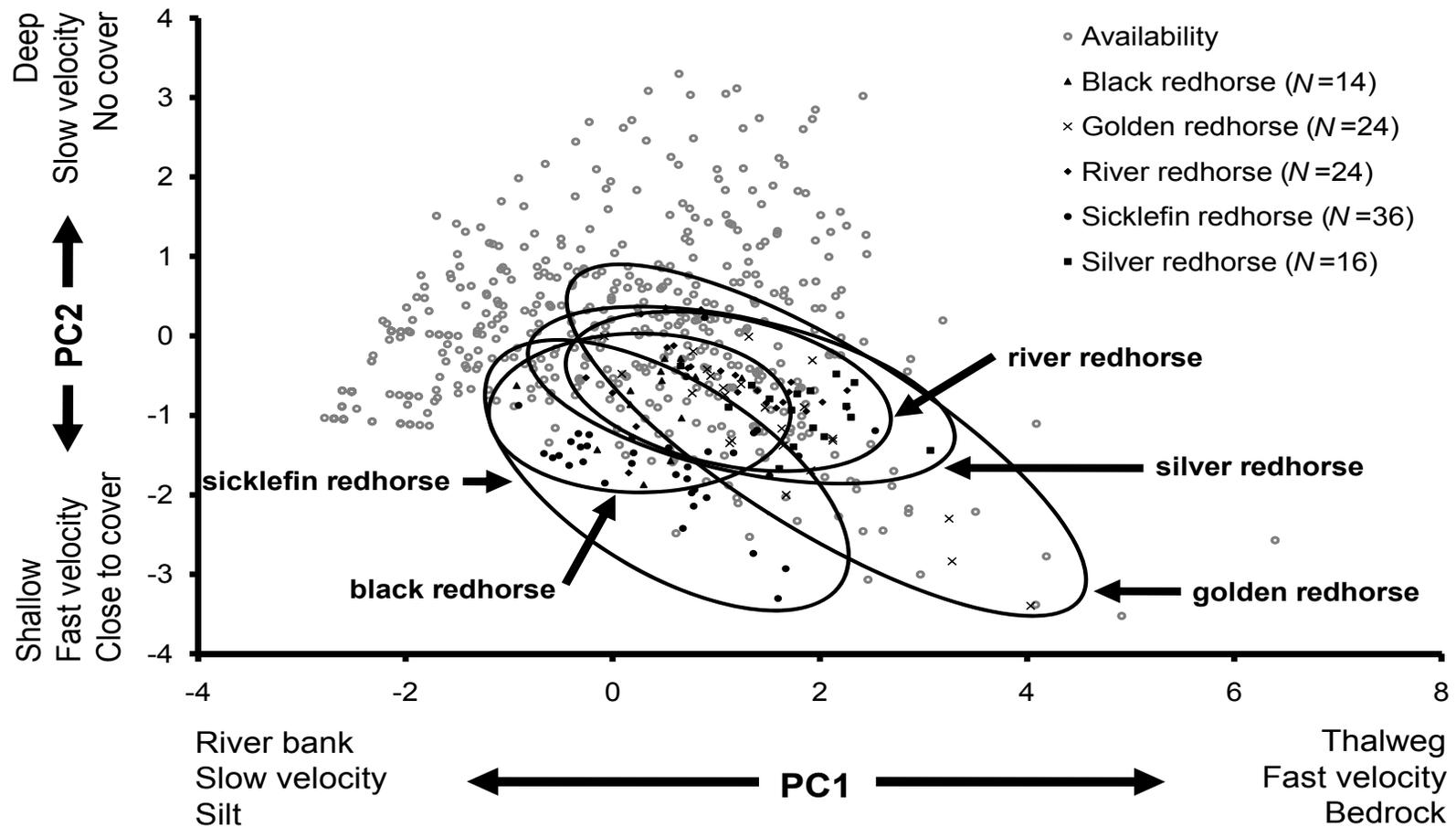


Figure 29.—Plot of redhorse principal component scores for microhabitat use and availability, describing microhabitat variables most important in defining suitable spawning macrohabitat.

**Chapter III – Comparison of Two-Way Weirs and Prepositioned Electrofishers for  
Sampling Potamodromous Fish Migration**

## **Abstract**

Potamodromy is common among many North American nongame riverine fishes, yet funding, research, and management efforts and results for potamodromous fishes are scarce. With the implementation of legislation protecting recreational and nongame fishes (e.g., Endangered Species Act (ESA), Committee on the Status of Wildlife in Canada, and the Species at Risk Act (SARA), Fisheries Act in Canada) and holistic fisheries management becoming more prevalent, methods of efficiently sampling previously unmanaged nongame potamodromous fishes will be essential. However, many nongame potamodromous fishes inhabit medium-sized streams during spawning migrations that are difficult to sample with conventional gears (e.g., nets and electrofishing). I compared an efficient salmonid migration passive sampling technique (resistance board weirs) and an active sampling technique previously used to obtain microhabitat use data (prepositioned areal electrofishers (PAEs)) to determine gear efficiencies for migrating potamodromous fishes.

Catch efficiency and composition for each gear were assessed by deploying both gears concurrently at two sites in Valley River, a North Carolina mountain stream in the Hiwassee drainage, from March through July during 2006 and 2007. Fish species richness and diversity were higher for PAE total catch, while weir catch had higher species dominance. Total PAE catch by number was much higher than total weir catch, but PAE total biomass was lower than weir total biomass, indicating that weirs sample larger fish. PAE catch biomass was initially higher than weir biomass over time, but both gears showed a substantial decrease in biomass during early-April suggesting a temporary termination of the

redhorse spawning migration. However, weir biomass was greater than PAE biomass in the following weeks, demonstrating that weirs are more efficient at sampling peak migration. Catch in weirs was greatly reduced after a peak in fish migration, while PAEs continued to collect potamodromous and resident fish. A sampling bias associated with water velocity was detected for PAEs, but not weirs. Neither weirs nor PAEs demonstrated a depth bias in wadeable reaches.

On average, PAE mortality was five times higher than weir mortality. My catch efficiency and composition comparisons indicate that weirs are more effective at documenting migration chronology, sample nocturnal migration, are not biased by flow or habitat, and maintain low mortality. Therefore, PAEs are a low-effort technique when sampling emphasis is seasonal occupancy, and weirs are most suitable when the emphasis is on sampling migratory riverine fish. My results will assist fisheries managers in selecting the most appropriate gear to address research and management objectives when sampling potamodromous fishes in medium-sized rivers.

## **Introduction**

Many fish species migrate seasonally for reproduction or other ecological functions (Lucas and Baras 2001). In North America over the last century, a barrage of factors contributing to the decline of many diadromous fishes has been broadly documented (Cada and Sale 1993), while the status of most inland nongame potamodromous species is commonly overlooked (Schmetterling and McEvoy 2000). Anthropogenic alterations in

fluvial ecosystems, such as migration barriers, degraded water quality, instream habitat degradation, altered flow and thermal regimes, and introduced species have been associated with declining native nongame potamodromous fish stocks (Cooke et al. 2005). Obtaining critical spatial and temporal population information associated with spawning migrations and stock size in conjunction with restoration programs is crucial toward enhancing the status of imperiled native fish populations that make significant migrations for spawning.

In addition to Salmonidae, select members of the Petromyzontidae, Acipenseridae, Polyodontidae, Lepisosteidae, Hiodontidae, Anguilliformes, Clupeidae, Cyprinidae, Catostomidae, Ictaluridae, Esocidae, Osmeridae, Gadidae, Moronidae, Centrarchidae, and Percidae families are reported to conduct upstream spawning migrations (Etnier and Starnes 1993; Jenkins and Burkhead 1993). Despite the fact that the vast majority of the fishes that make spawning migrations are not salmonids, a disproportionate level of attention and funding historically and currently is devoted toward salmonid migration management and research because these fishes are considered game fish (Sousa 1982). However, many salmonid species are considered detrimental exotics in the majority (e.g., rainbow trout *Oncorhynchus mykiss* and brook trout *Salvelinus fontinalis*) or entirety (i.e., brown trout *Salmo trutta*) of their current North American range (Gresswell 1988; Behnke 1992; Jenkins and Burkhead 1993). Potamodromy is a common life history characteristic for fishes in general, and upper stream reaches are primarily utilized for spawning purposes. Thus, collection techniques developed and refined to sample migratory salmonids (e.g., weirs and traps) should be considered when developing a monitoring program or research aimed at

sampling the spawning migration of a nonsalmonid species, population, or river system that previously received little attention.

Many migratory fishes utilize medium-sized streams during spawning migrations. Several obstacles, including water velocity, water depth, and stream width, can hinder fisheries biologists from effectively sampling medium-sized streams and rivers with conventional gears. Electrofishing is commonly applied to sample riverine fish, but medium-sized rivers are typically too deep and swift for backpack electrofishers, too shallow for boat-mounted electrofishers, and too wide for many mark-recapture and removal techniques to be efficient. Thus, I sought alternative gears for assessing the spawning migration of several native and non-native potamodromous species. I employed modified two-way resistance board weirs and prepositioned areal electrofishers (PAE) in a medium-sized Appalachian river to determine their relative gear efficiency.

Conventionally, two-way resistance board weirs are used to capture brood stock for terminal fisheries, gather age structure data, determine condition status, estimate adult sex ratio, determine spawning escapement, estimate abundance, estimate smolt production, and identify migratory patterns; weirs are often the standard against which other gears are evaluated (Hubert 1996; Zimmerman and Zabkar 2007). Prepositioned areal electrofishers are increasingly used to sample lotic habitats and are typically employed to collect microhabitat use data while eliminating fright bias (Bain et al. 1985; Aadland 1993; Fisher and Brown 1993), collect brood stock while maintaining natural selection integrity (Grabowski and Isely 2005), and estimate unbiased species richness (Bowen and Freeman

1998; Walsh et al. 2002). Since many potamodromous fishes (e.g., *Moxostoma* spp.) congregate near and display high site fidelity toward medium-sized river spawning grounds (Jenkins and Burkhead 1993), I desired to compare the efficiency of PAEs for sampling migrating nongame potamodromous fishes to that of an established salmonid migration sampling technique (i.e., resistance board weir) (Zimmerman and Zabkar 2007).

## **Objectives**

The goal of this research was to obtain spatiotemporally similar samples with PAEs and resistance board weirs to determine their efficiency at sampling potamodromous fishes conducting a spawning migration in a medium-sized Appalachian Mountain river. Specifically, my objectives were to assess gear efficiency by (1) comparing catch composition community indices and parameters for both gears; (2) comparing the capability of each gear to document migration chronology; (3) examining gears for velocity bias; (4) estimating mortality rates for each gear; and (5) determining construction cost and effort required for both gears.

## **Methods**

### *Site Description*

This study was conducted on Valley River, a tributary of the Hiwassee River in the upper Hiwassee Basin of the southern Blue Ridge Province in the southern Appalachian Mountains, western North Carolina (Figure 1). Valley River was selected to conduct this

gear comparison study because potamodromous species representing Clupeidae (i.e., blueback herring *Alosa aestivalis*), Cyprinidae (i.e., common carp *Cyprinus carpio*), Catostomidae (i.e., sicklefin redhorse *Moxostoma* sp.), Ictaluridae (i.e., channel catfish *Ictalurus punctatus*), Moronidae (i.e., white bass *Morone chrysops*), Centrarchidae (i.e., smallmouth bass *Micropterus dolomieu*), and Percidae (i.e., walleye *Stizostedion vitreum*) utilize this river as a spawning tributary within the upper Hiwassee Basin. Valley River is an unregulated, moderate-gradient river that drains approximately 303 km<sup>2</sup>. The confluence of Valley River and Hiwassee River is near Hiwassee Lake and during periods of high reservoir levels during the spring and summer, the confluence is inundated by Hiwassee Lake.

One downstream and one upstream site were selected on Valley River located 11.8 and 22.4 river km upstream from the Valley River and Hiwassee River confluence (Figure 1). At the downstream site, Valley River is a seventh order stream and drains 267.7 km<sup>2</sup>, and at the upstream site, it is a sixth-order stream draining 188.1 km<sup>2</sup>. During spring flows, when spawning migrations typically occur, upper Valley River substratum is generally composed of bedrock (19.7%), sand (17.5%), and small cobble (13.1%); lower Valley River substratum is generally composed of bedrock (37.2%), sand (21.9%), and fine gravel (10.2%). The upper Valley River site had an average depth of 0.24 m and mean column velocity of 0.16 m/s. And the lower Valley River site had an average depth of 0.39 m and mean column velocity of 0.22 m/s. From 1 March to 1 July, mean Valley River stream flow, obtained from U.S. Geological Survey gauging station 06020002 located 0.85 km downstream from the

downstream site, was 4.48 m<sup>3</sup>/s (SE, min – max; 0.14, 1.84 – 11.27) in 2006 and 3.77 m<sup>3</sup>/s (SE, min – max; 0.23, 1.25 – 19.37) in 2007.

### *Fish Sampling and Study Design*

Fish communities were sampled during 2006 (20 April – 30 June) and 2007 (27 March – 6 June). PAEs and two-way resistance board weirs were used concurrently two consecutive days per week for the duration of this study. PAEs were constructed based on the design of Fisher and Brown (1993) and Grabowski and Isely (2005), and modified resistance board weir construction and installation was based on methods described by Torbin (1994), Mogen (1996), and Stewart (2002, 2003).

PAEs consisted of paired 15-m lengths of 0.65 cm stainless steel cable, spaced 1.0 m apart using 7.0 cm polyvinyl chloride (PVC) pipe. Alternating current (AC) powered the PAEs employing a 3,500-W generator and converter unit that regulated amperage. The PAE device and converter unit were connected to the generator using a 30-m electrical cord so that the PAE could be energized without approaching it and introducing fright bias (Kinsolving and Bain 1993).

For each PAE sample, the bottom grid cable assembly was positioned within the stream perpendicular to flow and allowed to remain undisturbed for a minimum of 15 min to eliminate fright bias (Bain et al. 1985; Bowen and Freeman 1998; Freeman et al. 2001). Then a single netter was positioned on the stream bank approximately 15 m downstream of the PAE. The PAE was then charged and all stunned fish were collected as the netter moved

upstream. The PAE was energized for 30–60 s; however, on rare occasions when more than 25 individuals were observed stunned, the PAE was energized over 60 s to ensure that all stunned fish were netted. As a result of a low mean conductivity of 48.15  $\mu\text{S}/\text{cm}$  (SE, min–max; 0.48, 34.8–63.6) during 2006 and 2007, the effective electrical field was restricted to the area between the two electrodes resulting in minimized variability in sampling efficiency among PAE samples relative to coverage area (Bowen and Freeman 1998; Freeman et al. 2001). After the field was electrified, the stream substrate between the electrodes and immediately downstream of the PAE were agitated by the netter and then visually scanned to dislodge and collect any additional stunned fish. All fish were transported to a recovery container prior to release. Each fish was identified to species, weighed (g), measured for total length (mm), and assessed for mortality (dead or alive) prior to release. In addition, sex was determined for adult redhorses based on gamete expression, tubercle development, and criterion developed by R. E. Jenkins (personal communication). Fish that could not be positively identified in the field were preserved in a 10% formalin solution and identified in lab.

My modified resistance board weirs deviated from traditional construction and installation guidelines and included shortened picket lengths (1 m), no substrate apron, and the utilization of 2.1 cm outer diameter PVC pipe to construct the weir panels. Picket diameter and length was reduced to minimize cost and because estimation of absolute migration spawning stock size was not an objective of this comparison study. The substrate apron was excluded from the modified weir design to reduce cost and was feasible owing to

the coarseness of the underlying substrate (i.e., small and large cobble) and high degree of substrate embeddedness. Undermining beneath the weir was not an issue during this study despite several high flow events that submerged the entire weir. Smaller diameter and lengths of PVC pipe drastically reduced the cost of each weir and were used entirely for that reason.

Fish samples were obtained during near base flow conditions. During 2006, modified fyke nets were used as the upstream and downstream traps. Due to practical limitations associated with the fyke nets (i.e., holes and clogging) in 2006, angle iron and metal conduit live traps were installed in 2007 following the design illustrated by Stewart (2003). All weir panels and live traps were designed with a gap width of 3.8 cm.

For each two-way resistance board weir sample, the weir was allowed to fish for approximately 24 h prior to collection. Weirs were set and collected near sunrise for every sample to ensure that all weir samples equally sampled diurnal and nocturnal fish migration. Captured fish were removed from the traps and in addition to data collected for all PAE samples, migration direction was recorded. After each individual fish was processed, it was released in the direction in which it was migrating.

Both the upstream and downstream weir sites were selected based on stream lateral profile suitability as described by Torbin (1994). At the downstream site, a PAE location was selected 0.30 km upstream and 0.85 km downstream from the weir; at the upstream site, a PAE site was selected 0.30 km upstream and 0.25 km downstream from the weir resulting in four PAE and two weir samples per sampling event. At both the upstream and

downstream site, the upstream PAE location was positioned within a riffle and the downstream PAE location was positioned within the downstream margin of a pool. Water temperature (°C), dissolved oxygen concentration (mg/L), depth (m), bottom velocity (m/s), and mean column velocity (m/s) were measured with each fish sample. Conductivity (µS/cm) was measured for each PAE sample.

### *Statistical Analysis*

Site Microhabitat Evaluation.—I utilized the Wilcoxon rank sum test to assess habitat similarity between the two weir locations, riffle PAE locations, and pool PAE locations. Comparisons were also conducted between riffle and pool PAEs to test for a macrohabitat difference and between downstream and upstream PAEs to test for a spatial difference. Habitat differences between gears were also assessed. Microhabitat variables included in each comparison included depth, bottom velocity, mean column velocity, water temperature, and dissolved oxygen, for both gears, and conductivity for PAE assessments.

Catch Composition Parameters and Indices.—Catch species diversity was calculated using the Shannon-Wiener Index ( $H'$ ) (Shannon and Weaver 1949) as

$$H' = -\sum_{i=1}^S (p_i) (\log_e p_i),$$

where  $s$  = number of species, and  $p_i$  = proportion of the total sample represented by the  $i$ th species (Kwak and Peterson 2007). I did not employ the Shannon-Wiener Index to describe the fish assemblage within Valley River based on PAE and resistance board weir samples, but rather to describe the catch composition structure among gears.

Dominance ( $D_i$ ) is an assemblage structural index that is a measure of the relative abundance of the most frequently represented species within the catch, and was calculated for each gear type. I selected the three species most frequently represented within catches to calculate dominance ( $D_3$ ) as

$$D_3 = \sum_{i=1}^S p_i,$$

where  $p_i$  = proportion of the entire sample assemblage represented by the  $i$ th species (Kwak and Peterson 2007). In addition, total number of fish captured and associated biomass were calculated for each gear. All parameters and indices were computed for each sampling week during the 2006 and 2007 sampling intervals. Between 2006 and 2007, weeks that overlapped were averaged. For both gears, weekly species richness, species diversity, and dominance indices were compared using the Student's  $t$ -test to test for differences between gears. Weekly biomass values were compared between gears using the Wilcoxon-Mann-Whitney test.

To ascertain if both gears sampled available fish assemblages similarly with regard to size structure, comprehensive length-frequency distributions for both were compared using a Kolmogorov-Smirnov (K-S) two-sample test. Samples from 2006 and 2007 were pooled for length-frequency distributions and the subsequent K-S test.

Total catch composition (i.e., sedentary and potamodromous fishes) and that for adult potamodromous fishes for both gears was used for all analyses using criterion of Jenkins and Burkhead (1993) and Etnier and Starnes (1993) to stratify potamodromous species. Number

of individuals per gear, species richness, species diversity, biomass, and dominance were calculated for each gear and specified catch (i.e., total catch; adult potamodromous catch).

Catch Composition Similarity.—To directly compare the catch composition between PAE and resistance board weir samples, Morisita's resemblance coefficient (i.e., Morisita's index) (Morisita 1959; Kwak and Peterson 2007) was employed. Morisita's index was employed because quantitative resemblance coefficients are much more sensitive to obscure disparities between the compared fish assemblages as long as either or both of  $N_1$  and  $N_2$  are not prohibitively small (Wolda 1981; Kwak and Peterson 2007). For both catch comparisons, fish less than 20 mm long (i.e., young-of-year) were excluded from analyses (Karr 1981, Angermeier and Karr 1986, Angermeier and Schlosser 1987; Roset et al. 2007).

To determine if gear type and stream location affected catch composition, Morisita's index was calculated between and within each gear type. For PAEs, I tested for a spatial (i.e., upstream and downstream) and macrohabitat (i.e., riffle and pool) difference, while I tested for a spatial difference only pertaining to weirs. Additionally, Morisita's index was calculated comparing spatially similar catch compositions between 2006 and 2007.

Microhabitat.—Current velocity and depth have been reported to decrease the efficiency of PAEs (Bain et al. 1985; Larimore and Garrels 1985); it is unclear if velocity and depth affect the sampling efficiency of resistance board weirs. Thus, I used simple linear regression to assess if catch is influenced by stream velocity and depth for each gear. For PAEs, velocity and depth bias was investigated for total PAE catch and separately for riffle

and pool catch; for resistance board weirs, velocity and depth bias were investigated for total weir catch and separately for downstream and upstream weir catch.

Spawning Migration Chronology.—Spawning migration chronology between 2006 and 2007 was similar for all species consistently captured for both gears; thus, average weekly catch was calculated for overlapping dates for both gears during 2006 and 2007. Efficiency of documentation of spawning chronology by both PAEs and resistance board weirs was evaluated by comparing the average weekly total catch for both gears using a K-S test. K-S tests were performed on daily data when at least one consistently captured potamodromous species was sampled by at least one gear.

## **Results**

### *Gear Construction, Cost, and Effort*

A single PAE costs a total of \$5,360 including power source; a single modified resistance board weir cost a total of \$7,025 to construct and install. The vast majority of the cost associated with PAE materials was a generator (\$1,500) and amperage converter (\$3,500). Materials to fabricate the bottom grid cable assembly were acquired for \$150 per PAE. Resistance board weirs required an extremely high level of initial effort pertaining to construction and installation; PAEs required minimal effort. Two weirs required a total of 1,000 person-hours for construction and installation; four PAEs required 40 person-hours for construction and installation. However, once the resistance board weirs were installed, minimal effort was required for operation and maintenance; PAEs required initial setup effort

of approximately 30 min prior to the collection of each PAE sample. Conversely, each resistance board weir required three full days of effort by two individuals for installation, but no setup effort prior to the collection of each sample.

### *Site Microhabitat Comparison*

Excluding water temperature, microhabitat was generally not significantly different between corresponding macrohabitats (i.e., riffle and pool) sampled by PAEs; however, depth, bottom velocity, and mean column velocity were significantly different between riffles and pools, while water temperature, dissolved oxygen, and conductivity were not. Microhabitat was generally similar between downstream and upstream PAE locations, but was significantly different between downstream and upstream weir locations. All microhabitat variables (i.e., PAEs and weirs) were significantly different between gears. Specifically, Wilcoxon rank sum tests reveal that depth, bottom velocity, mean column velocity, temperature, and dissolved oxygen differed significantly between the downstream and upstream weir locations ( $P < 0.05$ ), while only depth and temperature differed significantly between downstream and upstream PAE locations ( $P < 0.05$ ). Bottom velocity, mean column velocity, dissolved oxygen, and conductivity did not differ significantly between riffle PAE locations ( $P > 0.05$ ), while depth and water temperature did ( $P < 0.05$ ). Depth, bottom velocity, dissolved oxygen, and conductivity did not differ significantly ( $P > 0.05$ ) between pool PAE locations, but mean column velocity and temperature did. Depth, bottom velocity, and mean column velocity differed significantly between riffle and pool

PAE locations ( $P < 0.05$ ). Depth, bottom velocity, mean column velocity, temperature, and dissolved oxygen were significantly different between cumulative PAE and resistance board weir samples ( $P < 0.05$ ).

#### *Catch Composition Parameters and Indices*

A total of 35 species were collected during 2006 and 2007; PAEs collected 32 species and the resistance board weirs collected 20 species (Tables 1-4). PAEs collected 13 species not present in resistance board weir samples representing families Centrarchidae, Cottidae, Cyprinidae, Percidae, Petromyzontidae, and Poeciliidae (Tables 1-4). Resistance board weirs collected 3 species (channel catfish, common carp, and walleye) not present in PAE samples (Tables 1-4). Species captured only by PAEs (e.g., gilt darter *Percina evides* and redbreast sunfish *Lepomis auritus*) were generally smaller, laterally compressed, or relatively sedentary and therefore not susceptible to capture by the weirs; however, only PAEs captured smallmouth redhorse *Moxostoma breviceps* ( $N=1$ ). Common carp and walleye were relatively rare in the weir catch; however, channel catfish were consistently present in weir samples from mid to late-April in 2006 and late-March to early-May in 2007 (Tables 1-4).

Pertaining to total catch, PAE species richness and diversity was relatively stable overtime and higher than that of resistance board weirs; weir species richness and diversity was consistently lower and peaked in late-April and steadily decreased through mid-June ( $P < 0.0001$ ; Figure 2-3). Both gears' dominance index values were relatively stable overtime; however, weir species dominance values were consistently greater than those for PAEs ( $P <$

0.0001; Figure 4). Resistance board weir and PAE biomass values were similar over time except that weir biomass was twice that of the PAEs in late-April and early-May during the peak of the redhorse spawning migration ( $P = 0.3702$ ). Biomass for both gears decreased toward zero in mid-April (Figure 5). Cumulative species length-frequency distributions for both gears revealed statistically significant differences between the two gears ( $P < 0.0001$ ; Figure 6). PAEs predominantly sampled smaller fishes ranging from 50 – 250 mm followed by a smaller peak of larger potamodromous fishes ranging from 350 – 600 mm; weirs predominately sampled larger potamodromous fishes ranging from 350 – 600 mm (Figure 6).

The differences between PAE and weir catch compositions became less evident when only adult potamodromous fishes were compared (Table 5). PAE total catch was approximately four times greater compared to PAE adult potamodromous catch ( $N = 1,653$ ,  $N = 428$ ; respectively); resistance board weir catch varied little between total catch ( $N = 494$ ) and adult potamodromous catch ( $N = 424$ ; Table 5). PAE species richness (32 species) and species diversity ( $H' = 2.71$ ) were greater than those of the resistance board weirs (19 species,  $H' = 2.22$ ) when calculated for total catch; however, PAE species richness (11 species) and species diversity ( $H' = 1.79$ ) was less than that of the resistance board weirs (13 species,  $H' = 1.84$ ) when calculated for adult potamodromous catch (Table 5). Biomass was similar between total catch and adult potamodromous catch for both gears (Table 5). Dominance indices, when total catch was analyzed for both gears, revealed that resistance board weirs ( $D_3 = 0.64$ ) generally were more efficient at sampling larger adult potamodromous fishes (i.e., black redhorse *Moxostoma duquesnei*, golden redhorse

*Moxostoma erythrurum*, sicklefin redhorse) than PAEs ( $D_3 = 0.42$ ), which primarily sampled smaller sedentary fishes (i.e., central stoneroller *Campostoma anomalum*, juvenile northern hog sucker *Hypentelium nigricans*, warpaint shiner *Luxilus coccogenis*). However, dominance indices when adult potamodromous catch was analyzed for both gears reveal that catch composition for both gears was similar (PAEs, weirs; 0.68, 0.74), indicating that the majority of the catch for both gears was predominately comprised of three gear-specific species (Table 5). Black redhorse was most frequently represented in the catch of both gears followed by golden redhorse, while the northern hogsucker was the third most common species for the PAEs and the sicklefin redhorse was the third most frequently sampled species by the resistance board weirs. This divergence suggests that adult sicklefin redhorse are an abundant migrant within Valley River during the spring migration, yet not effectively collected with PAEs. Additionally, adult northern hogsuckers appear to be common within Valley River although not frequently represented in the catch of the resistance board weirs.

Cyprinidae and Catostomidae were most frequently represented within the catch of both gears; however, Cyprinidae was the dominant family in the PAE catches (54.3%), and Catostomidae was dominant in the weir catch (77.2%). The majority of cyprinids within the weir catch was collected in 2006 when modified fyke nets with smaller mesh were employed, compared to the pickets of traps in 2007 that were employed as the upstream and downstream live traps.

### *Sex Ratio Comparison*

Sex was determined for a total 324 adult redhorses collected with PAEs and 365 adult redhorses collected with resistance board weirs. PAE redhorses consisted of 124 black redhorse, 83 golden redhorse, 32 river redhorse *Moxostoma carinatum*, 21 sicklefin redhorse, and 64 silver redhorse *Moxostoma anisurum*, while weir redhorses were comprised of 119 black redhorse, 102 golden redhorse, 17 river redhorse, 93 sicklefin redhorse, and 34 silver redhorse. The sex ratio for PAE redhorses was typically even (black redhorse, golden redhorse, sicklefin redhorse: 1:1) except for river redhorse which was biased toward females (2.6:1) and silver redhorse which was biased toward males (1:9.7). The sex ratio for weir redhorses was generally biased toward females (black redhorse: 2.1:1, golden redhorse: 2.6:1, sicklefin redhorse: 1.8:1) except for river redhorse which was biased toward males (1:1.8) and silver redhorse which was even (1:1).

### *Catch Composition Similarity*

Moderate to low Morisita's resemblance coefficients for both 2006 and 2007, relative to stream location, gear type, and macrohabitat, reveal little disparity among catch composition comparisons suggesting that all analyzed catch assemblages were unique and dissimilar (Table 6). For 2006, Morisita's resemblance coefficients reveal that downstream PAE catch was most similar to that of upstream PAE catch and downstream weir catch was most similar to that of upstream weir catch with resemblance coefficients of 0.63 and 0.62, respectively (Table 6). In 2006, for all other catch composition comparisons, Morisita's

index coefficients indicate that compared catch compositions were either nearly as dissimilar as they were similar (Table 6). For 2007, Morisita's resemblance coefficients suggest that downstream weir catch was similar to upstream weir catch with a resemblance coefficient of 0.65 (Table 6). For 2007, all other Morisita resemblance coefficients resulting from catch composition comparisons were less than 0.60 suggesting catch compositions were nearly as similar as dissimilar (Table 6).

Morisita's resemblance coefficients, comparing the catch compositions of 2006 to those of 2007, indicate a high degree of spatiotemporal similarity for both gears (Table 6). Morisita's resemblance coefficient of 0.61, resulting from comparing the 2006 catch composition of the upstream weir to that from 2007, suggests that there may be a temporal disparity between weir samples when sampling spring spawning migrations if the weir is positioned too far upstream from the confluence, as a low Morisita resemblance coefficient was not produced (i.e., 0.82) when the 2006 downstream weir catch was compared to that of 2007 (Table 6). All other catch composition comparisons between years and subsequent Morisita's resemblance coefficients were greater than 0.81 suggesting that between years, catch compositions for these gears at a particular stream location were similar (Table 6).

### *Spawning Migration Chronology*

Resistance board weir and PAE catch over time for larger potamodromous fishes (i.e., redhorses and channel catfish) was statistically significantly different ( $P < 0.05$ ; Figure 7). For black redhorse and golden redhorse, resistance board weirs documented effectively

migration chronology; PAEs documented effectively only seasonal occupancy (Figure 7). For sicklefin redhorse and river redhorse, resistance board weirs captured effectively migration chronology; PAEs documented effectively neither migration chronology nor seasonal occupancy. Silver redhorse migration chronology was documented effectively by both PAEs and resistance board weirs (Figure 7). However, 96.3% of the silver redhorse captured by the PAEs were sampled from spawning grounds, suggesting that fish collected were demonstrating spawning site fidelity and not actively migrating. PAEs were not effective at documenting channel catfish migration chronology, seasonal occupancy, or nocturnal movement (Figure 7). No channel catfish were collected with a PAE in either 2006 or 2007 despite weirs consistently collecting channel catfish from late-March to mid-May and not prior to and following this period, indicating a small channel catfish migration run (Figure 7).

#### *Microhabitat Effects*

Linear regression revealed that PAE total catch significantly decreases as stream velocity increases ( $P = 0.0038$ ; Figure 8), and weir total catch is generally not affected by stream velocity ( $P = 0.0667$ ; Figure 9). However, linear regression revealed that upstream weir catch significantly increased with increasing stream velocity ( $P = 0.0046$ ; Figure 9). Conversely, downstream weir catch was not significantly ( $P = 0.1533$ ) affected by stream velocity variation (Figure 9). Linear regression also revealed that PAE catches were not significantly affected by depth ( $P > 0.05$ ; Figures 10). However, linear regression revealed

that total weir catch significantly increased as depth increased ( $P = 0.0214$ ), despite stratified weir catches not being significantly affected by depth ( $P > 0.05$ ; Figure 11).

### *Sampling Mortality*

Gear-specific mortality rates were similar for 2006 and 2007 for both gears, but dissimilar between gears (Table 7). PAE total catch mortality rates were 50% in 2006 and 63% in 2007, and those for resistance board weir total catch were 20% in 2006 and 5% in 2007 (Table 7). Overall, redhorses experienced a lower mortality rate for weirs (4%) than for PAEs (21%) (Table 7). An elevated PAE mortality rate was predominately due to high mortality of central stoneroller (53%), northern hogsucker (53%), Tennessee shiner *Notropis leuciodus* (76%), warpaint shiner (85%), whitetail shiner *Cyprinella galactura* (88%), mirror shiner *Notropis spectrunculus* (90%), and silver shiner *Notropis photogenis* (91%). Redhorse collected by PAEs also demonstrated elevated mortality rates compared to those captured by weirs (Table 7). PAE mortality rates for redhorse species were 28% for black redhorse, 15% for golden redhorse, 25% for river redhorse, 9% for sicklefin redhorse, and 11% for silver redhorse.

### **Discussion**

This gear comparison reveals that these two gears sample different components of a fish assemblage, and each gear has associated attributes and shortcomings pertaining to efficacy of sampling migrating potamodromous fishes within a medium-sized river. PAEs

sample indiscriminately between migratory and sedentary fishes yielding seasonal occupancy, while weirs only sample larger adult mobile fishes, providing migratory information. Hubert (1996) noted that catch of passive capture techniques (weir) is dependent on stock size, season, water temperature, time of day, discharge, turbidity, velocity, and fish behavior, while Hayes et al. (1996) emphasized that catch of active sampling techniques (PAE) is complicated by gear avoidance, size selectivity, physical obstructions, deep water, and swift currents. Specifically, PAEs exhibited microhabitat bias (i.e., velocity), spatial bias (macrohabitat), temporal bias (i.e., diurnal), costly fabrication components, high sampling effort, migration direction obscurity, high mortality, and a tendency not to sample rare potamodromous fishes, but required low fabrication effort and catches exhibited minimum size bias. Weir traits were costly and high construction effort and produced size-biased samples, while lacking microhabitat bias, spatial bias, temporal bias, low sampling effort, migration direction information, and low mortality. Weir size selectivity against smaller fishes could be eliminated by incorporating narrower picket gap widths, but trade-offs exist, such as increased cost, increased effort, greater head differential, and increased debris collection. Generally, PAEs are a lower effort, efficient technique when the emphasis is seasonal occupancy; resistance board weirs are most efficient for sampling potamodromous fishes when migration is of primary concern.

Comparing PAE and weir catches by species, size, and sex selectivity is important in determining gear efficiency related to objectives, as maximum sampling efficiency is advantageous when sampling fish populations (Hubert 1996). Generally, my modified weirs

exhibited size selectivity for large fish and PAEs were generally unbiased for fish size. However, PAEs were less efficient at sampling large fish (e.g., redhorses) compared to small fish (e.g., minnows) due to larger fish readily escaping the effective electric field and recovering prior to collection, resulting in PAEs exhibiting minimal size selectivity against larger fish. Scarcity of sicklefin redhorse and complete absence of walleye, common carp, and channel catfish in PAE samples indicate PAE species selectivity against rare fishes and occupants of riffles. Grabowski and Isely (2007) suggested that PAEs demonstrate sex selectivity for male catostomids when sampling spawning areas, and this possibly explains why adult catostomid sex ratios are commonly biased toward male fish in the literature (Peterson et al. 2000; Vokoun et al. 2003). My findings on PAE adult silver redhorse catch and subsequent male biased sex ratio bolsters this idea, because this catostomid was the only species that occupied spawning grounds within a PAE sample site. Additionally, weir adult silver redhorse catch and subsequent sex ratio was unbiased (1:1), further supporting the notion that PAEs yield male biased sex ratios when samples are obtained from spawning areas. I found that PAEs generally produced even sex ratios (1:1) when not sampling spawning areas; however, weir sex ratios were typically biased toward females. Zimmerman and Zabkar (2007) declared that weirs are considered the most accurate sampling technique available for migrating salmonids, and catch is theoretically an absolute count (i.e., true sex ratios) (Cousens et al. 1982). I was unable to find any literature indicating that catostomids exhibit morphological and behavioral sexual dichotomies inconsistent with those of salmonids that would preclude weirs for yielding catostomid absolute catches. Thus, my

weir sex ratios indicate that Valley River redhorse migrants are generally female biased, and PAEs under-sampled female redhorses (i.e., sex bias) despite general sampling of non-spawning areas. It is likely that weir sex ratios accurately reflect population sex ratios, but weirs may have exhibited size selectivity for larger females and under-sampled smaller males (Page and Johnston 1990). Pertaining to large fishes, PAEs demonstrated species selectivity, sex selectivity, and moderate size selectivity, while weirs demonstrated only size selectivity; indicating that PAEs exhibit greater overall gear selectivity.

Smallmouth redhorse primarily utilize lower Valley River during the spawning season (R. E. Jenkins, personal communication) and therefore were not expected to be present for collection at either sampling site. Additionally, blueback herring, a nonindigenous species that is laterally compressed, was not a primary target species during this study as little was known about their utilization of Valley River during the spring spawning season, other than they utilize lower stream reaches while foraging on drifting eggs and larvae of other potamodromous fishes (Wheeler et al. 2004). My resistance board weirs were designed to sample extremely streamlined and more laterally compressed *Moxostoma* spp. (e.g., sicklefin redhorse and black redhorse) as these fishes appeared to be the smallest and most laterally compressed fishes that utilized the middle and upper reaches of Valley River (Jenkins 1999). PAEs documented that both smallmouth redhorse and blueback herring were present at the downstream site during this study, but were rare in all samples. Blueback herring were captured by the resistance board weirs in 2006 when modified fyke nets served as traps, and no blueback herring were captured by the weirs in 2007 when metal

conduit live traps were installed in the place of the modified fyke nets. This result was a function of picket gap width that was too large to collect severely laterally compressed fishes. Therefore, an extremely small gap width, substantially smaller than the minimum body width of target species, should be used during weir construction to ensure that untargeted species, exotic species, rare species, and extremely small adult specimens are not selected against as a result of excessive gap width.

Both gears were costly, and resistance board weirs required significant construction and installation effort. I constructed modified weirs that substantially reduced the total expenditures required for each weir and successfully addressed the objectives of this study. My 20-m modified resistance board weir design was successful at sampling low to moderate flows (i.e., base flow conditions); however, if spawning stock abundance is of concern or mark-recapture or other parameter estimating methods are applied and river flow fluctuates widely, then strict adherence to the designs of Torbin (1994) and Stewart (2002; 2003) would be optimal. Zimmerman and Zabkar (2007) estimated that materials needed to fabricate a 60-m resistance board weir with only one trap would cost approximately U.S. \$100,000 , which suggests approximately \$30,000 – \$40,000 for a 20-m weir with two traps if traditional gear designs are implemented. I fabricated two 20-m modified resistance board weirs, each with two live traps and two fish chutes for a total of \$14,050. However, if a generator and power converter are already in possession, a single PAE can be constructed for approximately \$150 with minimal construction effort. Gears utilized for this particular efficiency comparison were relatively comparable in cost; however, traditional resistance

board weir fabrication designs require substantially greater monetary expenditures as well as initial construction and installation effort and these aspects should be considered during gear selection to sample migratory fishes.

Other techniques commonly used to sample adult migratory fishes vary widely in cost. Materials needed to obtain tower counts are relatively inexpensive ranging from U.S. \$7,000 to 16,000; however, this technique requires clear water and is susceptible to error through observer variability, large migrations, and weather conditions (Woody 2007). Materials needed to acquire hydroacoustic estimates with dual-frequency identification sonar (DIDSON) approach \$110,000, but hydroacoustic methods require the presence of only a single species to successfully enumerate migrating fish or use of alternative sampling techniques (e.g., drift gill nets; fish wheels; boat electrofishers) to apportion the sonar estimate to species (Maxwell 2007). Materials required to construct PAEs are relatively inexpensive relative to traditional techniques employed to sample migratory fishes; while, the cost of a weir, depending on river width, design, and number of traps, can either be inexpensive or extremely costly. PAEs require considerable effort immediately prior to collection of each sample and after sample collection, while resistance board weirs generally require minimal maintenance following initial installation. Approximately equal time was needed to obtain a single resistance board weir sample and single PAE sample (i.e., 1 h). By comparison, snorkel surveys are generally expedient sampling techniques used to estimate fish abundance, distribution, size, and determine presence/abundance; however, are less effective during fish migration (Dolloff et al. 1996; O'Neal 2007). Effort required to operate

a rotary screw trap is variable and depends on the quantity of migrating fish. Screw traps can be checked daily during minimal migration periods (i.e., 24 h samples) however, generally require constant monitoring by multiple individuals during peak migration. Rotary screw traps are used to estimate abundance, timing, size, survival, and behavior of migratory fishes; however, gear design generally limits collection of fish to downstream migrating juveniles (Volkhardt et al. 2007). Similarly, effort needed to operate a fish wheel can vary from periodic monitoring (e.g., 3 times/d) to constant monitoring. Fishwheels are used to provide species composition, stock characteristics, migration timing, distribution, size, and abundance; however, require swift flow to operate efficiently and negatively impact subsequent upriver migration (Underwood et al. 2004; Bromaghin et al. 2007). PAEs and resistance board weirs, compared to other migratory fish sampling techniques, require minimum to moderate sampling effort.

Torbin (1994) originally designed the two-way resistance board weir as a portable sampling device to sample migrating adult Pacific salmon in the remote rivers in the backcountry of Alaska which is only assessable by boat or helicopter. The circumstances (i.e., backcountry, debris laden streams, highly variable spring discharge) that necessitated the conception of the resistance board weir are likely prohibiting characteristics to the practicality of employing a PAE to sample many remote potamodromous migratory runs. All PAE locations sampled during my study were easily accessible with a 4-wheel drive vehicle, which was necessary due to the considerable weight of the gear required to assemble a PAE (i.e., generator, converter, bottom grid cable assembly) and the necessity to sample multiple

different stream reaches. Weirs are excessively heavy and cumbersome compared to PAEs; however, once transported to and installed within the river, subsequent relocations and additional sampling sites are unnecessary. Small and medium-size rivers are too small to reach each PAE sampling location by boat and utilization of a helicopter to relocate between sampling sites is generally prohibitively impractical. Therefore, based solely on accessibility, a resistance board weir is the more feasible gear choice to sample the migratory run of a potamodromous species utilizing a medium-sized river in even a mildly remote location. However, use of PAEs to sample medium-sized rivers that are easily accessible by road is a feasible choice. In addition, large remote rivers that are boat accessible and wadeable are candidates for PAE sampling (Kinsolving and Bain 1993; Bowen and Freeman 1998).

Resistance board weirs generally, by design, sample migratory runs independent of microhabitat; PAE catch composition is influenced by microhabitat sampled. Passive gears (e.g., weir) collect fish as a function of behavior (e.g., migration) and migratory species generally migrate independent of macrohabitat (i.e., riffle, run, pool). Active sampling techniques (e.g., PAE) are microhabitat biased unless sampling effort is stratified equally among all possible microhabitats, and this is not practical. Biases associated with backpack and boat electrofishing in riverine systems include unnavigable stream reaches, deep water, swift currents, varying water temperature, varying substrates, varying discharge, high turbidity, and low conductivity (Bohlin et al. 1989; Reynolds 1996). Similarly, snorkel surveys are biased by deep depths, swift currents, turbidity (i.e., visibility), cold water

temperatures, and habitat complexity (e.g., cover) (Dolloff et al. 1996; O'Neal 2007). In addition to weirs, gears typically employed to sample migratory fishes (Johnson et al. 2007) that yield catches not or minimally biased by microhabitat include hydroacoustics, tower counts, rotary screw traps, tangle nets, and video techniques.

PAEs exhibited higher species richness and diversity than did resistance board weirs relative to total catch; however, weirs exhibited higher species richness and diversity among large adult potamodromous catch and were effective at sampling small migratory runs by nocturnal (i.e., channel catfish) and rare potamodromous species (i.e., common carp, walleye). Alternating current (AC) electrofishing has been successful at sampling channel catfish (Santucci et al. 1999; Vokoun and Rabeni 1999) and other ictalurids (Daugherty and Sutton 2005) in riverine systems. In addition, literature reveals that channel catfish conduct considerable spring spawning migrations (Dames et al. 1989; Pellett et al. 1998; Hubert 1999). Pellett et al. (1998) revealed that channel catfish spawn and occupy small home ranges in the Wisconsin River during spring and summer, while migrating downstream in autumn to the upper Mississippi River and remaining there through winter. In addition, channel catfish foraging activity was documented to increase at night in the Des Moines River, Iowa (Bailey and Harrison 1948), and the Vermilion River, Illinois (Kwak et al. 1992). Randolph and Clemens (1976) demonstrated in Oklahoma culture ponds that channel catfish feed from 2000 to 2400 h during August however; primarily feed between 1200 and 1600 h during March, suggesting foraging temporal variability. It is unclear if channel catfish in my weir catch were collected due to nocturnal behavior; however, channel catfish

catch primarily occurred from late-March to early-May and these fish generally expressed gametes. Thus, my weir channel catfish catch is likely indicative of a small spring spawning migration within Valley River, rather than solely attributable to increased nocturnal activity. PAEs neglected to detect a channel catfish spawning migration or seasonal occupancy. Nevertheless, due to the time integrated sampling of weirs and static sampling of PAEs, weirs are the more practical technique when sampling the spawning migration of nocturnal potamodromous fishes.

During my study, resistance board weirs primarily sampled mobile large potamodromous fishes, and similar to findings by Bowen and Freeman (1998), PAEs were generally efficient at sampling the entire fish assemblage of a medium-sized river. Environmental consequences of intentional and unintentional introductions of nonindigenous fishes (e.g., northern snakehead *Channa argus*, flathead catfish *Pylodictis olivaris*, striped bass *Morone saxatilis*, blueback herring *Alosa aestivalis*) are usually harmful, and occasionally catastrophic (Taylor et al. 1984; Fuller et al. 1999). As of 1999, 536 nonindigenous fish taxa were reported to occur within U.S. waters; 316 (61%) being native to the United States, 185 (35%) foreign fishes, and the remainder representing hybrids (Fuller et al. 1999). Interactions of nonindigenous and native fishes should be continuously examined to assess the need for management before a crisis situation is realized (Fuller et al. 1999). Understanding the affects that potamodromous nonindigenous fishes have on native resident fishes can initiate and guide management decisions designed to mitigate negative interactions. PAEs are an appropriate gear to obtain species richness, distribution,

abundance, and microhabitat use data of unmixed assemblages (pre-introduction or pre-migration) for comparison to mixed assemblages (post-introduction or post-migration) to determine the severity of any negative integration.

The polyvinyl chloride (PVC) pipes employed as weir panel pickets during 2007 in my weirs did not function perfectly. I utilized PVC pipe with an outer diameter of 2.1 cm to reduce cost however, used a narrower stringer width of 0.66 m compared to that of Torbin (1994) (i.e., 0.76 m). Despite utilization of a narrower stringer width, excessive litness was a concern regarding distance between the base stringer and first stabilization stringer (i.e., end stringer). Apparently, a combination of small PVC pipe diameter and insufficient compensatory stringer width created this unforeseen sampling phenomenon. This phenomenon was revealed in 2007 when frightened redhorses residing immediately downstream of the weir at the downstream site were observed swimming rapidly into the weir panels and squeezing through the picket gaps despite the gaps being considerably smaller than the fish's body width. In addition to these fish compressing laterally, the PVC pipe probably bent. It is unclear why this phenomenon was not observed in 2006 when anecdotal evidence (i.e., visual observations) revealed greater fish abundances congregated directly downstream of the weirs. Sunlight exposure and subsequent PVC pipe increased flexibility between 2006 and 2007 is likely not a contributing factor as electrical PVC conduit is UV resistant (Stewart 2002). Regardless, larger diameter PVC pipe (e.g., 3.3 cm outer diameter) similar to Torbin (1994) would mitigate this condition. However, with

appropriate compensatory stringer spacing width, 2.1 cm diameter PVC pipe would function effectively as weir panel pickets.

Morisita's similarity coefficients reveal that spatial variations did not affect catch composition similarity for resistance board weirs during 2006 and 2007, and PAEs during 2006, indicating that catch composition is spatially independent relative to resistance board weirs as long as the weirs are deployed within indiscriminately utilized stream reaches relative to potamodromous fishes and corresponding spawning migration. However, spatial variation appeared to affect catch composition similarity of the PAEs during 2007, suggesting that stream reaches sampled by PAEs affect catch composition independent of macrohabitat (i.e., riffle and pool) sampled since macrohabitats sampled were constant despite spatial variation (i.e., downstream site and upstream site). In addition, in both 2006 and 2007, riffle PAE samples were not statistically similar to pool PAE samples regardless of sample site, suggesting that individual PAE catch composition is generally characterizing the potamodromous fish community occupying the particular habitat being sampled rather than the entire potamodromous fish community utilizing the spawning tributary for migratory purposes. This relationship may hold implications and provide insight related to similar methods of electrofishing (boat electrofishing, backpack electrofishing, barge electrofishing) in relation to sampling spawning migrations (e.g., Coughlan et al. 2007). During my study, PAE catch composition was correlated with macrohabitat sampled rather than descriptive of migration characteristics and this relationship is likely universal relative to electrofishing and efforts to describe the spawning migration of potamodromous fishes. Resistance board weirs

are time-integrated, habitat independent, and migration dependent, and therefore not biased toward the particular time period (i.e., continuous sampling) and habitat being sampled.

Morisita's resemblance coefficient of 0.61 resulting from comparing the catch composition of the upstream weir between 2006 and 2007 compared to Morisita's resemblance coefficient of 0.82 when the catch composition of the downstream weir was compared between 2006 and 2007, shows that a weir installed at a significant distance from the mouth of a spawning tributary may have dissimilar catch assemblages from year to year as a result of environmental stochasticity. During this study, a significant drought occurred in the Hiwassee Basin in 2007 resulting in reduced flow potentially influencing catches in the upstream weir related to species that were consistently captured in both 2006 and 2007. PAEs neglected to detect this decrease in utilization of upstream reaches, exhibiting a Morisita's Index value of 0.83 when the catch compositions of the upstream PAEs were compared between 2006 and 2007. This result is significant if fisheries managers are considering PAEs, and possibly electrofishing in general, to monitor migratory runs as these techniques are less likely to detect spatiotemporal variations. In addition, resistance board weirs should be installed within spawning tributaries relatively close to the tributary confluence if objectives are to document the spawning migration independent of flow conditions.

The onset and duration of the spawning migration of numerous salmonid species (Banks 1969; Keefer et al. 2008) and non-salmonid potamodromous fishes (Northcote 1984; Lucas and Baras 2001) has been correlated with river discharge. Sampling efficiency of

PAEs has been reported to decrease with increasing stream velocities (Larimore and Garrels 1985). This is relevant because PAE catch may not be representative during periods of high flow (e.g., spring runoff) or when migration is likely to initiate or peak. PAE efficiency pertaining to sampling migratory fishes that utilize discharge as a migration cue should be considerable cause for apprehension if objectives are to sample during the spawning migration. For fishes that initiate or intensify migration with elevated discharges, a sampling method that is effective in swift, unwadeable currents is crucial to quantify the migration. Schwartz and Herricks (2004) recommend incorporating a seine into sampling to collect immobilized fishes downstream of an electrified PAE in high-velocity flows >1.0 m/s however, suggest that this collection technique will likely introduce a fright bias. In my study, resistance board weirs demonstrated no significant bias ( $P > 0.05$ ) associated with water velocity and therefore may be a more suitable option over PAEs, if spawning migration occurs under elevated stream discharges.

In my study, half of all fish collected using PAEs died, while nearly all potamodromous fish collected with a resistance board weir survived. Reynolds (1996) noted that sampling powered by AC is most injurious to fish and likely to result in significant fish mortality (Fisher and Brown 1993), while resistance board weirs have demonstrated proficiency at conducting mark-recapture studies (Zimmerman and Zabkar 2007) and thus low mortality rates. Thus, AC electroshock should be used only when water conductivity is not sufficient for DC electroshock (Reynolds 1996). In addition, minimization of PAE electrifying duration would reduce mortality (Reynolds 1996), but a reasonable duration is

required for effective sampling (about 30–60 s). Additionally, Fisher and Brown (1993) employed AC to discourage attraction of fish into the sampling field (i.e., electrotaxis) and ensure determination of sampling area size. During this study, large potamodromous fishes (e.g., redhorses) succumbed to electrofishing-induced mortality less frequently than did small fishes (e.g., warpaint shiner, tennessee shiner, central stoneroller). Field observations suggest that larger fish more often escaped the electric field, while smaller fish tended to remain within the electric field resulting in smaller fish being exposed to electric current for a longer duration. Larger fish exited the electric field by body displacement behavior (i.e., oscillotaxis or “tail thrashing”). Those fish were observed quickly leaving the electric field and avoided capture. The propensity of larger fish to flee a PAE AC electric field results in lower mortality but introduces bias against adult potamodromous fishes.

Environmental variables such as water temperature and stream discharge have been identified as releasing stimuli and governors of the spawning migration in potamodromous fishes (Northcote 1984; Kwak and Skelly 1992; Lucas and Baras 2001). The effects of hydroelectric dam discharges (i.e., regulated flows and manipulated water temperatures) negatively influence riverine potamodromous fishes that rely on stable and consistent hydrologic conditions to complete critical life history periods (Cooke et al. 2005). Comprehensive and reliable life history information on the reproductive ecology (e.g., spawning migration chronology) of potamodromous fishes is required to guide management plans for imperiled populations and ultimately ensure their persistence. Similarly, understanding spawning chronology in association with spawning microhabitat

characteristics, especially for imperiled fishes, is critical information for fisheries managers to manage hydrographs and thermal regimes below dams that mimic natural flows conducive to migration and subsequent spawning. Resistance board weirs in my research effectively documented redhorse spawning migration chronology and detected a probable channel catfish spawning run in Valley River. Generally, PAEs only documented seasonal occupancy of the potamodromous fishes consistently sampled and failed to elucidate specific spatiotemporal bounds and subsequent environmental stimuli that were associated with the initiation and termination of the associated spawning migrations.

Considering the scarce comprehensive knowledge that currently exists for the majority of nongame potamodromous fishes (Warren and Burr 1994) and that legislation (e.g., Endangered Species Act (ESA), Committee on the Status of Wildlife in Canada, and the Species at Risk Act (SARA), Fisheries Act in Canada) equally protects imperiled nongame and recreational fish species, natural resource agencies can justify management of previously ignored fishes (Cooke et al. 2005). Current regulation and subsequent degradation that occur in most rivers in the lower 48 states and increasing urbanization that is likely to necessitate the construction of additional reservoirs to meet increasing water requirements (Ward and Stanford 1979; Collier et al. 1996), necessitates a comprehensive understanding of critical life history requirements of all riverine fishes. New knowledge and comparisons of sampling techniques applicable for use within lotic systems that I present here will assist fisheries managers in selecting the most appropriate gear to address their objectives when sampling riverine fishes.

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Table 1.—PAE catch and biomass for 2006 from Valley River, North Carolina, including rare species.

| Species                | Downstream grids |             |        |             | Upstream grids |             |        |             |
|------------------------|------------------|-------------|--------|-------------|----------------|-------------|--------|-------------|
|                        | Riffle           |             | Pool   |             | Riffle         |             | Pool   |             |
|                        | Number           | Biomass (g) | Number | Biomass (g) | Number         | Biomass (g) | Number | Biomass (g) |
| Banded darter          | 1                | 4.0         | 0      | 0.0         | 0              | 0.0         | 4      | 4.0         |
| Bigeye chub            | 1                | 3.0         | 0      | 0.0         | 0              | 0.0         | 0      | 0.0         |
| Black redhorse         | 12               | 6,318.0     | 27     | 13,739.0    | 5              | 3,380.0     | 31     | 8,698.0     |
| Blotched chub          | 0                | 0.0         | 0      | 0.0         | 2              | 15.0        | 0      | 0.0         |
| Blueback herring       | 4                | 57.0        | 4      | 74.5        | 0              | 0.0         | 0      | 0.0         |
| Bluegill               | 0                | 0.0         | 10     | 417.0       | 0              | 0.0         | 1      | 80.0        |
| Brook trout            | 1                | 280.0       | 0      | 0.0         | 0              | 0.0         | 0      | 0.0         |
| Brown trout            | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 0      | 0.0         |
| Central stoneroller    | 59               | 881.5       | 7      | 158.0       | 40             | 542.5       | 4      | 154.0       |
| Gilt darter            | 1                | 2.5         | 0      | 0.0         | 9              | 23.5        | 0      | 0.0         |
| Golden redhorse        | 6                | 5,596.0     | 20     | 17,840.0    | 0              | 0.0         | 5      | 4,154.0     |
| Greenside darter       | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 1      | 1.0         |
| Largemouth bass        | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 0      | 0.0         |
| Mirror shiner          | 21               | 65.0        | 0      | 0.0         | 3              | 7.5         | 4      | 8.5         |
| Mottled sculpin        | 4                | 29.5        | 0      | 0.0         | 6              | 19.5        | 0      | 0.0         |
| Mountain brook lamprey | 0                | 0.0         | 0      | 0.0         | 1              | 4.0         | 0      | 0.0         |
| Northern hog sucker    | 39               | 2,202.0     | 16     | 1,395.5     | 17             | 1,777.0     | 75     | 5,492.5     |
| Rainbow trout          | 0                | 0.0         | 1      | 34.5        | 0              | 0.0         | 0      | 0.0         |
| Redbreast sunfish      | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 3      | 158.0       |
| River chub             | 21               | 870.5       | 6      | 214.5       | 0              | 0.0         | 0      | 0.0         |
| River redhorse         | 3                | 2,830.0     | 2      | 2,584.0     | 2              | 1,350.0     | 4      | 6,194.0     |
| Rock bass              | 1                | 79.0        | 2      | 213.0       | 0              | 0.0         | 0      | 0.0         |
| Sicklefin redhorse     | 2                | 2,444.0     | 5      | 5,560.0     | 4              | 3,790.0     | 3      | 3,420.0     |
| Silver redhorse        | 0                | 0.0         | 2      | 3,220.0     | 0              | 0.0         | 7      | 13,430.0    |
| Silver shiner          | 0                | 0.0         | 1      | 4.5         | 1              | 9.0         | 26     | 159.5       |
| Smallmouth bass        | 1                | 5.0         | 4      | 570.0       | 1              | 420.0       | 11     | 3,832.0     |
| Smallmouth redhorse    | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 0      | 0.0         |
| Tennessee shiner       | 42               | 136.5       | 2      | 14.0        | 7              | 19.5        | 1      | 5.0         |
| Warpaint shiner        | 97               | 574.5       | 18     | 149.0       | 4              | 44.5        | 19     | 114.5       |

Table 1.—(Continued).

| Species              | Downstream grids |             |        |             | Upstream grids |             |        |             |
|----------------------|------------------|-------------|--------|-------------|----------------|-------------|--------|-------------|
|                      | Riffle           |             | Pool   |             | Riffle         |             | Pool   |             |
|                      | Number           | Biomass (g) | Number | Biomass (g) | Number         | Biomass (g) | Number | Biomass (g) |
| Western mosquitofish | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 0      | 0.0         |
| Whitetail shiner     | 0                | 0.0         | 15     | 256.0       | 1              | 14.0        | 42     | 542.5       |
| Yellow perch         | 1                | 188.0       | 7      | 626.5       | 0              | 0.0         | 0      | 0.0         |
| Total                | 317              | 22,566.0    | 149    | 47,070.0    | 103            | 11,416.0    | 241    | 46,447.5    |

Table 2.—PAE catch and biomass for 2007 from Valley River, North Carolina, including rare species.

| Species                | Downstream grids |             |        |             | Upstream grids |             |        |             |
|------------------------|------------------|-------------|--------|-------------|----------------|-------------|--------|-------------|
|                        | Riffle           |             | Pool   |             | Riffle         |             | Pool   |             |
|                        | Number           | Biomass (g) | Number | Biomass (g) | Number         | Biomass (g) | Number | Biomass (g) |
| Banded darter          | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 0      | 0.0         |
| Bigeye chub            | 6                | 12.0        | 3      | 6.0         | 0              | 0.0         | 1      | 5.0         |
| Black redhorse         | 33               | 18,716.0    | 32     | 18,639.0    | 7              | 4,379.0     | 18     | 8,384.0     |
| Blotched chub          | 0                | 0.0         | 0      | 0.0         | 8              | 29.0        | 0      | 0.0         |
| Blueback herring       | 2                | 11.0        | 0      | 0.0         | 0              | 0.0         | 0      | 0.0         |
| Bluegill               | 0                | 0.0         | 4      | 242.0       | 0              | 0.0         | 1      | 79.0        |
| Brook trout            | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 0      | 0.0         |
| Brown trout            | 2                | 479.0       | 1      | 273.0       | 0              | 0.0         | 0      | 0.0         |
| Central stoneroller    | 37               | 211.0       | 1      | 2.0         | 40             | 360.0       | 5      | 150.0       |
| Gilt darter            | 3                | 6.0         | 0      | 0.0         | 14             | 36.0        | 1      | 4.0         |
| Golden redhorse        | 4                | 3,493.0     | 28     | 20,917.0    | 0              | 0.0         | 21     | 17,799.0    |
| Greenside darter       | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 0      | 0.0         |
| Largemouth bass        | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 2      | 558.0       |
| Mirror shiner          | 22               | 38.0        | 5      | 11.0        | 8              | 22.0        | 0      | 0.0         |
| Mottled sculpin        | 8                | 29.0        | 1      | 7.0         | 14             | 65.0        | 2      | 7.0         |
| Mountain brook lamprey | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 2      | 11.0        |
| Northern hog sucker    | 41               | 3,758.0     | 14     | 1,186.0     | 24             | 1,118.0     | 29     | 3,712.0     |
| Rainbow trout          | 0                | 0.0         | 0      | 0.0         | 2              | 43.0        | 1      | 45.0        |
| Redbreast sunfish      | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 0      | 0.0         |
| River chub             | 17               | 191.0       | 18     | 582.0       | 2              | 53.0        | 1      | 61.0        |
| River redhorse         | 9                | 11,267.0    | 2      | 3,023.0     | 1              | 1,248.0     | 9      | 14,246.0    |
| Rock bass              | 1                | 4.0         | 2      | 166.0       | 0              | 0.0         | 1      | 142.0       |
| Sicklefin redhorse     | 3                | 3,056.0     | 1      | 1,069.0     | 0              | 0.0         | 4      | 4,422.0     |
| Silver redhorse        | 0                | 0.0         | 2      | 3,630.0     | 0              | 0.0         | 52     | 82,914.0    |
| Silver shiner          | 0                | 0.0         | 1      | 3.0         | 0              | 0.0         | 50     | 219.0       |
| Smallmouth bass        | 0                | 0.0         | 2      | 116.0       | 0              | 0.0         | 5      | 36.0        |
| Smallmouth redhorse    | 1                | 583.0       | 0      | 0.0         | 0              | 0.0         | 0      | 0.0         |
| Tennessee shiner       | 14               | 19.0        | 0      | 0.0         | 8              | 24.0        | 2      | 5.0         |
| Warpaint shiner        | 67               | 274.0       | 32     | 238.0       | 2              | 26.0        | 1      | 8.0         |

Table 2.—(Continued).

|                      | Downstream grids |             |        |             | Upstream grids |             |        |             |
|----------------------|------------------|-------------|--------|-------------|----------------|-------------|--------|-------------|
|                      | Riffle           |             | Pool   |             | Riffle         |             | Pool   |             |
|                      | Number           | Biomass (g) | Number | Biomass (g) | Number         | Biomass (g) | Number | Biomass (g) |
| Western mosquitofish | 0                | 0.0         | 0      | 0.0         | 0              | 0.0         | 2      | 2.0         |
| Whitetail shiner     | 25               | 202.0       | 21     | 273.0       | 6              | 57.0        | 27     | 285.0       |
| Yellow perch         | 1                | 60.0        | 4      | 253.0       | 0              | 0.0         | 0      | 0.0         |
| Total                | 296              | 42,409.0    | 174    | 50,636.0    | 136            | 7,460.0     | 237    | 133,094.0   |

Table 3.—Modified two-way resistance board weir catch and biomass for 2006 from Valley River, North Carolina, including rare species.

| Species             | Downstream weir |             |               |             | Upstream weir   |             |               |             |
|---------------------|-----------------|-------------|---------------|-------------|-----------------|-------------|---------------|-------------|
|                     | Downstream trap |             | Upstream trap |             | Downstream trap |             | Upstream trap |             |
|                     | Number          | Biomass (g) | Number        | Biomass (g) | Number          | Biomass (g) | Number        | Biomass (g) |
| Black redhorse      | 0               | 0           | 23            | 19,225      | 1               | 484         | 43            | 33,490      |
| Blueback herring    | 0               | 0           | 12            | 190         | 3               | 46          | 3             | 449         |
| Bluegill            | 0               | 0           | 4             | 209         | 0               | 0           | 0             | 0           |
| Brook trout         | 0               | 0           | 0             | 0           | 0               | 0           | 2             | 438         |
| Brown trout         | 0               | 0           | 1             | 104         | 0               | 0           | 0             | 0           |
| Central stoneroller | 0               | 0           | 4             | 53          | 0               | 0           | 8             | 383         |
| Channel catfish     | 0               | 0           | 11            | 12,144      | 1               | 2,740       | 0             | 0           |
| Common carp         | 1               | 4,150       | 1             | 3,880       | 0               | 0           | 0             | 0           |
| Golden redhorse     | 1               | 890         | 38            | 42,722      | 1               | 986         | 7             | 7,156       |
| Northern hog sucker | 0               | 0           | 1             | 142         | 0               | 0           | 3             | 3,173       |
| Rainbow trout       | 0               | 0           | 0             | 0           | 0               | 0           | 3             | 328         |
| River chub          | 0               | 0           | 1             | 119         | 0               | 0           | 2             | 121         |
| River redhorse      | 0               | 0           | 12            | 19,950      | 0               | 0           | 0             | 0           |
| Rock bass           | 0               | 0           | 8             | 0           | 0               | 0           | 0             | 705         |
| Sicklefin redhorse  | 0               | 0           | 19            | 23,999      | 0               | 0           | 19            | 19,904      |
| Silver redhorse     | 0               | 0           | 5             | 7,936       | 0               | 0           | 0             | 0           |
| Smallmouth bass     | 0               | 0           | 0             | 0           | 0               | 0           | 0             | 0           |
| Walleye             | 1               | 2,480       | 0             | 0           | 0               | 0           | 0             | 0           |
| Warpaint shiner     | 0               | 0           | 14            | 224         | 0               | 0           | 10            | 131         |
| Whitetail shiner    | 0               | 0           | 2             | 53          | 0               | 0           | 1             | 19          |
| Total               | 3               | 7,520       | 156           | 130,948     | 6               | 4,256       | 101           | 66,296      |

Table 4.—Modified two-way resistance board weir catch and biomass for 2007 from Valley River, North Carolina, including rare species.

| Species             | Downstream weir |             |               |             | Upstream weir   |             |               |             |
|---------------------|-----------------|-------------|---------------|-------------|-----------------|-------------|---------------|-------------|
|                     | Downstream trap |             | Upstream trap |             | Downstream trap |             | Upstream trap |             |
|                     | Number          | Biomass (g) | Number        | Biomass (g) | Number          | Biomass (g) | Number        | Biomass (g) |
| Black redhorse      | 2               | 1,550       | 43            | 40,040      | 1               | 708         | 6             | 4,462       |
| Blueback herring    | 0               | 0           | 0             | 0           | 0               | 0           | 0             | 0           |
| Bluegill            | 0               | 0           | 0             | 0           | 0               | 0           | 0             | 0           |
| Brook trout         | 0               | 0           | 0             | 0           | 0               | 0           | 0             | 0           |
| Brown trout         | 0               | 0           | 0             | 0           | 0               | 0           | 0             | 0           |
| Central stoneroller | 0               | 0           | 0             | 0           | 2               | 115         | 0             | 0           |
| Channel catfish     | 0               | 0           | 17            | 18,109      | 0               | 0           | 0             | 0           |
| Common carp         | 0               | 0           | 3             | 11,294      | 0               | 0           | 0             | 0           |
| Golden redhorse     | 3               | 1,878       | 49            | 51,401      | 2               | 1,577       | 1             | 925         |
| Northern hog sucker | 1               | 556         | 3             | 1,627       | 1               | 761         | 0             | 0           |
| Rainbow trout       | 1               | 1,616       | 0             | 0           | 0               | 0           | 0             | 0           |
| River chub          | 0               | 0           | 0             | 0           | 0               | 0           | 0             | 0           |
| River redhorse      | 1               |             | 5             | 6,962       | 0               | 0           | 0             | 0           |
| Rock bass           | 0               | 0           | 0             | 0           | 0               | 0           | 0             | 0           |
| Sicklefin redhorse  | 3               | 2,432       | 34            | 42,940      | 1               | 1,160       | 18            | 21,414      |
| Silver redhorse     | 17              | 26,871      | 12            | 19,538      | 0               | 0           | 0             | 0           |
| Smallmouth bass     | 0               | 0           | 0             | 0           | 0               | 0           | 0             | 0           |
| Walleye             | 0               | 0           | 2             | 4,271       | 0               | 0           | 0             | 0           |
| Warpaint shiner     | 0               | 0           | 0             | 0           | 0               | 0           | 0             | 0           |
| Whitetail shiner    | 0               | 0           | 0             | 0           | 0               | 0           | 0             | 0           |
| Total               | 28              | 34,903      | 168           | 196,182     | 7               | 4,321       | 25            | 26,801      |

Table 5.—Cumulative catch by number, species richness ( $S_{\text{obs}}$ ), species diversity ( $H'$ ), biomass (kg), and dominance ( $D_3$ ) estimates for PAEs and two-way resistance board weirs for total catch and adult potamodromous fish catch by gear during 2006 and 2007 sampling seasons.

| Parameter and indices       | Total catch |       | Adult potamodromous fish |       |
|-----------------------------|-------------|-------|--------------------------|-------|
|                             | PAE         | Weir  | PAE                      | Weir  |
| $N$                         | 1,653       | 494   | 428                      | 424   |
| Species richness            | 32          | 19    | 11                       | 13    |
| Species diversity ( $H'$ )  | 2.71        | 2.22  | 1.79                     | 1.84  |
| Biomass (kg)                | 361.1       | 471.2 | 341.9                    | 468.8 |
| Species dominance ( $D_3$ ) | 0.42        | 0.64  | 0.68                     | 0.74  |

Table 6.—Morisita similarity index values comparing the catch compositions within and between PAEs and two-way resistance board weirs for 2006 and 2007. Similarity indexes were also calculated comparing spatially similar catch compositions between 2006 and 2007.

| Years and comparisons                                | Morisita's index |
|--|------------------|
| 2006   |                  |
| Downstream PAEs verses downstream weir               | 0.49             |
| Upstream PAEs verses upstream weir                   | 0.37             |
| Downstream PAEs verses upstream PAEs                 | 0.62             |
| Downstream weir verses upstream weir                 | 0.63             |
| PAEs verses weirs                                    | 0.54             |
| Riffle PAEs verses Pool PAEs                         | 0.53             |
| 2007   |                  |
| Downstream PAEs verses downstream weir               | 0.38             |
| Upstream PAEs verses upstream weir                   | 0.16             |
| Downstream PAEs verses upstream PAEs                 | 0.56             |
| Downstream weir verses upstream weir                 | 0.65             |
| PAEs verses weirs                                    | 0.40             |
| Riffle PAEs verses Pool PAEs                         | 0.56             |
| 2006 and 2007  |                  |
| Downstream PAEs (2006) verses downstream PAEs (2007) | 0.94             |
| Upstream PAEs (2006) verses upstream PAEs (2007)     | 0.83             |
| Riffle PAEs (2006) verses riffle PAEs (2007)         | 0.92             |
| Pool PAEs (2006) verses pool PAEs (2007)             | 0.84             |
| PAEs (2006) verses PAEs (2007)                       | 0.93             |
| Downstream weir (2006) verses downstream weir (2007) | 0.82             |
| Upstream weir (2006) verses upstream weir (2007)     | 0.61             |
| Weirs (2006) verses weirs (2007)                     | 0.84             |

Table 7.—Total catch and redhorse percent mortality for PAEs and two-way resistance board weirs for 2006, 2007, and total catch. PAE redhorse catch includes adults and juveniles.

| Years and Gear | Total catch | Total mortality | Total mortality (%) | Redhorse catch | Redhorse mortality | Redhorse mortality (%) |
|----------------|-------------|-----------------|---------------------|----------------|--------------------|------------------------|
| 2006           |             |                 |                     |                |                    |                        |
| PAEs           | 784         | 395             | 50.4                | 140            | 28                 | 20.0                   |
| Weirs          | 192         | 39              | 20.3                | 109            | 1                  | 0.9                    |
| 2007           |             |                 |                     |                |                    |                        |
| PAEs           | 820         | 520             | 63.4                | 227            | 49                 | 21.6                   |
| Weirs          | 223         | 12              | 5.4                 | 198            | 10                 | 5.1                    |
| Total          |             |                 |                     |                |                    |                        |
| PAEs           | 1,604       | 915             | 57.0                | 367            | 77                 | 21.0                   |
| Weirs          | 415         | 51              | 12.3                | 307            | 11                 | 3.6                    |

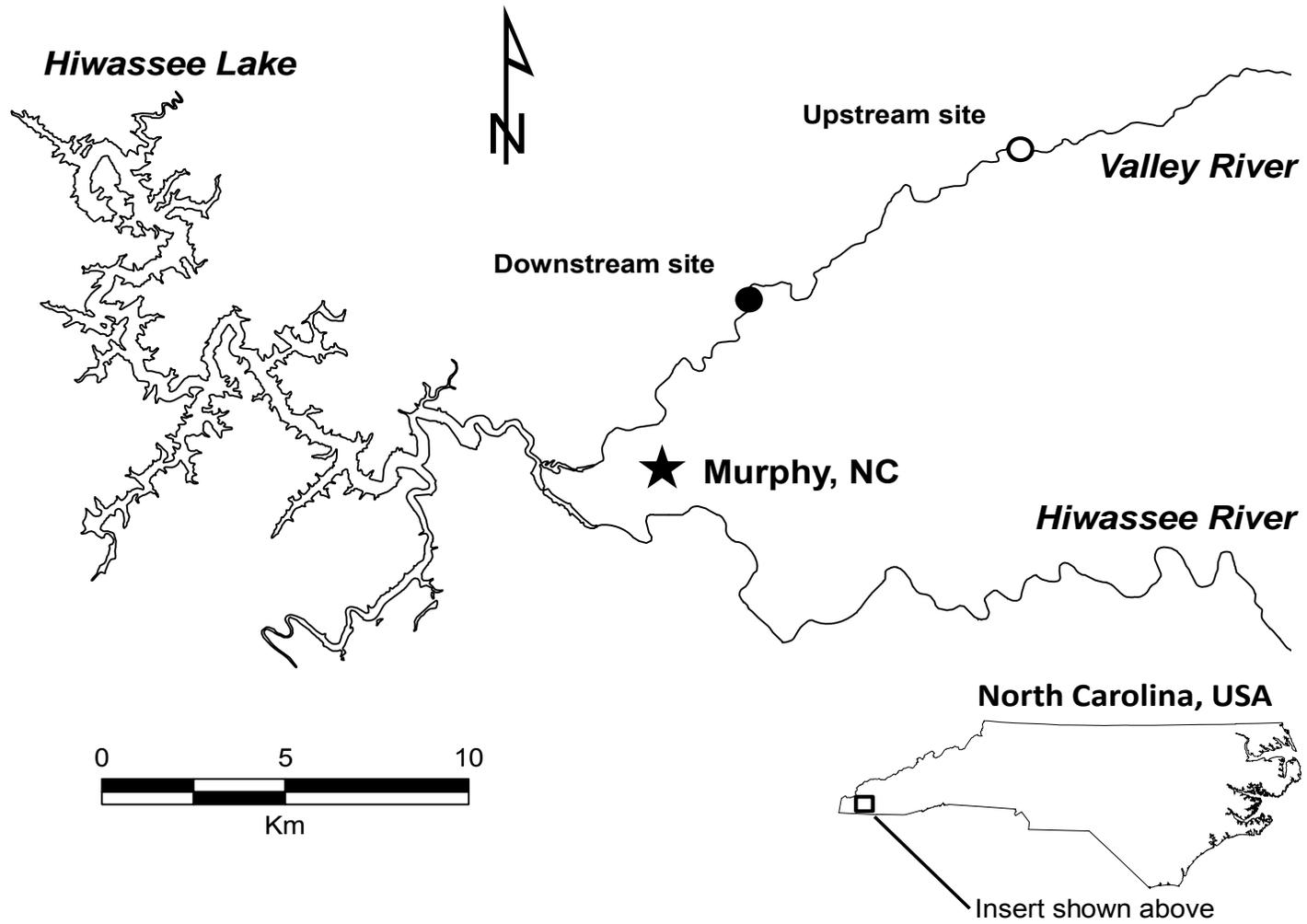


Figure 1.—Map of study area and sampling sites on Valley River, North Carolina.

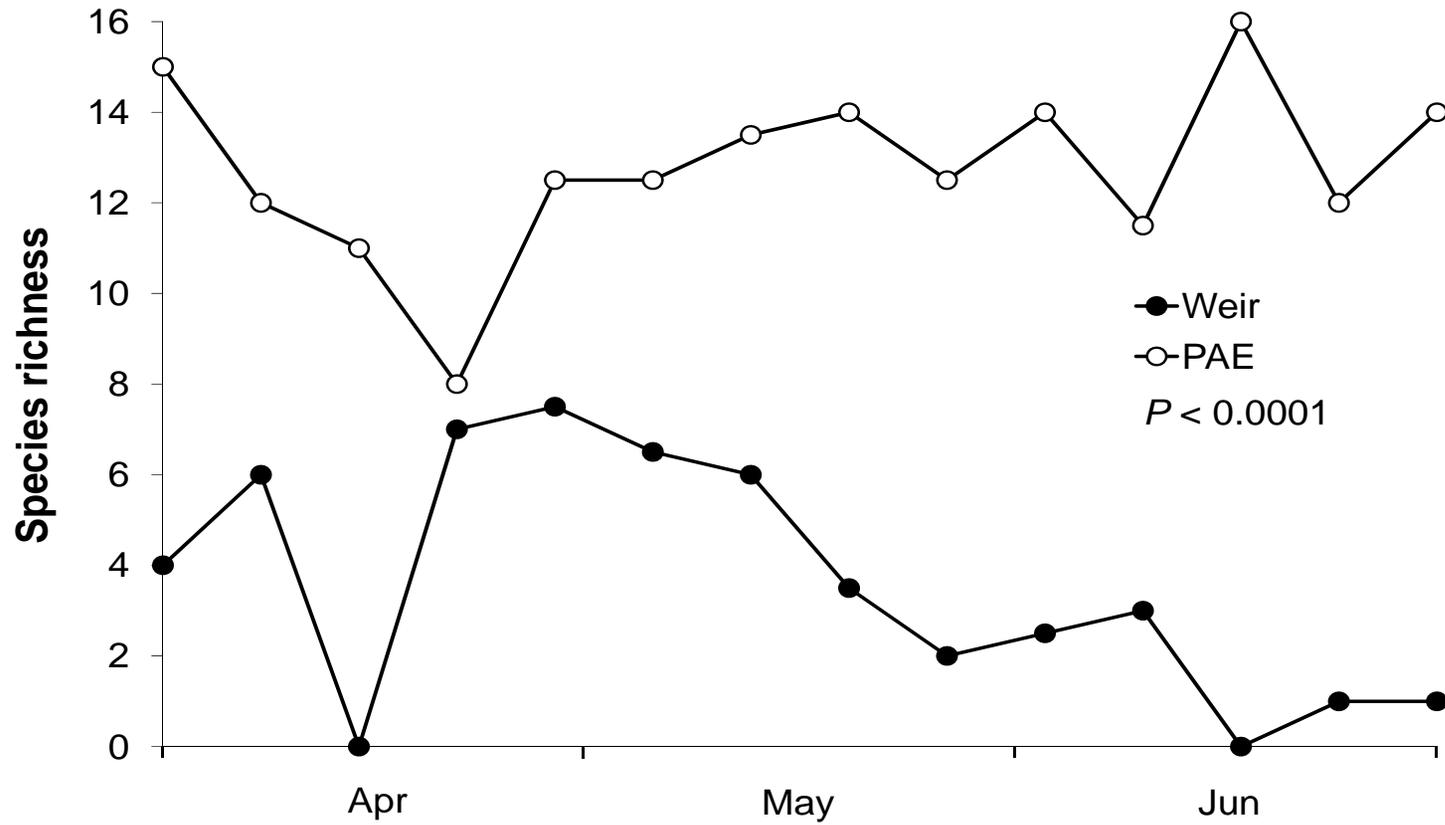


Figure 2.—Mean species richness between 2006 and 2007 for PAEs and two-way resistance board weirs. Gear specific weekly species richness estimates were compared using the Student's *t*-test.

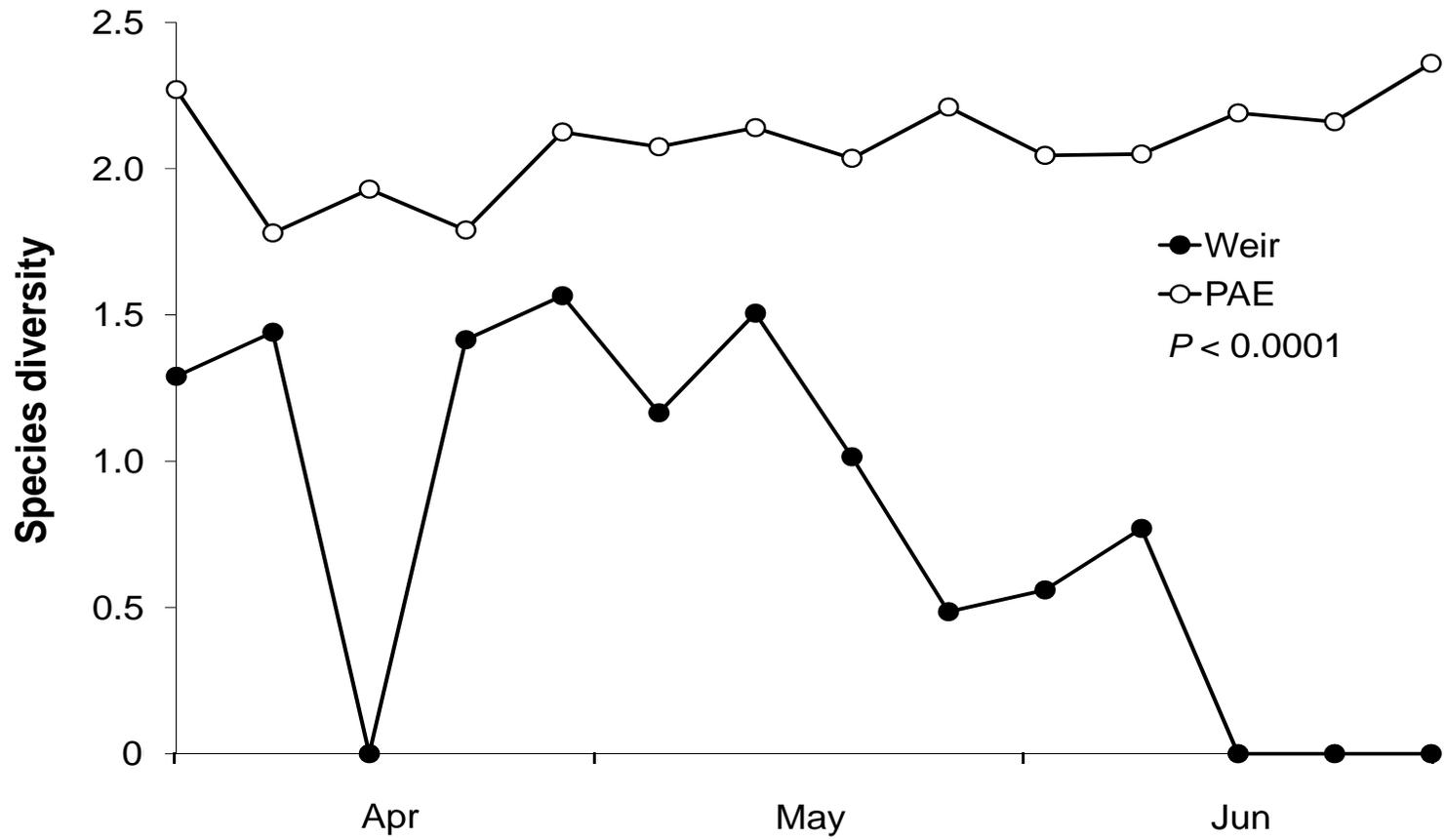


Figure 3.—Mean species diversity ( $H'$ ) between 2006 and 2007 for both PAEs and two-way resistance board weirs. Gear specific weekly species diversity estimates were compared using the Student's  $t$ -test.

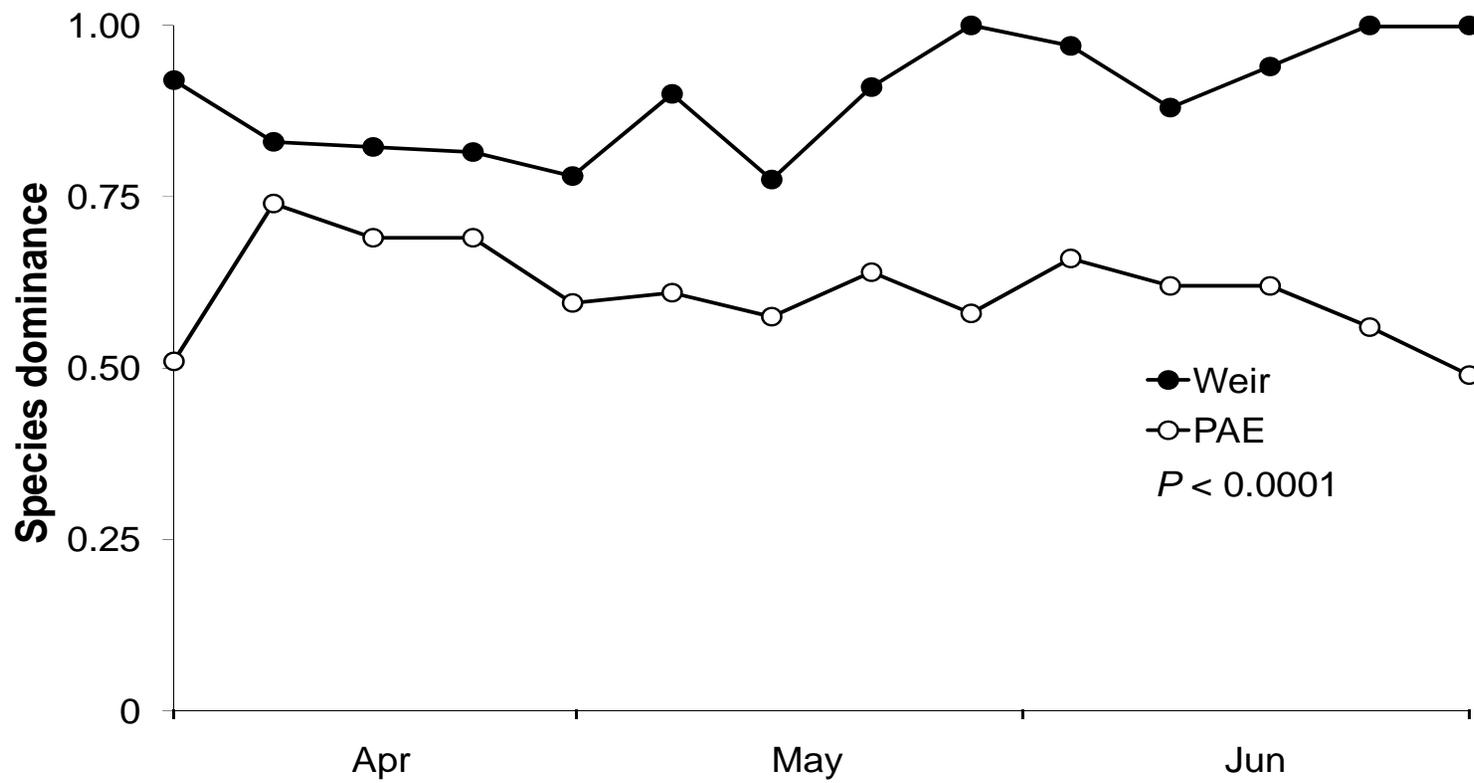


Figure 4.—Mean species dominance estimates ( $D_3$ ) between 2006 and 2007 for PAEs and two-way resistance board weirs. Gear specific weekly dominance estimates were compared using the Student's  $t$ -test.

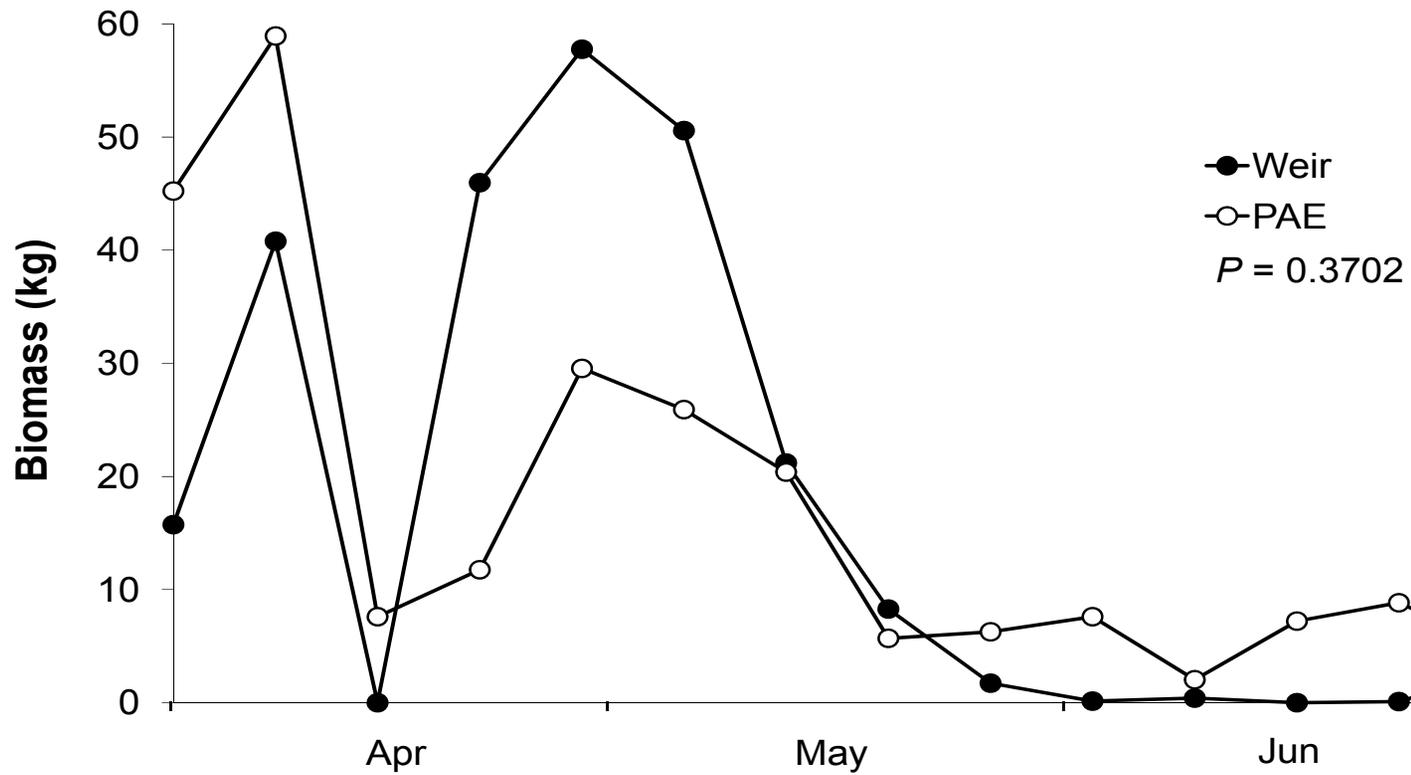


Figure 5.—Mean weekly biomass estimates between 2006 and 2007 for PAEs and two-way resistance board weirs. Gear specific weekly biomass estimates were compared using the Wilcoxon-Mann-Whitney test.

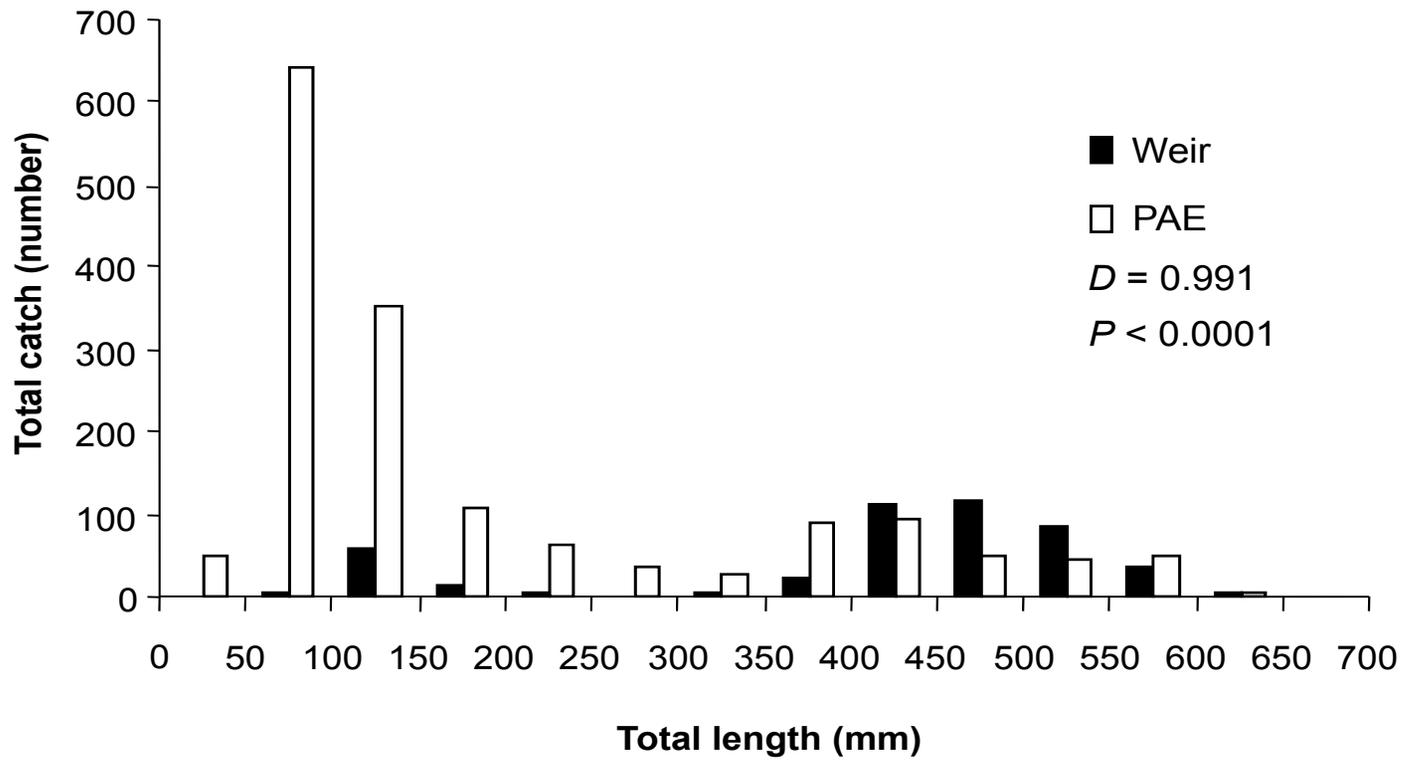


Figure 6.—Fish length frequency distributions for combined total catch from 2006 and 2007 for PAEs and two-way resistance board weirs. Length frequency distributions between gears were compared using a Kolmogorov-Smirnov two-sample test.

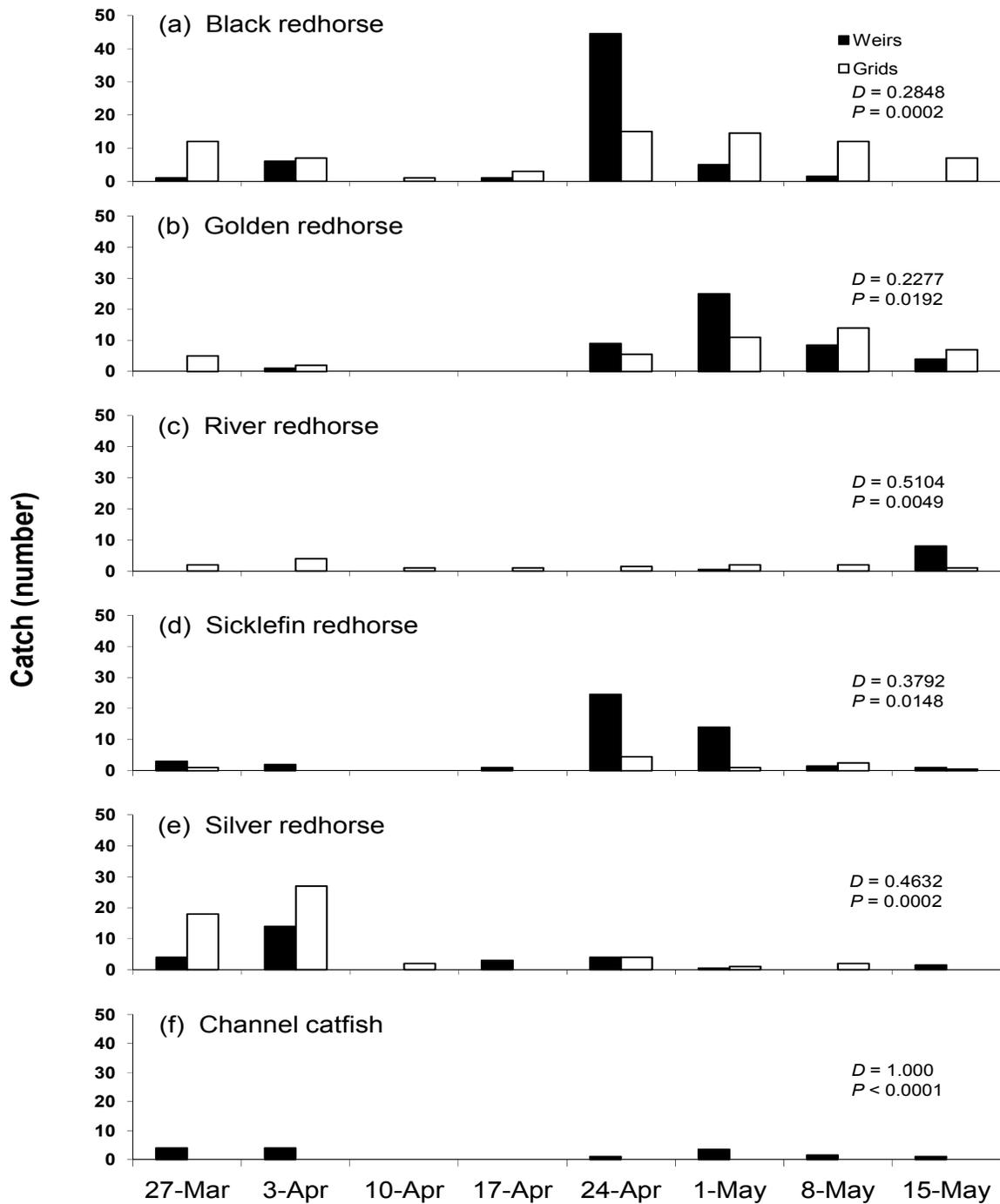


Figure 7.—Mean weekly frequency distributions of the most frequently sampled adult potamodromous fishes in 2006 and 2007 for both PAEs and two-way resistance board weirs. Gear frequency distributions were compared using a Kolmogorov-Smirnov two-sample test to determine which gear most efficiently documented spawning migration chronology.

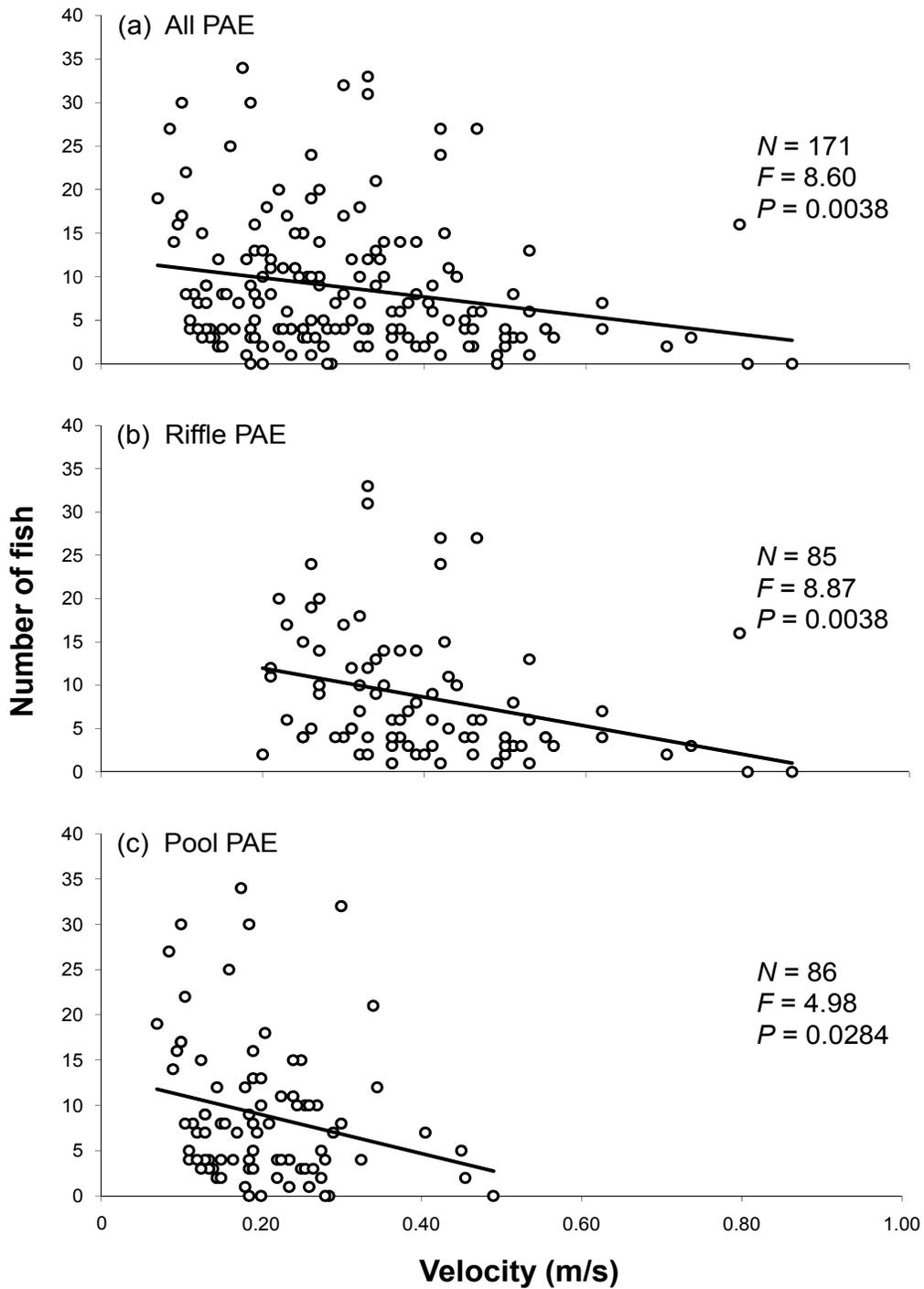


Figure 8.—Simple linear regression of daily catch and corresponding mean column velocity for all PAEs as well as PAE samples stratified by habitat type (i.e., riffle and pool).

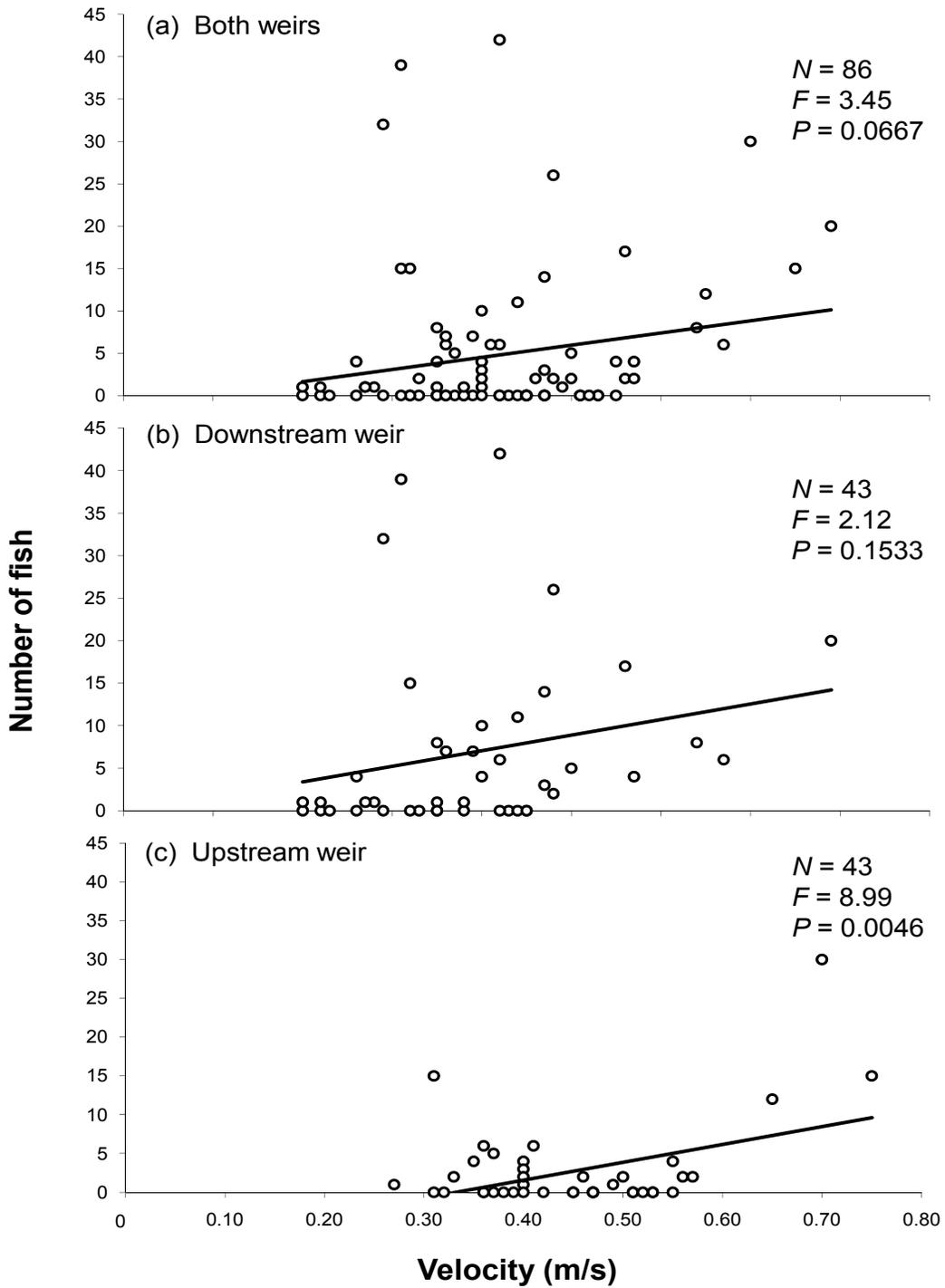


Figure 9.—Simple linear regression of daily catch and corresponding mean column velocity for cumulative two-way resistance board weir catch as well as resistance board weir samples stratified by sampling site (i.e., downstream and upstream).

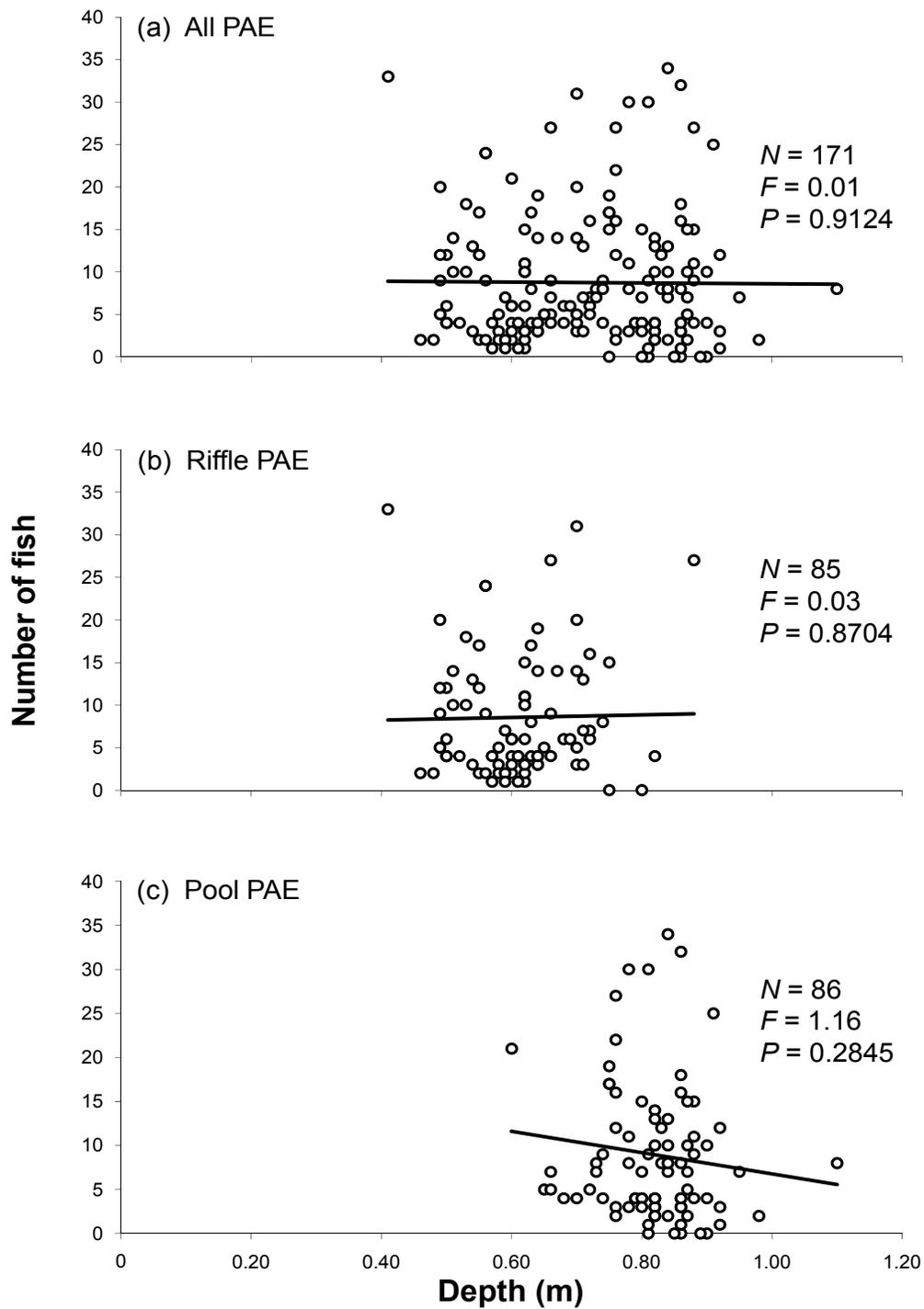


Figure 10.—Simple linear regression of daily catch and corresponding total depth for all PAEs as well as PAE samples stratified by habitat type (i.e., riffle and pool).

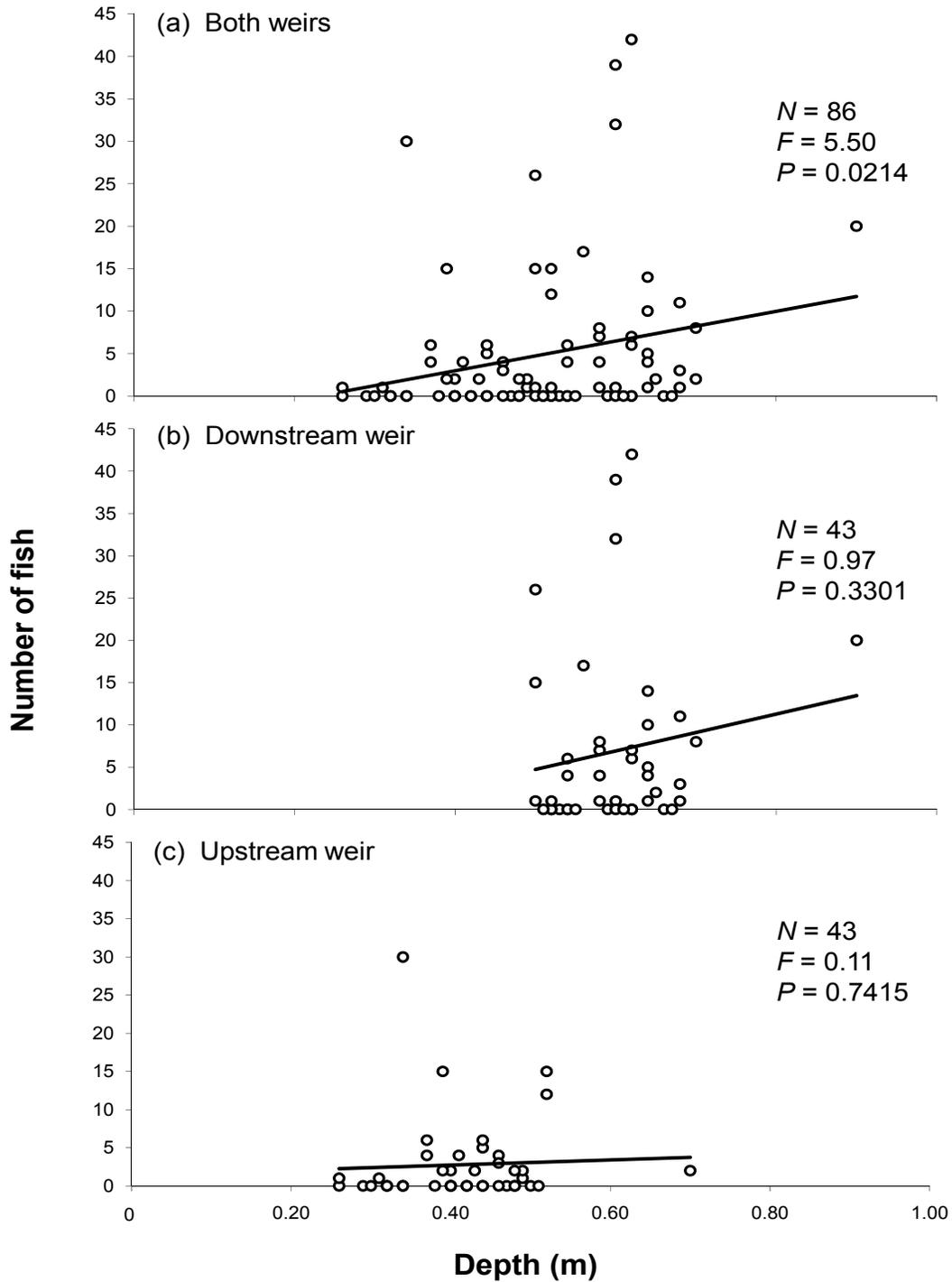


Figure 11.—Simple linear regression of daily catch and corresponding total depth for cumulative two-way resistance board weir catch as well as resistance board weir samples stratified by sampling site (i.e., downstream and upstream).