

**Differential stability of spawning microhabitats
of warmwater stream fishes**

by

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(Abstract)

I investigated streambed stability in spawning microhabitats of warmwater fishes in the upper Roanoke River, Virginia. Spawning microhabitats used by four reproductive guilds (egg-clusterers, mound-builders, egg-buriers, and pit-builders) were identified and ranges of 15 microhabitat variables used by each guild were compared to available microhabitat conditions to investigate habitat selection. Habitat usage by egg-clusterers was most characterized by selection for spawning rocks in the cobble size range, substrate roughness elements in the cobble to boulder size range, high roughness Reynolds number and moderate water column velocity. The mound-building bluehead chub (*Nocomis leptocephalus*) was distinct in its selection of substrate in the small gravel range, low water velocity and non-turbulent flow. Egg-buriers were the least distinct of the four guilds, exhibiting much variation in habitat use among the component species. However, all species used areas with small substrate (sand to gravel range), high velocity, and high turbulence. The pit-building central stoneroller (*Campostoma anomalum*) was distinct in its usage of areas with high velocity and turbulence, gravel sized substrate, and low embeddedness.

Stability of each guild's spawning microhabitats was empirically evaluated through analysis of tracer particle movement and repeated surveying of bed elevation along stream transects. Logistic regression equations developed from tracer particle data predicted that microhabitats selected by egg-clusterers are among the most stable of all available habitats during high flows. Microhabitats utilized by mound-builders, egg-buriers, and pit-builders are predicted to be less stable. Repeat transect surveying corroborates model predictions in that egg-burier habitats experienced changes in bed elevation in high flows, while egg-clusterer habitats did not.

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Introduction

Streambed movement in floods is a strong selective force on the life history traits of stream organisms that dwell in close association with the benthos (Townsend and Hildrew 1994). This may be the case for stream fishes, which are closely tied to the stream bottom, especially during the reproductive phase of life history. Most stream fishes place their eggs on, between, or under rocks, leaving them vulnerable to destruction during bed movement. Further, the frequency of bankfull flows (which are the flows at which bed movement occurs) is highest during the spring and early summer, the reproductive period for most temperate fishes. Hence, fishes must possess reproductive life history traits or habitat preferences that allow them to persist in this natural disturbance.

Habitat in gravel-bed streams is patchy (Townsend 1989, Hildrew and Giller 1994); there is much spatial variation in particle sizes, depth, velocity and other microhabitat parameters that influence bed movement (Hynes 1970). It follows that mobilization of the streambed is also spatially patchy (Naot 1984, Seal et al. 1993) with some areas experiencing more or less bed movement than others. I hypothesize that some fishes may survive natural bed movement disturbances by selecting spawning microhabitat patches that are less vulnerable to bed movement. These areas may be velocity 'dead zones' behind large substrate particles or areas of low turbulence and shear stress (Lancaster and Hildrew 1993). There is evidence of this type of adaptation in fishes; some fishes place their eggs on large, embedded cobbles (Page 1989) that likely do not move except during very high flows. Other species (e.g., catostomids and salmonids) bury their eggs in the hyporheos (Jenkins and Burkhead 1993, Kondolf and Wolman 1993) where they may be removed from the movement occurring on the stream bed surface (Montgomery et al. 1996). Use of such microhabitats allows fishes to avoid the effects of unpredictable flood disturbances and likely contributes to successful reproduction and the persistence of stream fish populations.

Channelization and riparian clearing in areas of heavy agriculture and urbanization alter stream morphology and may increase the amount of stream habitat that experiences bed movement during high floods (Petts 1984). Such an increase may result in severe alteration, destabilization and destruction of the areas in which certain fishes spawn. This alters the selective forces upon which the life histories of fishes have been forged, thereby resulting in new cycles of extirpations and extinction.

Managers responsible for mitigation for stream habitat alterations and restoration of stream channels need a well-developed knowledge of fish reproductive microhabitat requirements and their vulnerability to bed movement. Such knowledge would allow better interpretation of changes in stream fish communities and prediction of the effects of land use practices such as hydropower operation on stream fish reproduction and subsequent recruitment. The goal of this research was to develop such an understanding by quantitatively characterizing the spawning microhabitats used by several warmwater fishes of the upper Roanoke River drainage, Virginia, and to evaluate the relative stability of these microhabitats.

Literature Review

Streambed dynamics

Streams are a very dynamic system. They form as deposited or melted precipitation is transported downslope by gravity following the path of least resistance. With the water, sediment from the eroded hillslopes is transported downstream to alluvial floodplains and the ocean. The processes that occur in accord with this sediment transport determine the environment in which stream organisms dwell.

Stream channel characteristics are the result of many hydraulic and geomorphic processes operating at different scales, thus necessitating a hierarchical view of the stream (e.g., Frissell et al. 1986, Hawkins et al. 1993). At coarse levels such as the ecoregion or drainage basin, geology, climate, and land use characteristics interact to create general streambed materials, flow regime, and bedload (Leopold et al. 1964, Newbury 1996). At finer scales of resolution, such as the reach or mesohabitat unit (e.g., pool, riffle or run), hydraulic processes create the channel form (e.g., dunes, ripples, pool-riffle sequence) and maintain relatively stable sediment particle size distributions and patterns of variation in depth (Leopold 1994, Diplas 1994). Natural gravel bed streams are almost always dominated by a pool-riffle bedform with much variation in depth and velocity (Lisle 1982, Diplas 1994). Micro- and mesohabitat (e.g., pool, riffle, run, cascade) characteristics represent a dynamic equilibrium between the stream bed and flow regime mediated by channel morphology (Lisle 1982). At the finest scale, sediment particle size distributions are the result of the continuous processes of streambed erosion and subsequent deposition (Gordon et al. 1992, Leopold 1994).

Hydraulics and bed movement

Streambed materials and conditions are in a continual state of flux. A stream channel is continually transporting sediment inputs from hillslope runoff processes and erosion of channel materials (Leopold et al. 1964). During baseflow conditions this sediment is deposited in areas of little current such as stream edges, pools, and the downstream ends of point bars (Lisle 1982, Diplas 1994). In gravel bed streams, extensive bed movement and bedform change occurs, primarily at high flows (Leopold et al. 1964, Thorne et al. 1987, Newbury 1996).

These periodic bankfull flows often exert sufficient force to cause movement of (i.e., entrain) a majority of the bed particles including cobbles and even boulders (Thorne et al. 1987, Newbury 1996). Thus, bankfull flows represent a serious selective force on all stream dwelling organisms, particularly those that are benthically oriented (Townsend and Hildrew 1994).

Vulnerability of the streambed to turnover depends on a number of factors. The vulnerability of individual sediment particles to entrainment (i.e., being displaced and transported downstream) depends upon the amount of force being applied to those particles by the streamflow (Lane 1955). If the shear force (i.e., shear stress) being applied to the stream bed surface by the moving water reaches a certain level, known as the critical shear stress, the particle will be entrained (Lane 1955). The critical shear stress is determined by hydraulic conditions of the streambed, flow patterns and particle sizes. Small particles in areas of high turbulence, velocity and shear stress are most likely to be entrained (Carling 1992). Particles are also more likely to be entrained in areas with few large substrate particles providing stability to the bedform (Lancaster and Hildrew 1993). The spatial distribution of such factors is related to channel form, streambed roughness relative to depth, and stream slope, characteristics that are often heterogeneous in their spatial and temporal distribution (Leopold et al. 1964). As a result, vulnerability to entrainment is patchily distributed (Naot 1984, Seal et al. 1993).

Adaptations to bed movement

The primary importance of the physical habitat to stream organisms is widely accepted. In terms of life history, it is the most influential template upon which species traits are formed (Southwood 1988, Keddy 1992, Townsend and Hildrew 1993). Frequency and intensity of bed movement may be one of the most influential selective forces on benthic organisms because they are vulnerable to mortality or downstream displacement during floods (Vogel 1981, Townsend

and Hildrew 1993). Thus, only species with life history traits that allow them to avoid the deleterious effects of bed movement will persist.

The patchy nature of streambed stability has major implications for benthic organisms that are detrimentally impacted by bed movement (Vogel 1981, Townsend 1989). Stable microhabitat patches (i.e., those that experience less frequent and less intense bed movement) may act as refuges during high flow disturbances (Hildrew and Giller 1994, Robertson et al. 1995, Lancaster and Belyea 1997) by allowing avoidance of the effects of bed movement. During disturbances, organisms may utilize these stable microhabitats and persist to recolonize or provide recruits for surrounding populations (Robertson et al. 1997).

Use of stable microhabitats by macroinvertebrates and microcrustacea has been demonstrated (Hildrew and Giller 1994, Lancaster and Hildrew 1993, Robertson et al 1997). Macroinvertebrates avoid bed movement in areas with large substrate particles that are not entrained by high flows, areas with low shear stress at high flows, and areas with much hyporheic volume (Townsend et al. 1997). The importance of the hyporheos is even greater for epibenthic microcrustacea (Robertson et al. 1997). Certain shapes and configurations of bed particles may create localized streambed stability. Presence of tightly packed substrate clusters may stabilize bed roughness elements (Brayshaw et al. 1983). In addition, particles of certain shapes (e.g., disc or blade-shaped) require a higher shear stress to entrain (Panos Diplas, Dept. Civil Engineering, Virginia Tech, personal communication). Areas of low turbulence may also be more stable because of the lower force applied to the stream bottom (Statzner et al. 1988).

Similar to macroinvertebrates, the ecology and life history of many stream fishes are likely affected by bed movement. Egg and larval stages are most sensitive to bed movement because they are closely tied to the streambed and are physically weaker (Scheidegger and Bain 1995). Thus, the degree of hydrologic variability and frequency and intensity of high flow events are likely to be dominant influences on fish reproductive success (Schlosser and Toth 1984, Strange et al. 1993, Jennings and Philipp 1994). For example, Strange et al. (1993) found that the relative abundance of some fishes declined the year following high flows in their reproductive period.

Because of their apparent vulnerability to bed movement, fishes must possess reproductive life-history adaptations that minimize the likelihood of mortality due to bed movement. Fish possess several potential forms of adaptation to naturally occurring flow stress

and bed movement. The reproductive cycle of many stream species may coincide with temporal conditions minimizing danger of bed movement. For example, fishes may cue in on the falling limb of the hydrograph, which has lower shear stress relative to the rising limb at the same discharge (Song and Graf 1996). In addition, species that display fractional spawning or have multiple clutches per year (Heins and Rabito 1986) have more opportunities for successful reproduction. Alternatively, larval drift may mitigate the effects by allowing for recolonization from upstream reaches that may be more benign.

Another potential adaptation to bed movement is selection of stable microhabitats for spawning. Such stable spawning microhabitats are likely to occur in areas that have low shear stress (Townsend and Hildrew 1993), particle sizes that are not moved in flood events, and relatively smooth near-bed flow (Davis and Barmuta 1989). Some species (e.g., suckers) that bury eggs in well-sorted sediment with inter-gravel flow may be utilizing hyporheic zones removed from the effects of surface bed movement. Stability may also be enhanced behind large particles or clasts (Brayshaw et al. 1983). Further, less exposed particles are more likely to be stable. Areas of enhanced streambed stability likely occur at various spatial scales ranging from dead zones behind large substrate particles to highly meandering reaches (Sedell et al. 1990; Fausch and Bramblett 1991; Pearsons et al. 1992; Lancaster and Belyea 1997).

Potential selection of stable spawning microhabitats by fishes

Despite the descriptive reproductive life history information that exists for most stream fishes, little work has quantitatively characterized reproductive microhabitats. Even less attention has been given to hydraulic conditions and stability of spawning microhabitats. Most quantitative characterization of spawning habitats has been in response to a perceived need to predict changes resulting from human manipulations or to assess flow needs of fishes below hydropower dams. As a result, most studies have focused on economically important sport fishes such trout, bass, and sunfishes, neglecting the spawning microhabitat requirements of the majority of fishes. However, this body of work has elucidated some cases of strong habitat selection that may make these fishes more or less vulnerable to bed movement. Further, there is evidence that differences in habitat selection among fishes with varying reproductive strategies (Page 1989, Page and Johnston 1985) may result in differential vulnerability to bed movement (see below).

Redd-building salmonids display substrate size preferences in spawning microhabitat selection. Though particle size diameter in redds is related to fish size, almost all salmonids select substrate in the gravel range (Chapman 1981, Kondolf and Wolman 1993, Kondolf et al. 1993) with few cobbles present to strengthen the channel. These substrates are likely to be vulnerable to bed turnover. However, the redd building process concentrates larger sediments and the resulting bedform may be less vulnerable to scour than the surrounding area (Montgomery et al. 1995).

Most characterization of spawning habitat of warmwater fishes has centered on saucer nest-building centrarchids in instream flow studies. These studies have elucidated clear selection for nest spawning sites. Lukas and Orth (1993) found that redbreast sunfish (*Lepomis auritus*) use pool habitat with sand and gravel, low water velocity (bottom and average), and water deeper than 0.5 m. Smallmouth bass (*Micropterus dolomieu*) have been found to occur in similar areas (Lukas and Orth 1995), also preferring sand and small gravel. These areas have been found to be vulnerable to bed movement in high flows (Lukas and Orth 1995).

The little work that has been done on non-game fishes has been centered on the mound-building *Nocomis* chubs. *Nocomis* chubs manipulate the streambed during spawning by constructing large mounds of gravel (Miller 1964, Lobb and Orth 1988, Vives 1990), which are utilized as spawning habitat by many other fishes, most notably the cyprinid genera *Luxilus*, *Lythrurus*, *Clinostomus*, *Notropis*, and *Phoxinus* (Wallin 1992, Jenkins and Burkhead 1993). These mounds are frequently built in edge and pool habitats with moderate depth (30-50 cm) and low velocity (< 0.20 m/s; Miller 1964, Leonard et al. 1986, Lobb and Orth 1988). This behavior has been hypothesized to create hydraulically advantageous reproductive sites for the nest builder (Lobb and Orth 1988). However, the apparent vulnerability of mounds to destruction in high flows (Miller 1964) may still necessitate use of stable microhabitats in site selection for mound building.

Additional qualitative reproductive habitat descriptions of other reproductive guilds indicate potential for selection of stable microhabitats. For example, egg-clustering and egg-clumping darters and sculpins attach adhesive eggs to large cobbles in runs dominated by cobbles and boulders (Page 1985, Jenkins and Burkhead 1993). These nesting particles and surrounding bedform may be a very stable microhabitat (Lancaster and Hildrew 1993). Nesting rocks are also frequently disc-shaped and part of sediment clasts (personal observation),

configurations that enhance streambed stability (Brayshaw et al. 1983). Members of the egg-attaching cyprinid genus *Pimephales* often attach eggs to the underside of large stones (Johnston and Page 1992) that may also be stable. Crevice-spawning *Cyprinella* minnows often deposit eggs in crevices between large boulders and tree roots (Page and Johnston 1993) that are not likely to be entrained even in large floods.

In contrast, many reproductive strategies do not seem to allow selection of stable microhabitats. For example, egg-burying suckers and darters bury their eggs in submerged gravel bars or fast, turbulent riffles (Curry and Spacie 1984, Jenkins and Burkhead 1993), areas that are likely to experience bed movement, even in low flows. However, some species (e.g., Moxostomatine suckers) may bury eggs up to 25cm deep in gravel (Robert Jenkins, Dept. of Biology, Roanoke College, personal communication). Hence, they may bury their eggs deep in areas that likely have high hyporheic volume and intergravel flow which may shelter them from surface bed movement (Lancaster and Hildrew 1993). Depression pit-building central stonerollers (*Campostoma anomalum*) spawn in gravel bars similar to egg-buriers, but construct small pits (Page and Johnston 1993) that may create localized velocity refugia. Species that broadcast eggs over vegetation (e.g., Esocidae) or gravel (e.g., cyprinid genera *Notropis* and *Rhinichthys*) are likely to be even more vulnerable than those species that do not bury eggs.

These examples provide many interesting hypotheses as to the potential for differential vulnerability to bed movement by species of differing reproductive strategies. However, an evaluation of use of stable areas will require characterization of spawning microhabitats and an evaluation of the vulnerability to bed movement of these microhabitat conditions relative to those of all available habitats. Because bed movement is related to substrate and hydraulic conditions, spawning microhabitat characterization for investigation of microhabitat stability should be based on these parameters. The goal of this research was to characterize the substrate and hydraulic conditions of spawning microhabitats and to evaluate the relative stability of these habitats to determine if fish use stable microhabitats.

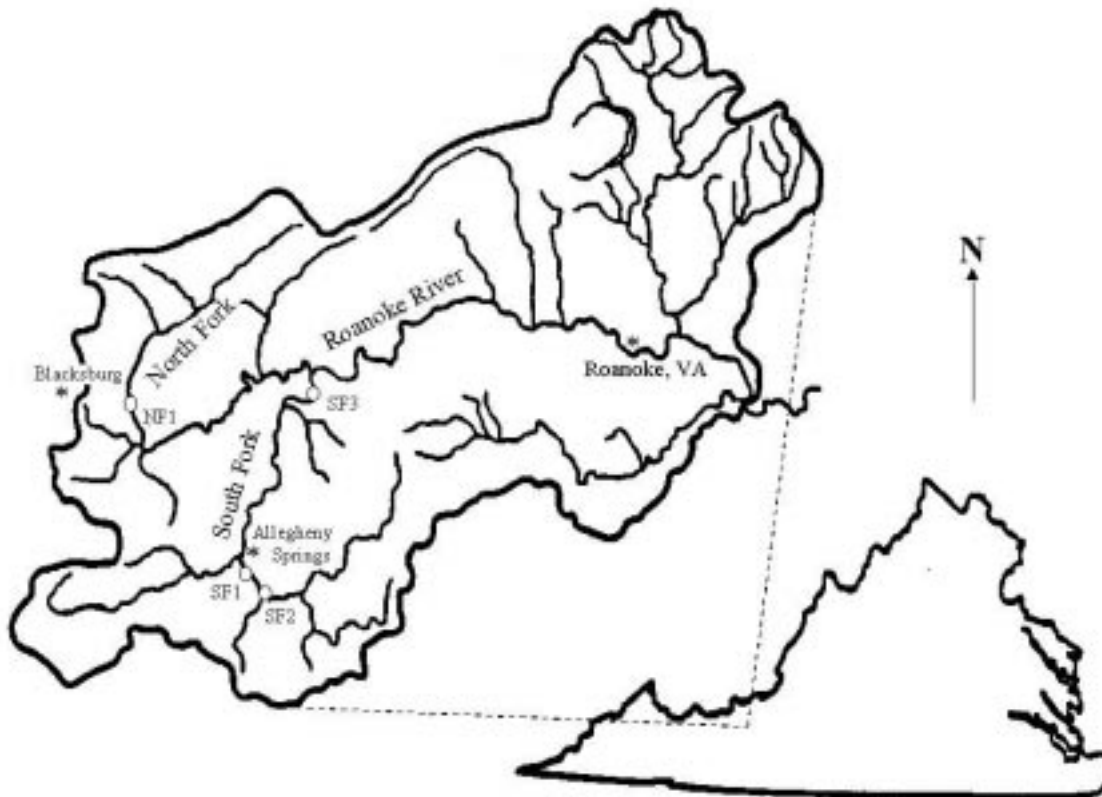
Study Drainage and Species

The North and South Forks of the Roanoke River above their confluence near Lafayette, Virginia (Figure 1.1) were selected as the study area for investigating the reproductive microhabitat selection of warmwater stream fishes. Both streams are spring fed and flow

through primarily hardwood forests of the Ridge and Valley physiographic province. Both forks have stream channels that are moderately meandering with coarse streambed materials, thus they exhibit regular pool-riffle sequences and the associated microhabitat heterogeneity. Upstream from the confluence, average wetted stream widths range from 6-30m and gradient is 6 m/km on average, but ranges from 2-20 m/km.

Baseflow discharge ranges from 50-80 cfs and 100-200 cfs at the North Fork and South Fork sites, respectively. The annual hydrograph is typical of montane streams in a region experiencing periodic thunderstorms. Groundwater flow maintains a stable baseflow, but thunderstorms and seasonal rains in spring result in higher flood peaks (Figure 1.2). Bankfull flow events are common from March to July, averaging 1 event per year but ranging up to as many as 8 events per year during 1961-1996 (Figure 1.3). Further, the primary land use of the

Figure 1.1. Location of study sites (open circles) in the upper Roanoke River watershed, southwest Virginia.



North Fork is agricultural pasture.

Heavy grazing has caused riparian destruction and subsequent bank failure and sediment input. The South Fork is also moderately agriculturalized, but has a more continuous riparian zone and stable banks (Matthews et al. 1982). Both streams have likely experienced an increase in the frequency of bed movement and changes in particle size distributions because of widespread bank failure and downcutting. Thus, it is likely that fishes in the upper drainage are faced with additional selection pressure to adapt to high flow events.

Figure 1.2. Typical annual hydrograph (1988) for the South Fork Roanoke River at Shawsville, Virginia showing flood peaks from March to May and low flows in late summer into fall.

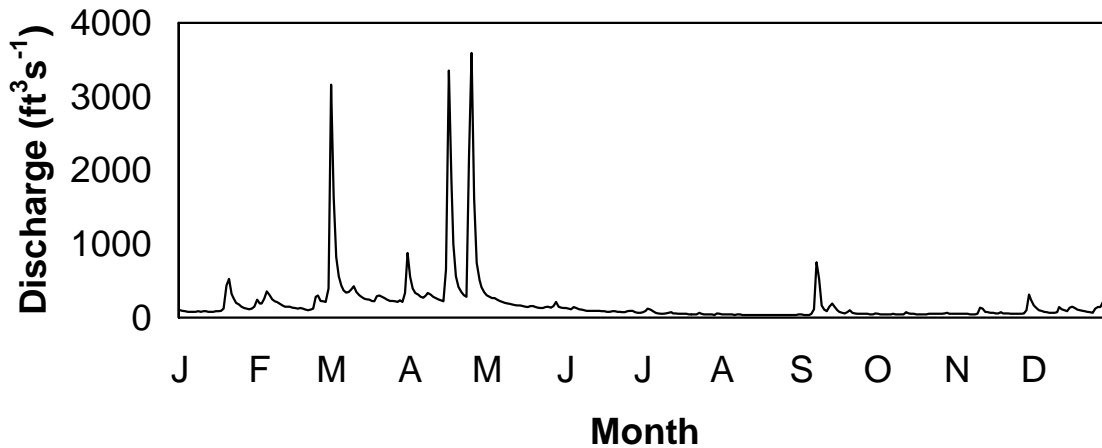


Figure 1.3. Annual frequency of bankfull flood events during March to May from 1961 to 1996 for South Fork Roanoke River at Shawsville, Virginia.

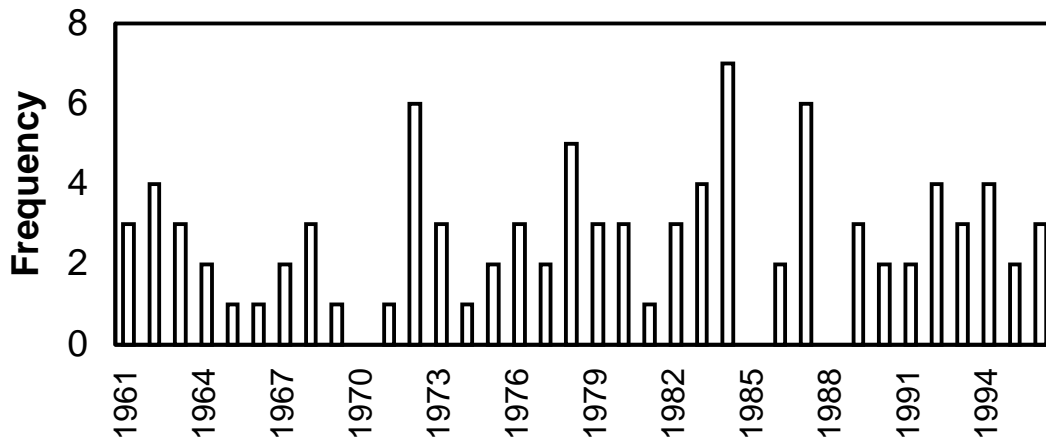


Table 1.1. Reproductive guilds and species representatives from the upper Roanoke River drainage, Virginia. Guild names are after Page (1989) and Johnston and Page (1992).

Parental Care	Guild	Roanoke Representatives
Non-Guarding	Broadcasters	<i>Notropis hudsonius</i> , <i>N. procne</i> , <i>N. volucellus</i> , <i>Rhinichthys atratulus</i>
	Broadcasters (nest associates)	<i>Clinostomus funduloides</i> , <i>Luxilus albeolus</i> , <i>L. cerasinus</i> , <i>Lythrurus ardens</i> , <i>Phoxinus oreas</i>
	Crevice spawning	<i>Cyprinella analostana</i>
	Egg Buriers	<i>Scartomyzon ariommus</i> , <i>S. cervinus</i> , <i>Thoburnia rathoeca</i> , <i>Hypentelium nigricans</i> , <i>H. roanokense</i> , <i>Moxostoma collapsum</i> , <i>M. erythrurum</i> , <i>M. pappillosum</i> , <i>Catostomus commersoni</i> , <i>Percina rex</i> , <i>P. roanoka</i> ,
	Egg attachers (vegetation)	<i>Esox niger</i>
Guarding	Saucer-nest builders	<i>Ambloplites rupestris</i> , <i>L. auritus</i> , <i>L. macrochirus</i> , <i>L. cyanellus</i> , <i>Micropterus dolomieu</i> , <i>M. salmoides</i>
	Pit Builders	<i>Campostoma anomalum</i>
	Mound Builders	<i>Exoglossum maxillingua</i> , <i>Nocomis leptcephalus</i> , <i>N. raneyi</i>
	Egg Attachers (rocks open)	<i>Pimephales notatus</i>
	Egg Clumpers (cavities)	<i>Ameiurus nebulosus</i> , <i>Noturus gilberti</i> , <i>N. insignis</i>
	Egg Clusterers (rock cavities)	<i>Cottus bairdi</i> , <i>Etheostoma flabellare</i> , <i>E. nigrum</i> , <i>E. podostemone</i>

Fish communities of the Roanoke River have been well studied, allowing description of life history and community ecology of most species (Matthews et al. 1982; Surat et al. 1982; Matthews 1990; Jenkins and Burkhead 1993). The upper Roanoke River fish fauna totals

approximately 40 species with representatives of 11 reproductive guilds (Table 1.1). These include representatives of guilds of potentially varying vulnerability to bed movement. Two egg-clustering darters [riverweed darter (*Etheostoma podostemone*) and fantail darter (*E. flabellare*)] are common, as are representatives of mound-builders (bluehead chub), egg-buriers [Roanoke darter (*Percina roanoka*), torrent sucker (*Thoburnia rathoeca*), and black jumprock (*Scartomyzon cervinus*)], and pit-builders (central stoneroller).

Chapter 1. Characterization of spawning microhabitats

Objective

The first objective of this research was to quantitatively characterize substrate and hydraulic conditions of spawning microhabitats of stream fishes by comparing microhabitats used to those available. I wanted to identify the ranges of each microhabitat variable (e.g., substrate size) and variable combinations (e.g., low depth and high shear stress) used by each guild to determine the essential features of their spawning microhabitats. This procedure tested the following general null hypothesis:

H₀: Spawning microhabitats of all species and guilds will be uniformly distributed among all available microhabitats

Based on literature descriptions of spawning habitats, I expected each guild to select distinct ranges of the available distribution of habitat conditions. Some specific alternate hypotheses were:

H_a: Egg-clusterers spawning microhabitats will be characterized by large substrate particle sizes.

H_a: Mound-builder spawning habitats will be characterized by small particle sizes and low velocity.

H_a: Egg-burrier and pit-builder spawning microhabitats will be characterized by small substrate particles and high velocities.

Methods

Spawning microhabitat location

Several sites in the upper Roanoke River drainage were electrofished during late March through early May 1997 and 1998 to identify areas of aggregation of species of interest and to find males in reproductive coloration and ripe females. Electrofishing was focused on the North and South forks, the two largest upper Roanoke River tributaries. Sites at which reproductive individuals were located were then snorkeled to verify locations of reproductive adults.

Reproductive microhabitats of various fishes were located at four sites from mid spring to early summer 1997 and 1998, three on the South Fork and one on the North Fork.

All sites were intensively surveyed by two or three snorkelers. Snorkelers moved upstream in a zigzag pattern covering the entire width of the stream, and covering all habitats with equal effort to avoid habitat bias. When fish reproduction was observed, the specific location was marked and divers continued until a longitudinal distance of approximately 200 m of stream was covered. This distance was selected because it allowed divers to cover at least two of each mesohabitat type (i.e., pools, riffles, and runs).

Spawning areas were located for a total of five guilds and 15 species in the two years. Criteria for assessing reproduction varied among spawning guilds. Brilliantly colored nuptial male egg-clusterers clean out the underside of a cobble and attract females which then invert and deposit eggs which the male fertilizes and guards. Egg-clusterer reproduction was determined by overturning rocks under which a male darter was positioned. If eggs were found on the underside of rocks with a male tending the nest, the nest was included as a reproductive site for the species of the male. Reproduction of mound builders and associates were assessed by presence of reproductively colored males and ripe females in the vicinity of active chub mounds. In most cases reproductive acts were also observed. Egg-burier reproduction was verified when a ripe female and one or more nuptial males aligned and quivered. It was assumed that this constituted selection of that microhabitat for spawning, although no eggs were visible. We assessed pit-builder reproduction by noting presence of males over a pit. In most cases, reproductive acts were observed, but eggs were not.

Spawning microhabitat use

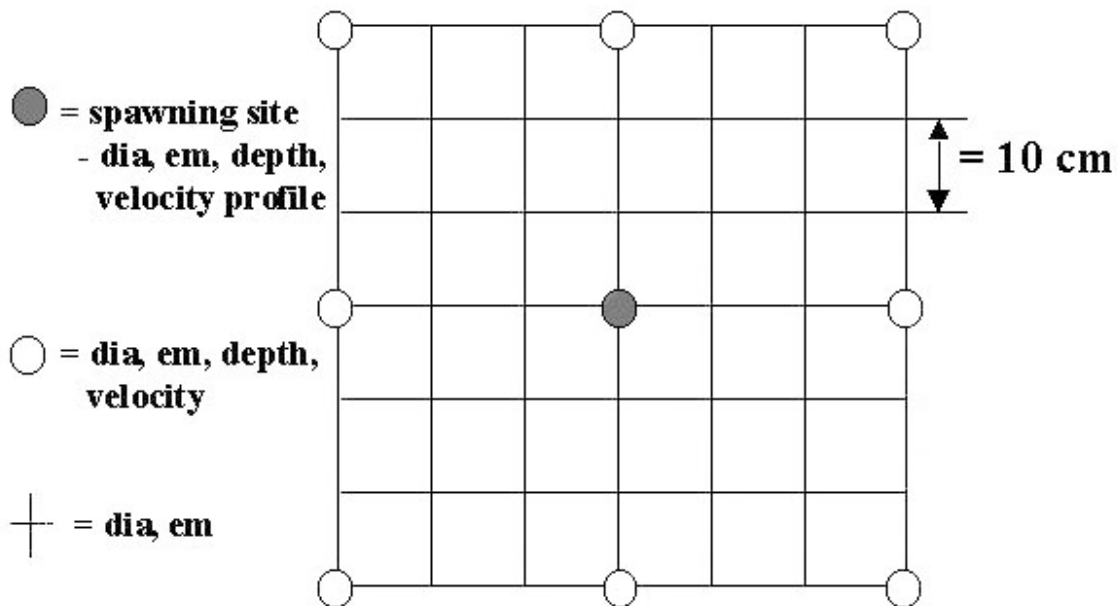
Spawning microhabitat use and overall microhabitat availability were sampled to investigate and characterize microhabitat selection by each species and guild. Several microhabitat variables were measured at all reproductive sites and compared to those available in the snorkeled reach. These variables represented several habitat factors by which species may select spawning microhabitats. I then examined the distribution of microhabitat conditions to the available distribution to examine patterns of selection by each guild.

To determine the ranges of each microhabitat variable used by each guild (i.e., microhabitat use), several microhabitat variables were measured at every marked spawning location using a grid (see Figure 1.4 for details of grid configuration) that was centered over the egg deposition area. To fabricate grid points, a 60-cm length of lead core rope with marks at 10-

cm increments was anchored parallel to flow with the center mark directly over the egg deposition site. A 60-cm length of rebar with marks at 10-cm increments was then centered over and perpendicular to the upstream end of the rope and measurements were made at each of the 10-cm marks on the rebar. The rebar was then moved downstream to the other lead rope marks and measurements were repeated. This method allowed measurements of substrate diameter and embeddedness, depth and velocity along a grid configuration without using a complex grid structure that would alter flow patterns.

Substrate particle diameter (intermediate axis) and degree of embeddedness (expressed as a percentage of the rock covered with sand or silt to the nearest 10%) were measured at all 49 intersections of the grid. In addition, I measured all 3 axes of spawning rocks for nest spawners to assess shape of these particles. I measured 49 particles because this number is considered minimum for a relatively precise estimate of the median (D50) and 90th percentile (D90) of

Figure 1.4. Microhabitat sampling grid configuration showing positions of 49 measurements of substrate diameter (dia) and embeddedness (em), nine measurements of depth-averaged velocity (velocity) and depth, and vertical velocity profile.



a surface sediment particle size distribution on a microhabitat scale (Panos Diplas, personal communication). Surface substrate was measured as opposed to a bulk measurement because it is the heavily armored bed surface that most determines the stability of that microhabitat

(Montgomery et al. 1995). In addition, water depth (cm) and water column velocity (m/s) were measured at 9 locations on the grid (Figure 1.4). Water column velocity was measured at 0.4 depth in areas < 50 cm deep and was the average of velocity at 0.2 and 0.8 depth in areas > 50 cm deep. Finally, the vertical velocity profile was delineated at the center of the grid, to be used for shear stress calculations. To elucidate the velocity profile, I measured velocity from as close to the bottom as possible (most often within 2 cm) to the surface in 10 equal increments.

Substrate particle measurements were made by hand. The index finger was placed at the grid intersection and the particle that was contacted was picked up and measured to the nearest mm. If one particle could not be determined, the intersection would be characterized as sand or silt. In all subsequent calculations, the mid-point of the Wentworth range (Gordon et al. 1993) corresponding to sand (1 mm) or silt (0.05 mm) were used. All velocity measurements were made with a factory calibrated Marsh-McBirney Model 2000 electromagnetic flow meter.

From these measurements, several summary variables were used to characterize the grid microhabitat (Table 1.2). These summary variables allowed assessment of habitat selection at two scales, the grid scale (median sediment particle size, sediment roughness, embeddedness, areal silt coverage, mean depth, mean velocity, Reynolds number, Froude number, roughness Reynolds number, relative depth, and thickness of laminar sublayer) and the deposition site (sediment particle size at center of grid, embeddedness at center of grid, and velocity at stream bottom). They also allowed characterization of hydraulic and turbulence conditions at the site, flow patterns, and forces being applied to the stream bottom.

Summary variables are grouped into four categories (Table 1.2). Roughness variables were calculated from particle diameter measurements. Median sediment diameter (D_{50}) was calculated as the median particle diameter of the 49 grid measurements (in mm). Diameter at center (D_{cen}) is the diameter of the particle at the egg deposition site. For nest spawners, this was the diameter of the particle to which eggs were attached. For all other species, this was the diameter of one of the particles under which eggs were buried. Roughness height (k) was calculated as the 90th percentile of the surface sediment size distribution constructed for each grid from the 49 sediment diameter measurements (in mm). Roughness height is included as a variable because it is important in determination of microhabitat conditions experienced by benthic organisms (Davis and Barmuta 1989, Carling 1992). Areas with high roughness height

(i.e., areas with large substrate particles) may have lower near-bed flow velocities under the same mean flow velocity conditions than areas dominated by sand or silt because of formation of velocity refuges behind cobbles or boulders (Carling 1992). In addition, the value of k relative to other microhabitat variables (e.g., depth and thickness of laminar sublayer) determines flow patterns and degree of turbulence of near-bed flows (see discussion of relative depth and laminar sublayer thickness below) (Davis and Barmuta 1989).

Table 1.2. Variable categories and summary variables calculated from grid measurements to characterize spawning microhabitat.

Variable Category	Variable
Roughness	Median substrate particle size (D50) Substrate particle size in grid center (Dcen) Substrate roughness (k)
Fine Sedimentation	Mean embeddedness (Em) Embeddedness in center (Emcen) Percent areal silt coverage (Silt)
Hydraulic – mean flow	Mean depth (D) Mean water column velocity (V) Froude number (Fr) Reynolds number (Re)
Hydraulic – near bed flow	Demersal velocity (Vbot) Shear stress (Shear) Roughness Reynolds number (rRe) Relative depth (Rel) Thickness of laminar sublayer (δ)

Siltation variables were embeddedness, embeddedness at grid center, and percent areal coverage of silt. Mean embeddedness was the mean of the 49 embeddedness measurements. Embeddedness at center of the grid was the embeddedness (to the nearest 10%) of the center particle (i.e., the rock on which eggs were placed or buried under). Embeddedness was used as a variable for two purposes. First, it allowed detection of high levels of sedimentation. Second, it allowed assessment of anchoring and exposure to flow for the sediment particles. Sediment particles embedded in sand may be less exposed to flow and more difficult to entrain. Percent

silt areal coverage (Silt) was a visual estimation of percent of the grid area covered by silt, measured to the nearest 10%.

Mean flow hydraulic variables were those variables that describe the depth, velocity and turbulence conditions in the grid as influenced by gravity and channel shape (Davis and Barmuta 1989). Depth (cm) was calculated as the mean of the nine grid measurements. Water column velocity (m/s) was calculated as the mean of the nine depth averaged velocity measurements from the grid. Reynolds number (Re) is the unitless ratio of inertial to viscous forces and characterizes mean-flow turbulence. It was calculated using the formula: (water column velocity \times depth) / ν where ν is kinematic viscosity (1×10^{-6} for water temperatures near 20°C). Flow is considered laminar if Re is less than 500, transitional if Re is between 500 and 2000, and turbulent if Re is greater than 2000 (Gordon et al. 1993). Degree of turbulence in spawning microhabitats is important because turbulent flow applies heavier forces to the stream bottom (Statzner et al. 1988), resulting in bed instability and scour. Hence, bed stability is not likely enhanced in areas of high localized Re. Froude number (Fr) is the unitless ratio of gravity to viscous forces and gives a characterization of surface turbulence characteristics. It was calculated using the formula: depth averaged velocity / $(g \times \text{depth})^{0.5}$ where g is the acceleration due to gravity (981 cm/s^2). Flow is considered subcritical (or tranquil) if Fr is less than 1, critical (flow characterized by standing waves) if Fr is equal to 1, and supercritical if Fr is greater than 1 (Gordon et al. 1993).

The near-bed flow conditions are described by the demersal velocity, shear stress, roughness Reynolds number, relative depth, and thickness of laminar sublayer. Flow conditions near the streambed are markedly different than the mean flow due to the influence of roughness elements. Velocity at the stream bottom (demersal velocity) is usually lower, shear stresses are higher, and flow may be more turbulent, but with a small layer of laminar flow forming along the roughness elements (Davis and Barmuta 1989). Demersal velocity was measured as the velocity at the center of the grid as close to the stream bottom as was possible to measure (usually within 2 cm). Shear stress, an estimate of the shear forces being applied to the stream bottom by the water, was measured using the vertical velocity profile method (Gordon et al. 1993). The slope (b) of log depth versus velocity profile was obtained for each grid and shear stress ($\text{Nm}^{-1} \text{s}^{-2}$) was calculated from the formula: $\rho \times (b / 5.75)^2$ where ρ is the density of water (998 kg/cm^3 at 20°C). In these units ($\text{Nm}^{-1} \text{s}^{-2}$) critical shear stress, the shear stress at which substrate particles are

moved, is directly proportional to substrate size in cm (Lane 1955). In other words, as a ‘rule of thumb,’ a shear stress of $23 \text{ Nm}^{-1}\text{s}^{-2}$ would be expected to move a substrate particle 23 cm in diameter. Thus, by comparing shear stress to substrate roughness measurements (D_{50} , D_{cen} , and k), the vulnerability to bed movement of grid particles may be predicted.

Roughness Reynolds number (rRe) characterizes bottom turbulence and was calculated using the formula: $(\text{water column velocity} \times \text{substrate roughness}) / [6 \times v \times \log_e(30 \times \text{depth} / \text{substrate roughness})]$ (Davis and Barmuta 1989). Streambeds are considered hydraulically smooth if rRe is less than 5, transitional if rRe is between 5 and 70, and hydraulically rough if rRe is greater than 70 (Gordon et al. 1993). Generally, organisms on the upstream side of substrate particles will experience much higher velocities than those on the downstream side. This effect is more pronounced at high levels of rRe (Nowell and Jumars 1984). Thus, flows with high rRe may allow good protection from predators, competition, and bedload if the low velocities behind or under roughness elements are utilized.

Relative depth and δ were measured because they describe near-bed flow conditions (Davis and Barmuta 1989). Relative depth is the ratio of depth to k and gives a measure of protrusion of substrate elements into the water column (Davis and Barmuta 1989, Gordon et al. 1993). Thickness of the laminar sublayer (δ) estimates the width of the laminar portion of the boundary flow layer near stream roughness elements. It was calculated using the formula: $(11.8 \times v / V_*)$ where V_* is the shear velocity calculated as $(b / 5.75)$. Flow pattern is highly dependent upon roughness height relative to depth; flow velocity is less when k is high relative to depth. Benthic organisms are more exposed to flow and vulnerable to displacement when relative depth is high. The degree of near-bed flow turbulence is determined by the thickness of δ relative to k . If δ is greater than k flow will be laminar near the bottom and hydraulically smooth (Davis and Barmuta 1989). Hydraulically smooth conditions are rare in a gravel stream (Gordon et al. 1993), only occurring in smooth bedrock outcrops (Davis and Barmuta 1989). More often k is much higher than δ and near bed flow is turbulent. However, smooth conditions may occur in localized areas behind boulders along stream edges.

Microhabitat availability

After the 1997 spawning season, one site on each fork (NF1 and SF1) was selected for an intensive investigation of spawning microhabitat selection the following year. At these two sites,

microhabitat availability would be sampled to allow characterization of microhabitats selected by each guild. These sites were chosen according to ease of repeated accessibility and presence of as many spawning strategies as possible. Sites were roughly 20 mean stream widths long to include one meander bend and at least two of each mesohabitat type. The site on the North Fork Roanoke (NF1) was approximately 170m long (MSW=8m) while the site on the South Fork (SF1) was roughly 240m long (MSW=12m) (see Figure 1 for site locations).

At each site, I set up a network of transects perpendicular to flow, spaced parallel to each other every 10 m from the bottom of the reach to the top of the reach. This 10-m spacing resulted in accurate representation of all habitat types. Because repeat measurements of the same habitats were important to various portions of my study, rebar stakes were placed at the ends of the transects and the locations of the stakes surveyed in relative to two benchmarks. Thus, the locations of transects could be reset following any loss of stakes in floods. Transect ends were placed off the floodplain wherever possible to minimize losses.

Along these transects, locations were selected from which to make availability measures. Depending on stream width, two to four locations per transect were randomly selected for location of grid measurements. The same grid measurements as for spawning locations were made at these points on the availability transects. For analysis, number of grids needed to be approximately equal to the total number of use observations. Number of grids per site was selected to meet this consideration and to adequately characterize available conditions. Density of grids was held constant at approximately one grid per 35m² across the two sites. NF had 2-3 grids per transect and SF had 3-4 grids per transect. These grids were then compared to the spawning microhabitat grids to determine microhabitat selection by species and spawning guild.

Microhabitat data analysis

To test the hypothesis that spawning microhabitat use of all reproductive guilds is uniformly distributed among all available microhabitats, spawning use and microhabitat availability data from NF1 and SF1 in 1998 were compared using Kolmogorov-Smirnov tests and Multiple Response Permutations Procedures (MRPP; Mielke 1981, McClure and Mefford 1995).

Where sample sizes were adequate, analysis was performed for individual species. However, because of low sample sizes of spawning sites for most species and to enhance

relevance of the data, analysis was performed for reproductive guilds. Species were assigned to guilds a priori according to existing classifications (Page 1989, Johnston and Page 1992) and behavioral descriptions (Jenkins and Burkhead 1993) (Table 1.1).

I pooled used and available microhabitat data from the two sites (NF1 and SF1) for analysis to increase sample sizes for all analyses. The inclusion of data from two sites decreased the likelihood that observed patterns of selection would be artifacts of site specific factors (e.g., absence of optimal spawning habitat in the snorkeled reach or high density of predators). It also decreased the likelihood of undersampling of all habitat conditions potentially available in the upper Roanoke River drainage.

I considered this to be a valid procedure (i.e., not introducing site bias or increasing variation of habitat variables) if there were no site differences in microhabitat conditions used by the various guilds that corresponded to differences in available conditions. Site differences in single variables and multivariate patterns were investigated before performing spawning habitat analyses with Kolmogorov-Smirnov tests for each individual variable and MRPP (see below for description of procedure) to determine the validity of the pooling. MRPP showed that multivariate microhabitat availability was significantly different between the two sites, though the test was only slightly significant ($P = 0.04$). This difference was likely attributable to site differences in availability of D50, Dcen, embeddedness at grid center, and relative depth (K-S tests, $P < 0.05$), all of which were higher at NF1 except relative depth. The only differences in available habitats that correspond with site differences in microhabitat use were higher D50, silt, and relative depth for egg-buriers at the North Fork site. These differences are discounted because species composition of egg-buriers was different across sites. The only other guild differences between sites were for mound-builders in siltation and relative depth, and their differences were in the opposite direction as those for the available (i.e., relative depth higher at NF1 and siltation higher at SF1). Thus, I considered the pooling of sites to be valid.

For the purposes of analysis, guilds were considered to have selected for a microhabitat variable if the distribution of used microhabitat was distinct from the available microhabitat for that variable. Comparison of use and available distributions was performed for each individual variable (univariate) and for all variables considered together (multivariate) to determine if any variable combinations were preferred. Univariate distribution differences were evaluated using two-sample Kolmogorov-Smirnov tests by each guild for all individual variables. Multivariate

distributions were tested with MRPP (Mielke et al.1981). This procedure is functionally analogous to MANOVA, but does not require the many assumptions about data structure (e.g., multivariate normality and homogeneity of variance). It is a procedure designed to detect concentration within a priori groups. The MRPP test statistic (Δ) is the average Euclidian distance between observations within two or more a priori groups. A smaller Δ indicates tighter clustering within groups. The procedure calculates all possible Δ 's for all possible combinations (i.e., the permutations) of assignments of the observations to the a priori groups. The output is in the form of a p-value which represents the probability of observing a Δ equal to or less than the observed value. In this analysis, MRPP was used to detect pairwise differences in multivariate distributions of all microhabitat variables between the available microhabitat and microhabitat used by each of the four guilds and each of the component species.

In addition to testing for univariate and multivariate differences between use and available distributions, I needed to identify the distinct ranges of each variable selected by each guild. This information is needed to evaluate the relative vulnerability of various portions of used and available microhabitat distributions to determine if species were selecting ranges of variables that were more or less stable than other available microhabitats (see Chapter 2). Patterns in microhabitat selection were investigated with univariate histograms of expected and observed frequencies for each guild and variable and with principal components analysis (PCA).

To construct histograms, I divided the range of variability into several classes for each variable and evaluated which of these ranges that each guild or species most strongly selected. I determined the expected use frequencies for each variable class by calculating the percentage of available observations in each class and multiplying the percentages by the sample size of each guild. I then compared the expected frequencies to the observed frequencies to determine which ranges of each variable that each guild preferred.

Categories for all substrate roughness variables were constructed according to a modified Wentworth scale (Gordon et al. 1993). Class widths were as follows: 0.05mm (corresponding to the silt category), 1mm (sand), 2-16mm (small gravel), 17-63mm (large gravel), 64-128mm (small cobble), 129-256mm (large cobble) and greater than 256mm (boulder). Class intervals for embeddedness variables were 10% increments. Depth was divided into 5 classes representing 20% increments of the overall available distribution. This created depth classes of 0-20, 21-31, 32-40, 41-51 and greater than 51 cm. Mean water column velocity and demersal velocity were

divided into 0.2m/s intervals from 0 to greater than 0.7m/s. The classes were ≤ 0.10 , 0.10-0.29, 0.30-0.49, 0.50-0.7 and greater than 0.7 m/s. Class intervals corresponding to the Wentworth classification were also used for shear stress histograms. However, because the shear stress measurement (in $\text{Nm}^{-1}\text{s}^{-2}$) is directly proportional to substrate diameter in cm, the class intervals were 0.05 (shear stress adequate to entrain particles in the silt class), 0.1 (sand), 0.2-1.6 (small gravel), 1.7-6.3 (large gravel), 6.4-12.8 (small cobble), 12.9-25.6 (large cobble) and greater than 25.6 $\text{Nm}^{-1}\text{s}^{-2}$ (boulder). Category selection for Re, Fr, and rRe did not correspond to the common categorizations for these variables (i.e., turbulent and non-turbulent ranges, critical and non-critical ranges). All available Re's were well into the turbulent range, Fr's were most frequently below critical flow, and rRe was always well into the rough range. Thus, class intervals for these variables were also 5 increments of 20% of available values, allowing observation of trends in use of differing levels of turbulence. Class intervals for relative depth and thickness of laminar sublayer also were in 20% increments of available values.

In addition to determining overall differences in distributions between use and available microhabitat, I wanted to assess how narrow the selected ranges of each microhabitat variable were for each guild. To do this I calculated the ratio of variance of the use observations for a given variable for a guild versus the overall variance of the available observations ($s^2_{\text{guild}}/s^2_{\text{available}}$) and performed F-tests of equality of variance to identify significant differences.

Principal components analysis was used to investigate patterns in multivariate microhabitat selection among the four guilds. The combined data set (available microhabitats and those used by each guild) was subjected to PCA of the correlation matrix. Principal components, or linear combinations of all microhabitat variables, that had eigenvalues of >1 were included for interpretation. Principal components were interpreted according to the correlation of each variable to the principal component. I considered any variable loading above 0.30 to be useful for interpretation of a principal component.

Standardized factor scores for all available and use grids were plotted on bivariate scatter plots of principle components. This resulted in a 'picture' of the available microhabitat conditions in the reach in multivariate space and the portion of that space selected by each guild or species. Patterns in location of guilds and species in multivariate space gave information on the combinations of variables that guilds or species selected. Because my data violated the

assumption of multivariate normality, results will not be used for statistical inference, but only to interpret patterns of microhabitat use.

Results

Location of spawning microhabitats

During spring and early summer 1997 and 1998, spawning microhabitats were identified at various sites for 15 Roanoke River species, including representatives from five reproductive guilds (Table 3). Nine species were observed in both 1997 and 1998, including riverweed darter (*Etheostoma podostemone*), fantail darter (*E. flabellare*), Roanoke darter (*Percina roanoka*), bluehead chub (*Nocomis leptcephalus*) and its nest associates. Black jumprock (*Scartomyzon cervinus*) was only observed in 1997 and johnny darter (*Etheostoma nigrum*), Roanoke logperch (*Percina rex*), torrent sucker (*Thoburnia rathoeca*), golden redhorse (*Moxostoma erythrurum*) and central stoneroller (*Campostoma anomalum*) were only observed in 1998.

Reproductive microhabitats were located for the broadcaster/nest associates white shiner (*Luxilus albeolus*), crescent shiner (*L. cerasinus*), rosefin shiner (*Lythrurus ardens*), rosyside dace (*Clinostomus funduloides*), and mountain redbelly dace (*Phoxinus oreas*). They were not considered individually but are summarized in the data for bluehead chub because they were observed spawning only on chub mounds.

Eighty-seven spawning sites were located for egg-clustering darters (58 riverweed darters, 24 fantail darters and 5 johnny darters; Table 1.3). Egg-clusterers spawned from early April to mid June in 1997 and from late April to late June in 1998. Fantail darters were the first of the three species to be observed both years, commencing spawning in early to mid April in water temperatures 15°C and above. Riverweed darters and johnny darters spawned in water temperatures between 17 and 24°C. Riverweed darters spawned from mid May to late June in both years. Johnny darter spawned in early June in 1998. Fifty bluehead chub mounds were observed during 1997 and 1998. Chubs were observed building mounds from mid to late May until mid July in both years and spawned in water temperatures from 19 to 25°C. Thirty-eight egg-burier spawning sites were located (23 Roanoke darters, two Roanoke logperch, three torrent suckers, nine golden redhorse and one black jumprock). There was more variation in spawning timing and water temperatures for egg-buriers. Roanoke logperch was the earliest spawner

observed in 1998, spawning on 15 April at a water temperature of 14°C. Torrent sucker was observed on 30 April 1998 at a water temperature of 15°C. The black jumprock spawned on 14 May 1997 at a water temperature of 12°C. Roanoke darters spawned from late April 1998 until mid May in water temperatures from 12 to 19°C. Golden redhorse spawning was observed from 29 May to 2 June 1998 in water temperatures of 20-21°C. Nine pit-building central stonerollers were observed in mid May 1998 at water temperatures between 17 and 20°C.

Table 1.3. Reproductive guilds and associated species for which spawning microhabitats were located, the sites at which their spawning microhabitats were located, number of spawning observations, and the year(s) in which they were observed. Broadcasters/nest associates are not considered individually. See Figure 1.1 for site locations.

Reproductive Guild	Species Representative(s)	1997			1998		
		NF1	SF1	SF2	NF1	SF1	SF3
Egg-clusterers	<i>Etheostoma podostemone</i>	8	4	13	13	20	
	<i>E. flabellare</i>	2	2	4	10	6	
	<i>E. nigrum</i>				2	3	
Egg-buriers	<i>Moxostoma erythrurum</i>				2	7	
	<i>Thoburnia rhothoeca</i>				3		
	<i>Scartomyzon cervinus</i>		1				
	<i>Percina roanoka</i>		1	7		15	
	<i>Percina rex</i>						2
Broadcasters/ Nest Associates	<i>Luxilus albeolus</i> , <i>L. cerasinus</i> , <i>Lythrurus ardens</i> , <i>Clinostomus funduloides</i> , <i>Phoxinus oreas</i>	2		14	11	23	
Mound-builders	<i>Nocomis leptocephalus</i>	2		14	11	23	
Pit-builders	<i>Campostoma anomalum</i>					9	

Spawning Microhabitat Characterization

Comparison of used and available microhabitats revealed patterns of microhabitat selection for all guilds (Figures 1.5-1.22). Most Kolmogorov-Smirnov distribution tests were statistically significant (Table 1.4), indicating that all four guilds selected a different distribution of most variables. In addition, most F-tests were significant for most guilds, indicating selection of a narrow range of the available microhabitat conditions (Table 1.5).

Reproductive microhabitats were located for three egg-clusterers: riverweed darter, fantail darter, and johnny darter. They exhibited consistent microhabitat preferences and these preferences were different from the available microhabitats. Kolmogorov-Smirnov tests were significant for all variables for egg-clusterers (Table 1.4). Microhabitat selected by egg-clusterers was also significantly different from the available in multivariate space (MRPP; $p < 0.05$).

Patterns of selection in histograms of used and available microhabitats indicated that egg-clusterer spawning microhabitats were most distinct from available microhabitats for substrate roughness variables. They exhibited patterns of use with significantly lower variability relative to available microhabitats for all three roughness variables (Table 1.5). Microhabitat use of all egg-clusterer species both individually and as a group was characterized by use of relatively large substrate particles (Tables 1.4, 1.6). They selected for areas with substrate roughness (k) in the cobble range (Figure 1.7a) and had a very low variance for this variable relative to the available (Table 1.5). In addition, Dcen was most often in the cobble range (Figure 1.6a). These particles were also most often disc- or blade-shaped (Figure 1.8), shapes with at least one flat side. All species selected nest rocks that were significantly larger than the average particle size in the grid (D50; Mann-Whitney test of equality of median; $p < 0.0001$), indicating further selection of large particles for egg deposition (Figures 1.9, 1.10).

Egg clusterers were also distinct in their preference of certain ranges of siltation variables. Like most species, they used areas dominated by particles that were slightly embedded. Embeddedness of the nest rock and average embeddedness in the grid were both below 20% on average (Tables 1.4, 1.6), though they were the highest of any of the four guilds. They showed a strong tendency to deposit eggs on substrate particles 0 or 10% embedded (Figure 1.12a), showing low variation in this variable (Emcen) relative to the available microhabitats (Table 1.5). Despite the significant Kolmogorov-Smirnov test, the distribution of

Table 1.4. Mean \pm standard deviation of spawning microhabitat variables for available microhabitats and spawning microhabitats of the four guilds and P-value ranges (in parentheses) for 2-sample Kolmogorov-Smirnov distribution tests comparing available microhabitats to the microhabitats used by each of the four guilds. Significant P-values (based on a large sample approximation) are underlined. Variable abbreviations follow Table 1.2.

Microhabitat Variable	Available	Egg-clusterers	Mound-builders	Egg-buriers	Pit-builders
N	146	54	34	27	9
D50 (mm)	52 \pm 86	49 \pm 30 (<u>0.02-0.05</u>)	19 \pm 3 (<u><0.01</u>)	31 \pm 18 (<u>0.02-0.05</u>)	39 \pm 5 (0.05-0.1)
Dcen (mm)	52 \pm 96	138 \pm 38 (<u><0.01</u>)	19 \pm 5 (<u><0.01</u>)	21 \pm 36 (<u>0.01-0.02</u>)	28 \pm 13 (0.1-0.2)
k (mm)	129 \pm 127	141 \pm 37 (<u><0.01</u>)	82 \pm 62 (<u>0.02-0.05</u>)	95 \pm 37 (<u>0.02-0.05</u>)	100 \pm 27 (>0.2)
Em (%)	12 \pm 8	15 \pm 6 (<u><0.01</u>)	6 \pm 5 (<u><0.01</u>)	10 \pm 5 (>0.2)	5 \pm 4 (<u>0.01-0.02</u>)
Emcen (%)	11 \pm 21	1 \pm 4 (<u><0.01</u>)	0 \pm 0 (<u><0.01</u>)	4 \pm 14 (<u><0.01</u>)	1 \pm 3 (<u><0.01</u>)
Silt (%)	23 \pm 19	15 \pm 12 (<u><0.01</u>)	25 \pm 14 (<u><0.01</u>)	15 \pm 8 (<u><0.01</u>)	1 \pm 0 (<u><0.01</u>)
D (cm)	37 \pm 18	37 \pm 8 (<u>0.02-0.05</u>)	28 \pm 13 (<u><0.01</u>)	32 \pm 16 (>0.2)	22 \pm 5 (<u><0.01</u>)
V (m/s)	0.41 \pm 0.26	0.45 \pm 0.17 (<u><0.01</u>)	0.21 \pm 0.06 (<u><0.01</u>)	0.56 \pm 0.22 (<u><0.01</u>)	0.56 \pm 0.16 (0.05-0.1)
Re ($\times 10^3$)	143 \pm 114	151 \pm 80 (<u>0.02-0.05</u>)	46 \pm 302 (<u><0.01</u>)	179 \pm 117 (0.1-0.2)	113 \pm 63 (>0.2)
Fr	0.22 \pm 0.17	0.23 \pm 0.09 (<u><0.01</u>)	0.14 \pm 0.07 (<u>0.02-0.05</u>)	0.30 \pm 0.12 (<u><0.01</u>)	0.30 \pm 0.09 (0.1-0.2)
Vbot (m/s)	0.20 \pm 0.17	0.25 \pm 0.04 (<u>0.01-0.02</u>)	0.15 \pm 0.07 (0.05-0.1)	0.26 \pm 0.10 (<u><0.01</u>)	0.25 \pm 0.14 (>0.2)
Shear ($\text{Nm}^{-1}\text{s}^{-2}$)	6.7 \pm 11	4.5 \pm 7 (<u>0.02-0.05</u>)	0.5 \pm 1 (<u><0.01</u>)	8.9 \pm 9 (0.05-0.1)	9.8 \pm 9 (>0.2)
rRe ($\times 10^2$)	88 \pm 90	81 \pm 54 (<u><0.01</u>)	13 \pm 13 (<u><0.01</u>)	80 \pm 60 (>0.2)	86 \pm 52 (>0.2)
Rel	48 \pm 136	3 \pm 0.87 (<u><0.01</u>)	5 \pm 5 (>0.2)	4 \pm 2 (0.1-0.2)	3 \pm 1 (0.1-0.2)
δ ($\text{m} \times 10^{-5}$)	4 \pm 7	3 \pm 3 (<u><0.01</u>)	12 \pm 1 (<u><0.01</u>)	3 \pm 2 (<u>0.02-0.05</u>)	2 \pm 1 (>0.2)

Table 1.5. Ratios of variance of use observations to variance of available observations (s_u^2/s_a^2) and p-values of F-tests of equality of variance (in parentheses) between use and available observations for all 15 habitat variables for egg-clusterers, mound-builders, egg-buriers, and pit-builders. Significant P-values are underlined. Variable abbreviations follow Table 1.2.

Habitat	Egg-clusterers	Mound-builders	Egg-buriers	Pit-builders
Variable				
D50	0.119 (<u><0.0001</u>)	0.001 (<u><0.0001</u>)	0.045 (<u><0.0001</u>)	0.004 (<u><0.0001</u>)
Dcen	0.154 (<u><0.0001</u>)	0.003 (<u><0.0001</u>)	0.153 (<u><0.0001</u>)	0.017 (<u><0.0001</u>)
K	0.087 (<u><0.0001</u>)	0.242 (<u><0.0001</u>)	0.087 (<u><0.0001</u>)	0.045 (<u><0.0001</u>)
Em	0.387 (<u><0.0001</u>)	0.265 (<u><0.0001</u>)	0.311 (<u>0.0005</u>)	0.143 (<u>0.003</u>)
Emcen	0.047 (<u><0.0001</u>)	0 (na)	0.462 (<u>0.01</u>)	0.026 (<u><0.0001</u>)
Silt	0.418 (<u>0.0003</u>)	0.793 (0.22)	0.185 (<u><0.0001</u>)	0.030 (<u><0.0001</u>)
D	0.216 (<u><0.0001</u>)	0.534 (<u>0.02</u>)	0.773 (0.22)	0.075 (<u>0.0003</u>)
V	0.444 (<u>0.0005</u>)	0.054 (<u><0.0001</u>)	0.723 (0.17)	0.386 (0.07)
Re	0.484 (<u>0.003</u>)	0.070 (<u><0.0001</u>)	1.043 (0.42)	0.300 (<u>0.04</u>)
Fr	0.315 (<u><0.0001</u>)	0.175 (<u><0.0001</u>)	0.498 (<u>0.02</u>)	0.260 (<u>0.02</u>)
Vbot	0.710 (0.08)	0.194 (<u><0.0001</u>)	0.348 (<u>0.001</u>)	0.656 (0.27)
Shear	0.368 (<u><0.0001</u>)	0.012 (<u><0.0001</u>)	0.671 (0.12)	0.576 (0.20)
Rre	0.354 (<u><0.0001</u>)	0.020 (<u><0.0001</u>)	0.438 (<u>0.008</u>)	0.329 (0.05)
Rel	4×10^{-5} (<u><0.0001</u>)	0.001 (<u><0.0001</u>)	2×10^{-4} (<u><0.0001</u>)	5×10^{-5} (<u><0.0001</u>)
δ	0.155 (<u><0.0001</u>)	2.584 (<u>0.0002</u>)	0.105 (<u><0.0001</u>)	0.040 (<u><0.0001</u>)

Table 1.6. Mean and standard deviation of 15 spawning microhabitat variables for 11 species from the upper Roanoke River in 1997 and 1998. Standard deviation is in parentheses below the mean. Species abbreviations are: RIV = riverweed darter, FAN = fantail darter, JON = johnny darter, ROA = Roanoke darter, LOG = Roanoke logperch, TOR = torrent sucker, GRE = golden redhorse, BLJ = black jumprock, BHC = bluehead chub, and CST = central stoneroller. Variable abbreviations follow Table 1.2.

Guild	Species	Year	n	D50 (mm)	Dcen (mm)	<i>k</i> (mm)	Em (%)	Emcen (%)	Silt (%)	D (cm)	V (m/s)	Vbot (m/s)	Shear (Nms ⁻¹ s ⁻²)	Re (×10 ³)	Fr	RRe (×10 ²)	Rel	δ (μm)
Egg-clusterers	RIV	97	25	64 (32)	155 (35)	164 (45)	21 (7)	2 (4)	30 (26)	35 (6)	0.41 (0.1)	0.17 (0.1)	28 (35)	130 (53)	0.21 (0.1)	76 (44)	2 (1)	4 (6)
		98	33	47 (31)	146 (42)	145 (43)	14 (7)	0 (0)	13 (9)	38 (8)	0.41 (0.1)	0.22 (0.1)	4 (4)	137 (68)	0.2 (0.1)	76 (38)	3 (1)	3 (1)
	FAN	97	8	48 (42)	129 (20)	161 (26)	16 (8)	0 (0)	14 (20)	29 (10)	0.41 (0.2)	0.18 (0.2)	28 (29)	117 (55)	0.24 (0.2)	79 (48)	2 (1)	3 (1)
		98	16	62 (24)	125 (22)	136 (28)	16 (5)	0 (0)	12 (10)	35 (9)	0.6 (0.2)	0.15 (0.2)	7 (11)	190 (93)	0.32 (0.1)	101 (76)	3 (1)	2 (0.7)
	JON	98	5	20 (9)	123 (37)	126 (28)	14 (6)	10 (13)	36 (21)	40 (11)	0.25 (0.1)	0.06 (0.1)	3 (2)	111 (64)	0.13 (0)	59 (47)	3 (1)	6 (0.8)
	Egg-buriers	ROA	97	8	55 (20)	6 (7)	161 (36)	22 (6)	0 (0)	6 (4)	29 (5)	0.5 (0.1)	0.11 (0.1)	80 (54)	133 (57)	0.28 (0.1)	133 (54)	2 (0.4)
98			15	41 (18)	32 (48)	117 (29)	13 (5)	8 (18)	12 (6)	45 (5)	0.67 (0.1)	0.3 (0.1)	11 (7)	268 (57)	0.29 (0.1)	117 (51)	4 (1)	1 (0.4)
LOG		98	2	66 (14)	11 (15)	129 (14)	12 (3)	0 (0)	5 (7)	39 (0.2)	0.87 (0.04)	0.32 (0.06)	32 (9)	312 (25)	0.38 (0.01)	23 (6)	3 (0.2)	6.5 (0.9)
TOR		98	3	27 (1)	12 (12)	51 (8)	3 (3)	0 (0)	7 (6)	21 (4)	0.8 (0.1)	0.31 (0.1)	22 (14)	163 (40)	0.56 (0.1)	74 (35)	4 (1)	0.9 (0.3)
GRE		98	9	15 (6)	7 (3)	72 (29)	7 (3)	0 (0)	22 (7)	13 (3)	0.3 (0.1)	0.18 (0.1)	1 (2)	36 (24)	0.22 (0.1)	20 (11)	3 (2)	5 (2)
BLJ		97	1	32 (-)	9 (-)	77 (-)	23 (-)	60 (-)	5 (-)	29 (-)	0.63 (-)	0.18 (-)	53 (-)	178 (-)	0.31 (-)	56 (-)	4 (-)	2 (-)
Mound-builders	BHC	97	16	19 (4)	16 (8)	98 (140)	6 (6)	0 (0)	75 (21)	36 (13)	0.17 (0.1)	0.11 (0)	2 (3)	53 (40)	0.09 (0)	12 (11)	8 (6)	81 (3)
		98	34	19 (3)	18 (5)	81 (62)	6 (5)	0 (0)	25 (17)	28 (13)	0.21 (0.1)	0.15 (0.1)	0.5 (1)	46 (30)	0.14 (0.1)	13 (13)	5 (5)	12 (1)
Pit-builders	CST	98	9	39 (5)	28 (13)	100 (27)	5 (4)	1 (3)	1 (3)	22 (5)	0.56 (0.2)	0.25 (0.1)	10 (9)	113 (63)	0.3 (0.1)	86 (52)	3 (1)	2 (1)

Figure 1.5. Frequency of expected and observed use of median grid particle size (D50) classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.

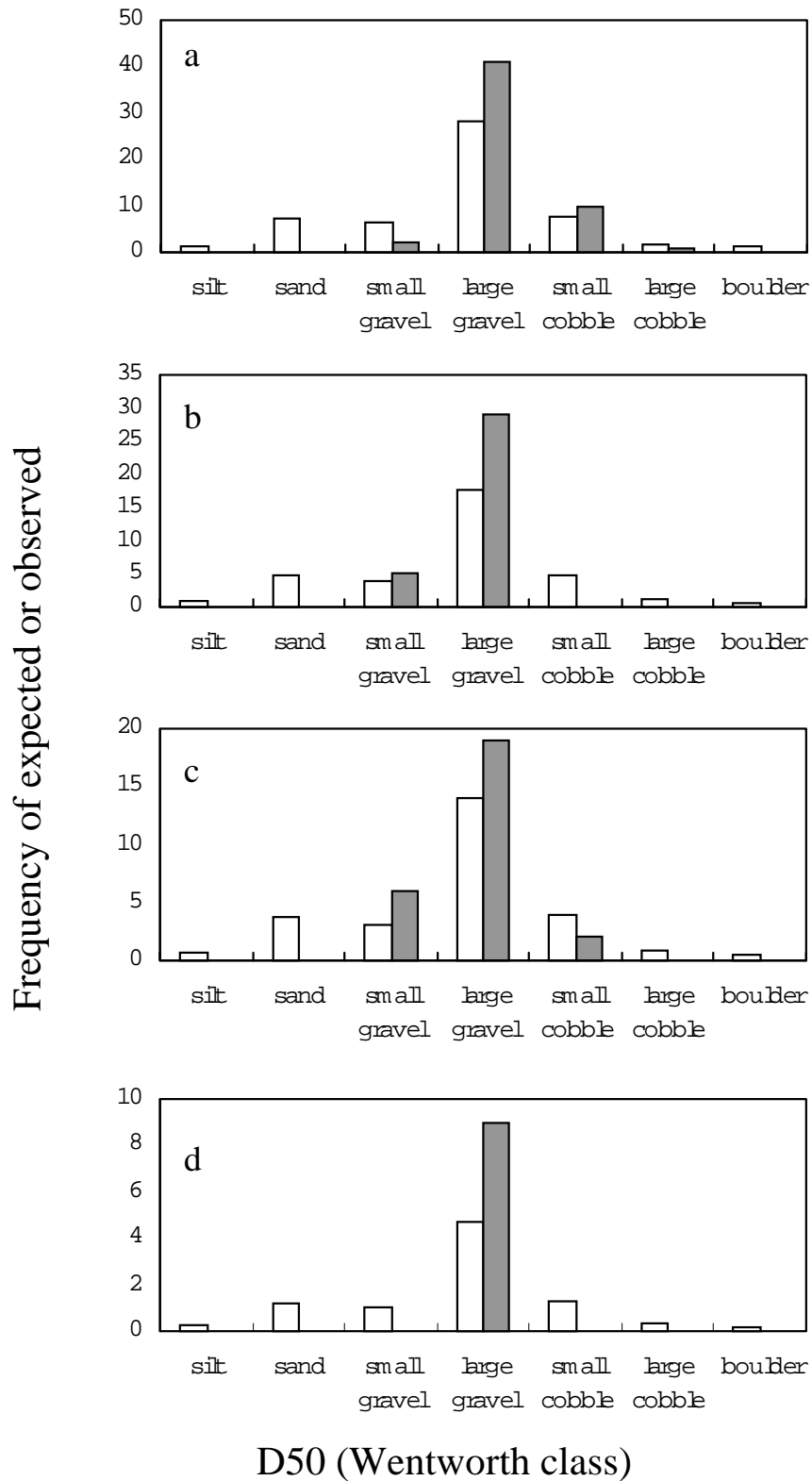


Figure 1.6. Frequency of expected and observed use of grid center particle (Dcen) size classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.

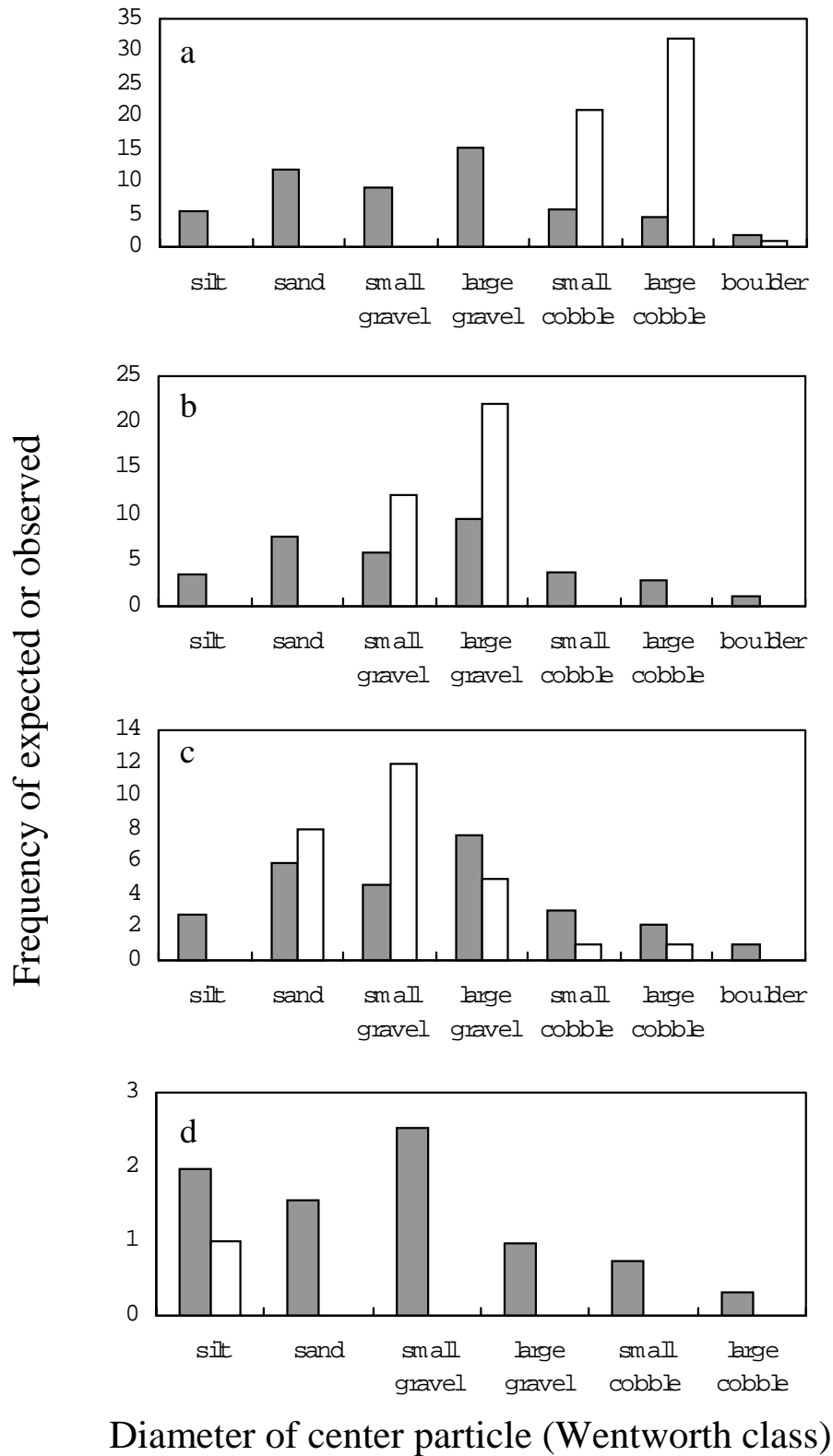
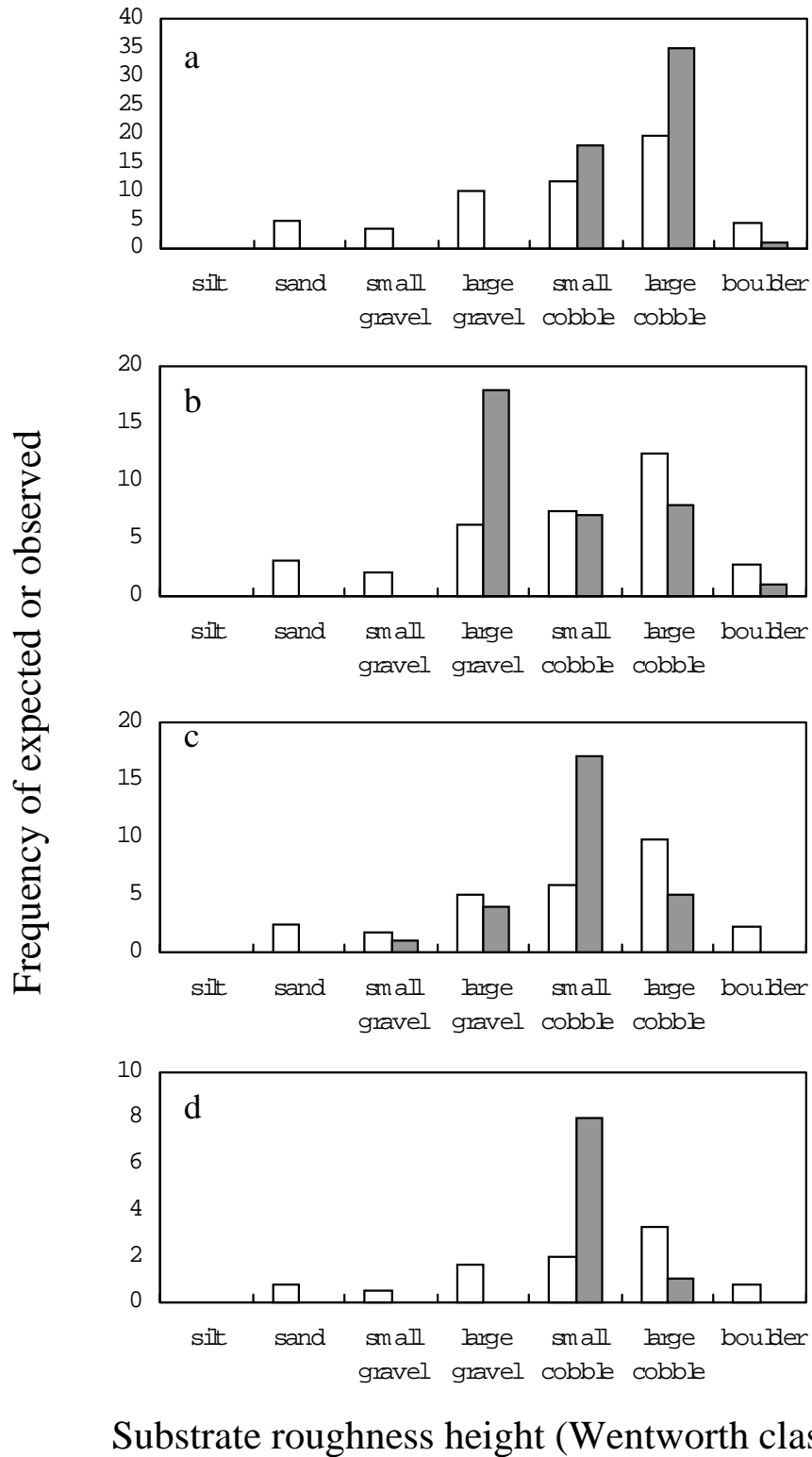


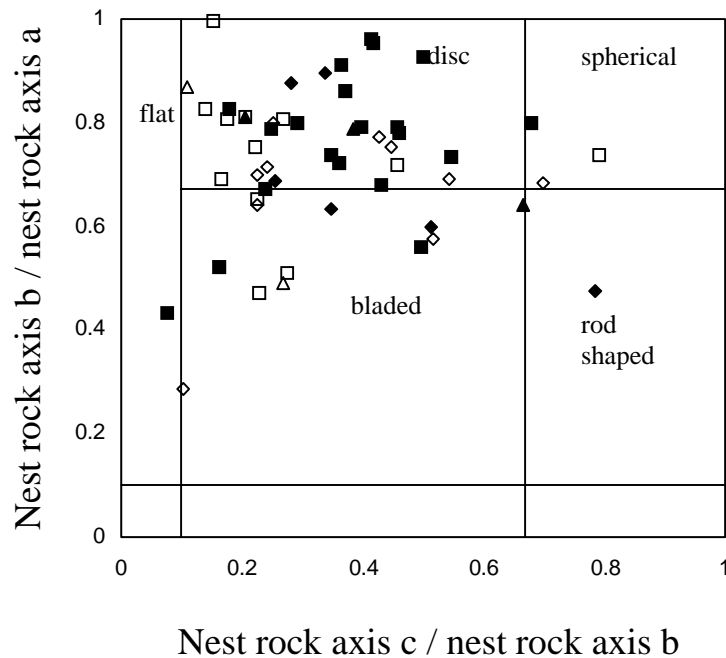
Figure 1.7. Frequency of expected and observed use of substrate roughness height (k) classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.



percent areal silt coverage by egg-clusterers was largely congruent with available microhabitats (Figure 1.13a). However, percent areal silt coverage was moderate for both johnny and riverweed darters (Table 1.6). These darters often spawned in edge habitats and slow runs that did experience slight aggradation.

Egg-clusterers also showed selection for distinctive mean flow hydraulic characteristics. They consistently utilized depths in the midrange of the available distribution, most commonly in the 30-50 cm range (Figure 1.14a). Fantail darters were different from the guild average, often using areas less than 25 cm deep (Table 1.6). As a guild, the egg-clusterers showed a preference for velocity between 0.30 and 0.70 m/s (Figure 1.15a). However, there were differences among species in velocity selection. Fantail darters used the highest velocities, johnny darters the lowest, and riverweed darters intermediate (Table 1.6). These differences in depth and velocity use were consistent with the observed usage by fantail darters of shallower, faster riffles, johnny

Figure 1.8. Shape of egg-clusterer nesting rocks at NF1 and SF1 in 1997 and 1998. Shape categories (modified from Gordon et al. 1993) are listed in plot regions that correspond to their diameter dimensions. Open and closed symbols are nest rocks at SF1 and NF1 (respectively). Diamonds are fantail darters, boxes are riverweed darters, and triangles are johnny darters.



darters in slower, shallower edge microhabitats near stream banks in runs and pools, and riverweed darters in riffles and runs with moderate depth and velocity. All three darter species used microhabitats with a very high Re and Fr in the middle range of the sub-critical zone (Tables 1.4, 1.6), selecting higher ranges of the available microhabitats of these variables (Figures 1.16a, 1.17a).

Near-bed hydraulic characteristics of egg-clusterer spawning microhabitats did not diverge from the available microhabitat for several variables. They used demersal velocities in proportion to their availability, though there was a suggestion of selection for mid-ranges of this variable (Figure 1.18a). Johnny darters used much lower demersal velocity than the other two darters (Table 1.6). The distribution of use of shear stress by egg-clusterers was very similar to available, with the strongest selection for areas of moderate shear stresses able to move up to large gravel substrate (Figure 1.19a). This trend was most evident for fantail darters that selected the highest shear stress of the three species (Table 1.6). Egg-clusterers used rRe well into the turbulent range (Tables 1.4, 1.6), indicating a strong preference for a hydraulically rough streambed. Egg-clusterers used moderate relative depths, with depths most often 2-4 k (Figure 1.21a). They selected for the midrange of available laminar sublayer thickness (δ ; Figure 1.22a), commonly in the $11-50 \times 10^{-5}$ m (0.11-0.5 mm) range. This range was much less than k , indicating very hydraulically rough streambed conditions. This was the observed pattern for all three species, though johnny darter had the highest average δ of the egg-clusterers (Table 1.6).

The only mound builder for which nests were located was the bluehead chub. It is possible that bull chubs (*Nocomis raneyi*) may have used some of the same mounds, but none were positively identified during nest activity and very few were ever collected at either site. No *N. raneyi* collected were large adults of spawning age.

The bluehead chub used distinct ranges of most variables, with nearly all K-S tests [with the exception of demersal velocity ($p=0.05-0.1$) and relative depth ($p = 0.5-1$); Table 1.4] and F-tests (Table 1.5) significant and with significant difference in multivariate microhabitat use (MRPP; $p = 0.000017$). Bluehead chub mounds were always dominated by small gravel,

Figure 1.9. Substrate roughness variables in microhabitats used by four reproductive guilds in the upper Roanoke River drainage in 1997 and 1998. Bars heights reflect means and error bars heights reflect one standard error.

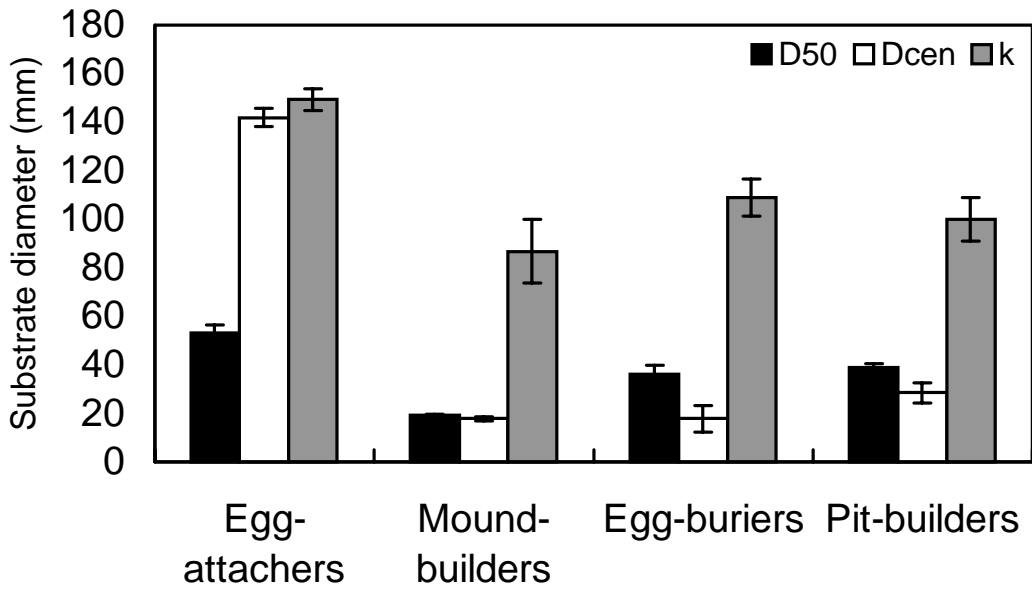
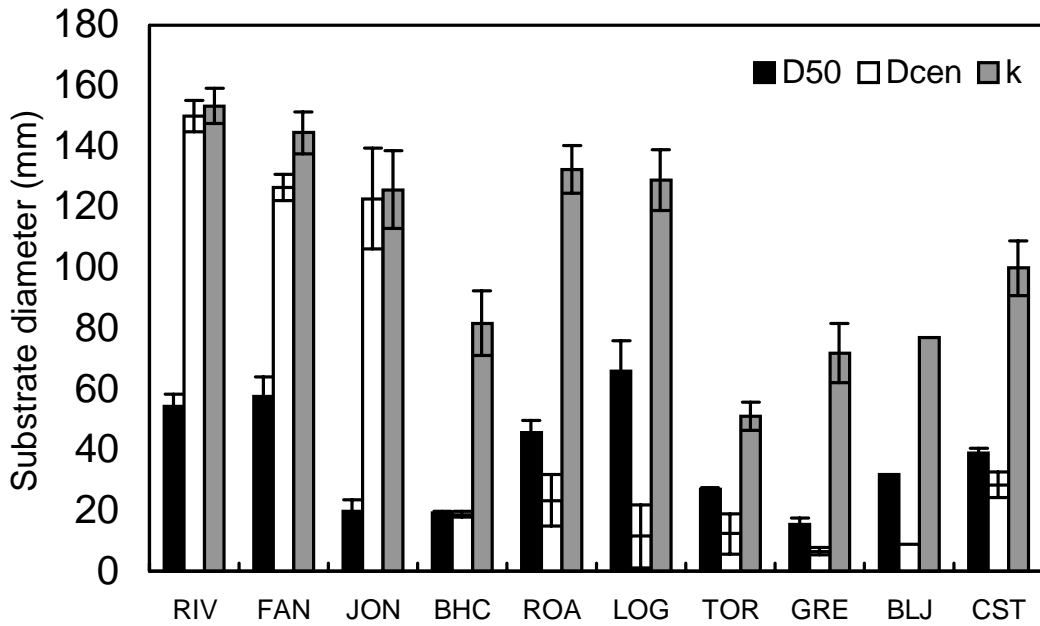


Figure 1.10. Substrate roughness variables in microhabitats used by ten species in the upper Roanoke River drainage in 1997 and 1998. Bars heights reflect means and error bar heights reflect one standard error. Species abbreviations are the same as in Table 1.6.



as indicated by their use of D50 and Dcen in the small gravel range (Tables 1.4, 1.6; Figures 1.5b, 1.6b) and low variance relative to the available habitats for this variable. Substrate roughness varied among mound locations; mounds were placed among substrate sizes ranging from sand and silt to large boulders, reflected in the moderate mean roughness value (Tables 1.4, 1.6; Figure 1.7b). Dcen was similar to D50 but lower than k (Figures 1.9, 1.10).

In terms of siltation of spawning microhabitats, the bluehead chub was atypical. They selected areas of a wide range of silt coverage, ranging from 10 to 100 percent (Figure 1.13b). However, the actual mounds always had very low embeddedness and siltation while active (Figures 1.11b, 1.12b). Mean embeddedness was driven higher by particles that were measured outside of the mound. Most mounds did experience a high level of silt deposition as incubation went on, though this was not quantified.

Bluehead chub used the most hydraulically benign mean flow of all four guilds. They used a wide range of depth, and were the only species to use very deep microhabitats (>40cm) (Figure 1.14b). Almost all mounds were in areas of low water column velocity (Tables 1.4, 1.6; Figure 1.15b), so they had a low variance for this variable relative to the available (Table 1.5). They also used areas with low and consistent Re (Figure 1.16b, Table 1.5) and low Fr (Figure 1.17b), indicating use of low turbulent, non-critical flow.

Near bed hydraulics were also relatively benign. Bluehead chub used the lowest demersal velocities and shear stress of any of the four guilds (Table 1.4; Figures 1.18b, 1.19b). They also strongly selected for low ranges of rRe (Figure 1.20b), using as near smooth near-bed flow as any of the four guilds. Bluehead chub also selected levels of relative depth and δ that were on the high end of the available distribution (Figure 1.21b, 1.22b) and that were the highest as any of the four guilds (Table 1.4).

As a guild, egg-buriers were more variable in microhabitat use than egg-clusterers or mound-builders. This resulted in fewer significant differences in univariate distribution and variance tests (Table 1.4, 1.5) and no difference between multivariate microhabitat used and that available (MRPP; $p = 0.3009$). MRPP of individual species indicated that though there was no difference between multivariate microhabitat use and

Figure 1.11. Frequency of expected and observed use of mean embeddedness classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.

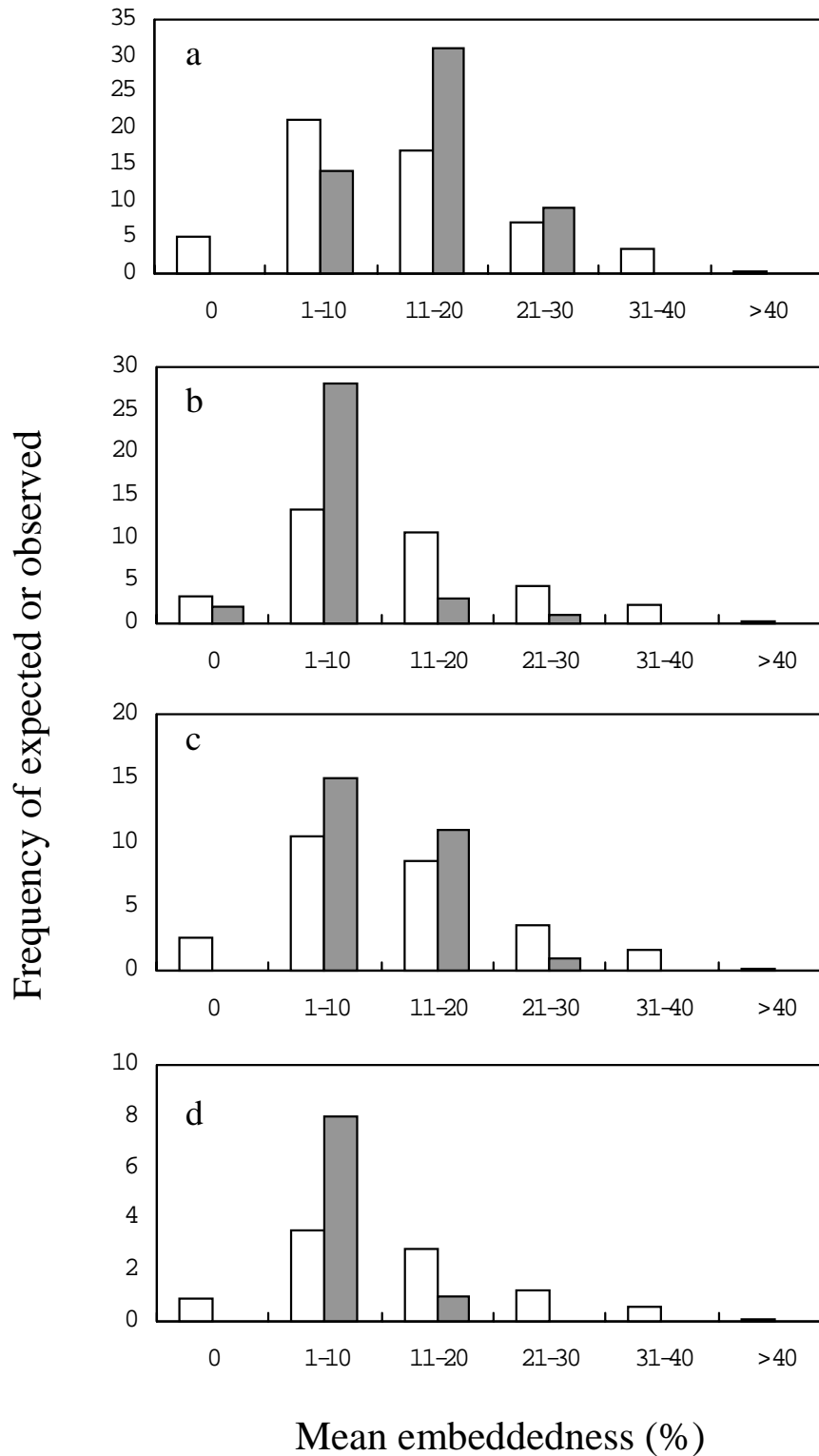


Figure 1.12. Frequency of expected and observed use of embeddedness of particle at grid center classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.

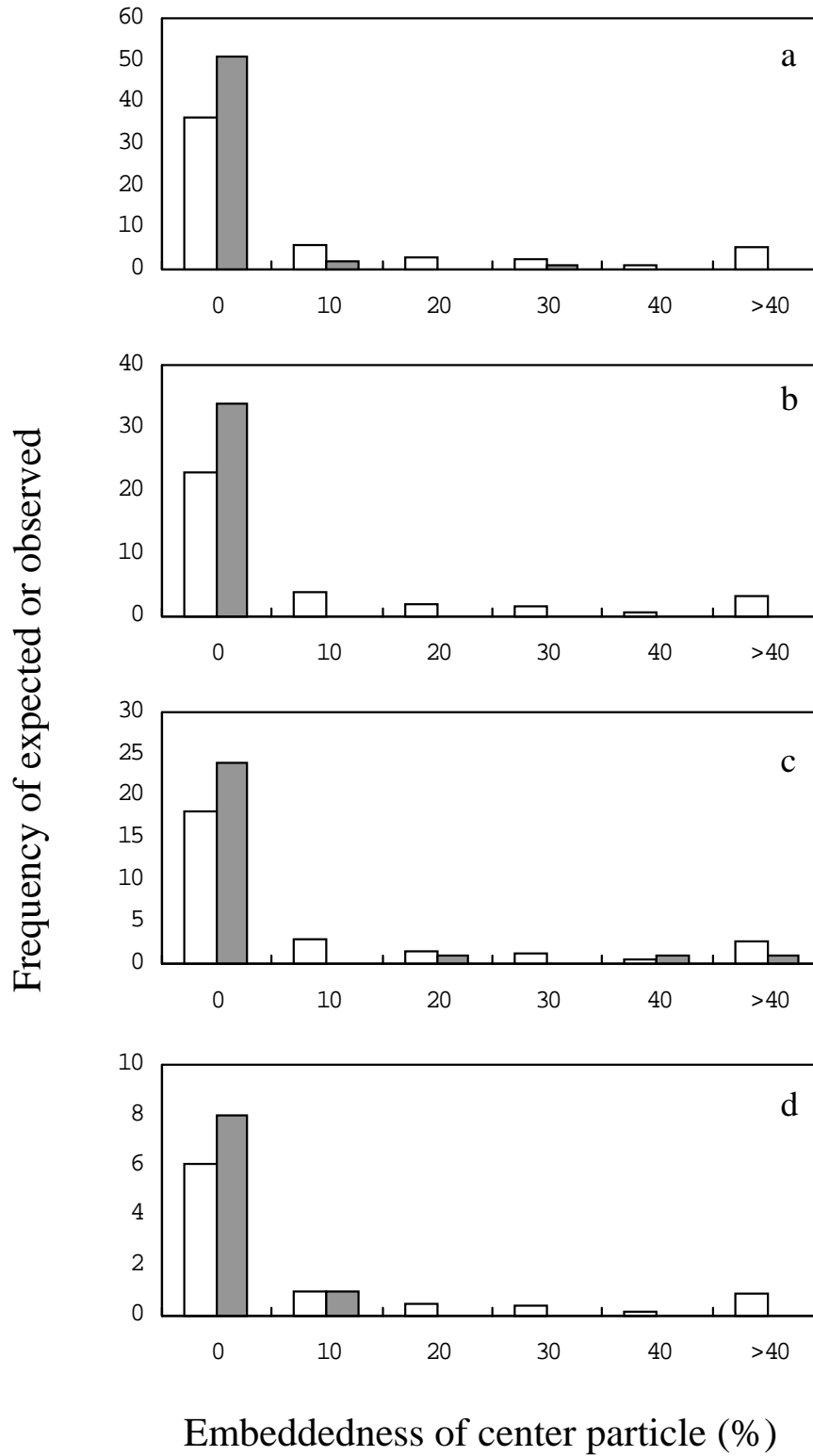
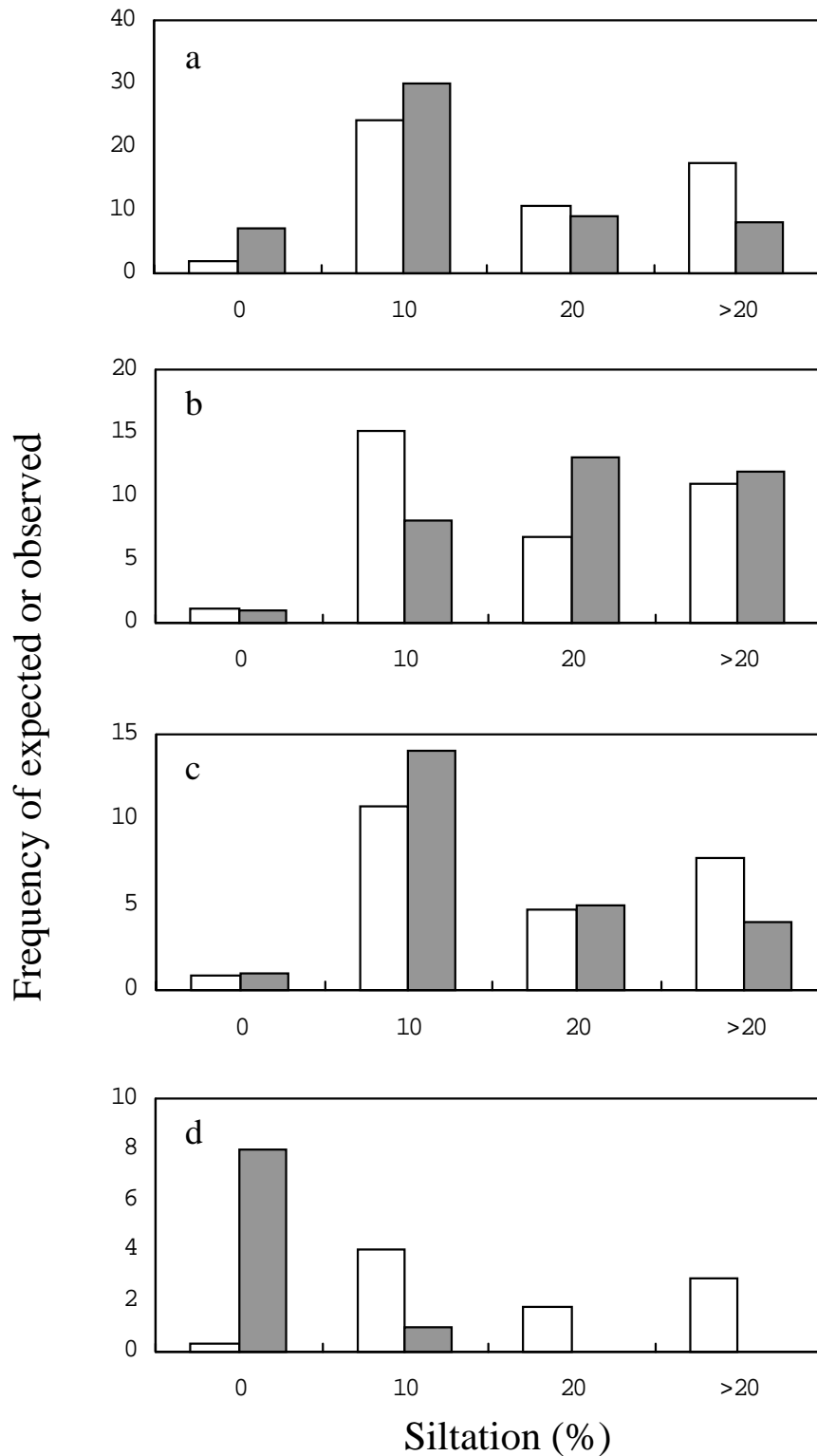


Figure 1.13. Frequency of expected and observed use of percent areal siltation coverage classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.



available microhabitats for the guild, there was a difference for two egg-burying species, Roanoke darter ($p = 0.0007$) and golden redhorse ($p = 0.0007$).

The greatest consistency among egg-buriers was their use of small substrate for egg depositional sites, as shown by the low diameter of rocks under which they spawned (D_{cen}) (Table 1.4, Figure 1.6c) and the low variance of this variable relative to the available (Table 1.5). As expected D_{cen} was lower than D_{50} for the guild and for all species (Figures 1.9, 1.10). This trend was strongest for the Roanoke darter, which spawned in large sand, interspersed among larger cobbles. The species varied in k of the selected spawning microhabitats, with golden redhorse, torrent sucker, and black jumprock using k in the small cobble range, while the Roanoke darter and Roanoke logperch used k in the large cobble range (Table 1.6). As a group, they showed a selection pattern similar to the available distribution, but showed a slight tendency toward k in the small cobble range (Figure 1.7c). The two *Percina* used the cover of the cobbles and buried their eggs in the intervening sand and small gravel. In contrast, the catostomids spawned in pool tails and gravel bar riffles that had no such cobble cover and were very exposed to flow.

Most egg-buriers used erosional areas or areas of sufficient velocity to eliminate most siltation. Thus, substrate in which eggs were buried had low percent areal silt coverage, with the exception of golden redhorse, which had an average coverage of 22% (Table 1.6). However, their agitation did appear to clear out much of the fine sediment in the egg deposition sites. Torrent sucker spawning was observed only at the more heavily sedimented NF1, but they deposited eggs in a riffle where fine sedimentation was low. The one observation for black jumprock suggested a similar strategy. Egg-buriers also used microhabitats with mean embeddedness and embeddedness of the center particle below 10% (Tables 1.4, 1.6).

Within-guild variation for egg-buriers was most evident in mean flow hydraulic variables. Golden redhorse used pool-tails and edge-habitats with low depth (<15 cm) and depth averaged velocity (0.30 m/s; Table 1.6). However, black jumprock and torrent sucker used areas with shallow water (<0.30 cm) and high velocity (>0.60 m/s; Table 1.6). Roanoke darter used similar microhabitats in 1997, but in 1998 the suitable substrate had been flushed from the spawning riffle by a large flood. In 1998 they

Figure 1.14. Frequency of expected and observed use of depth classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.

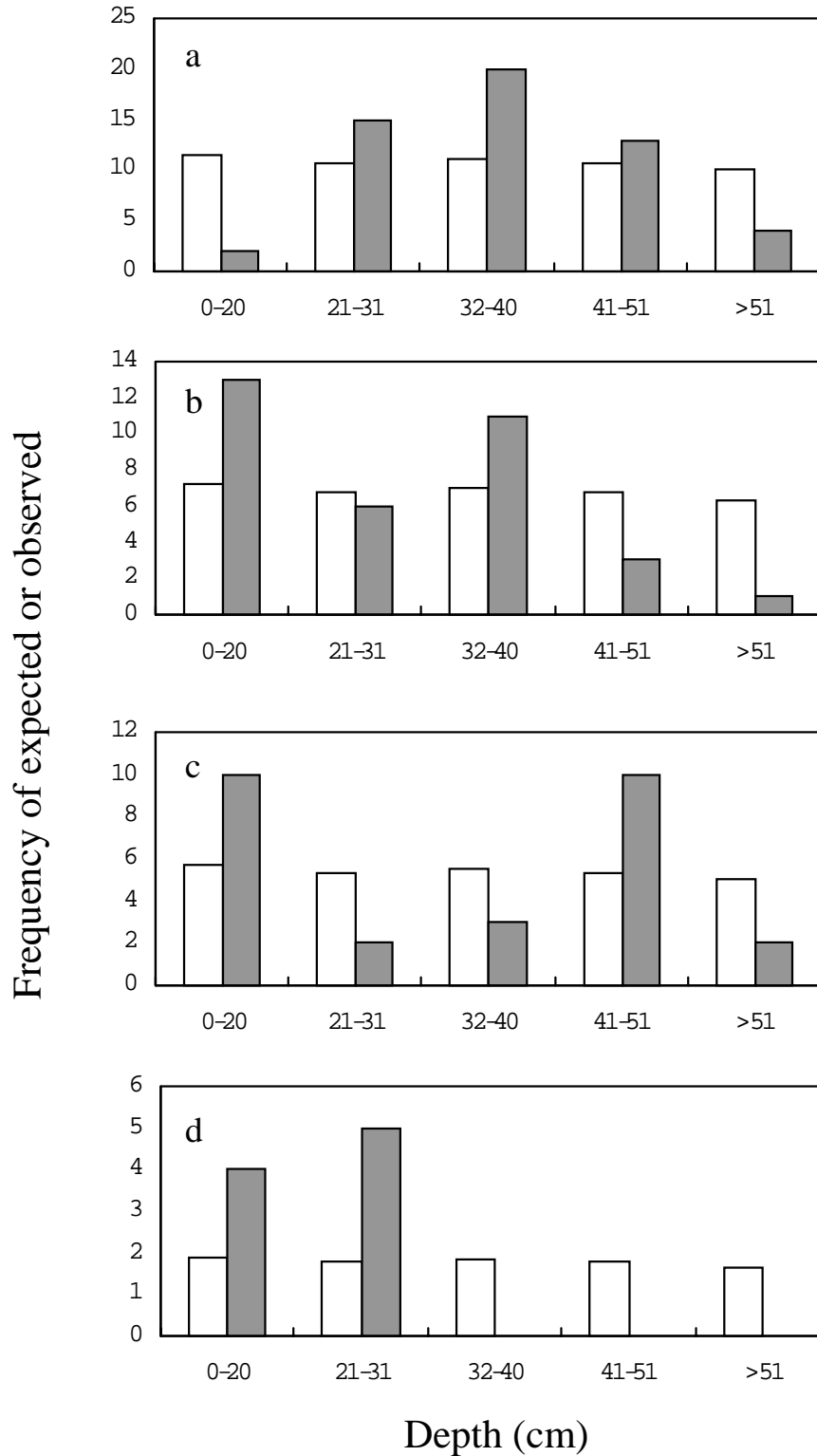


Figure 1.15. Frequency of expected and observed use of water column velocity classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.

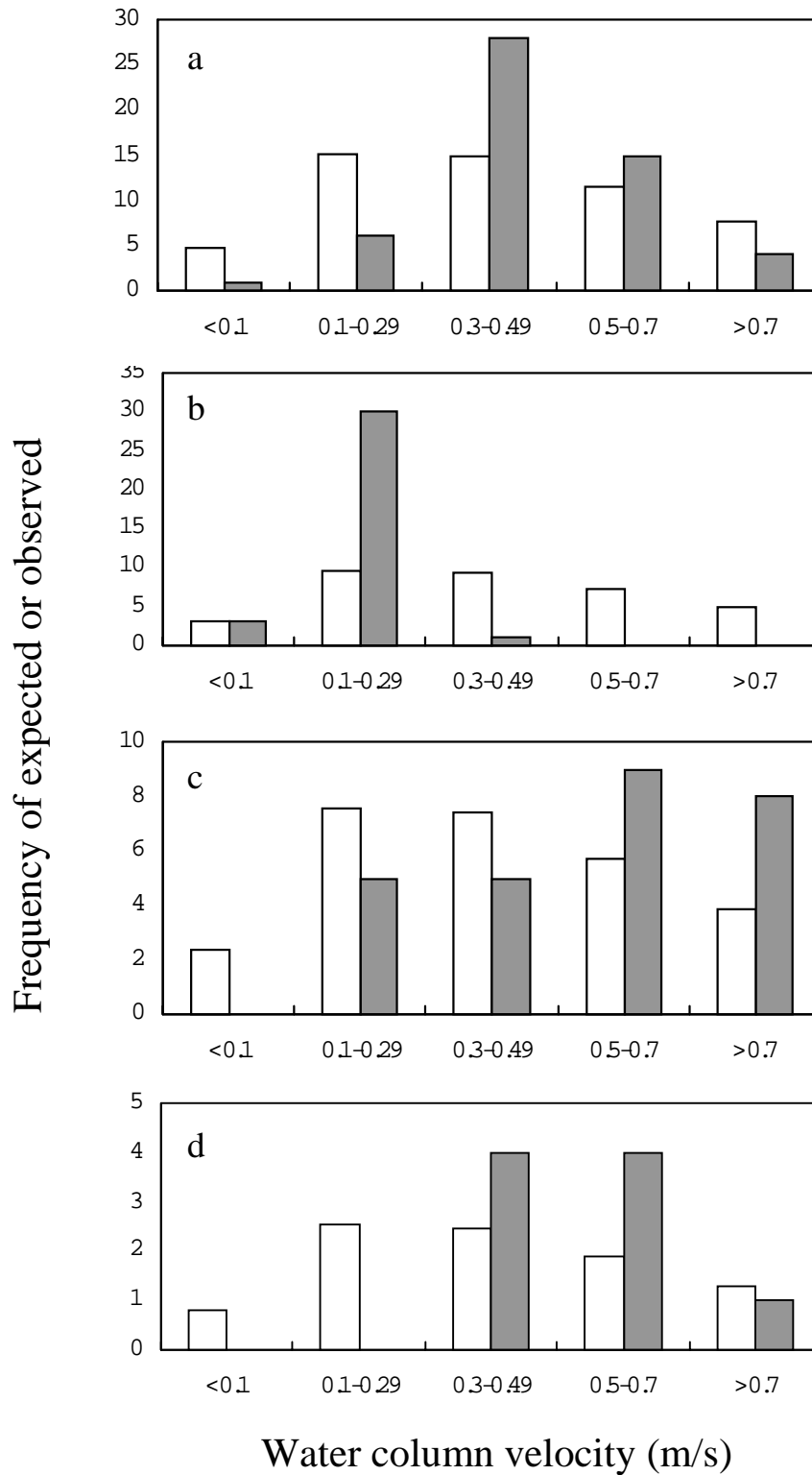


Figure 1.16. Frequency of expected and observed use of Reynolds number (Re) classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.

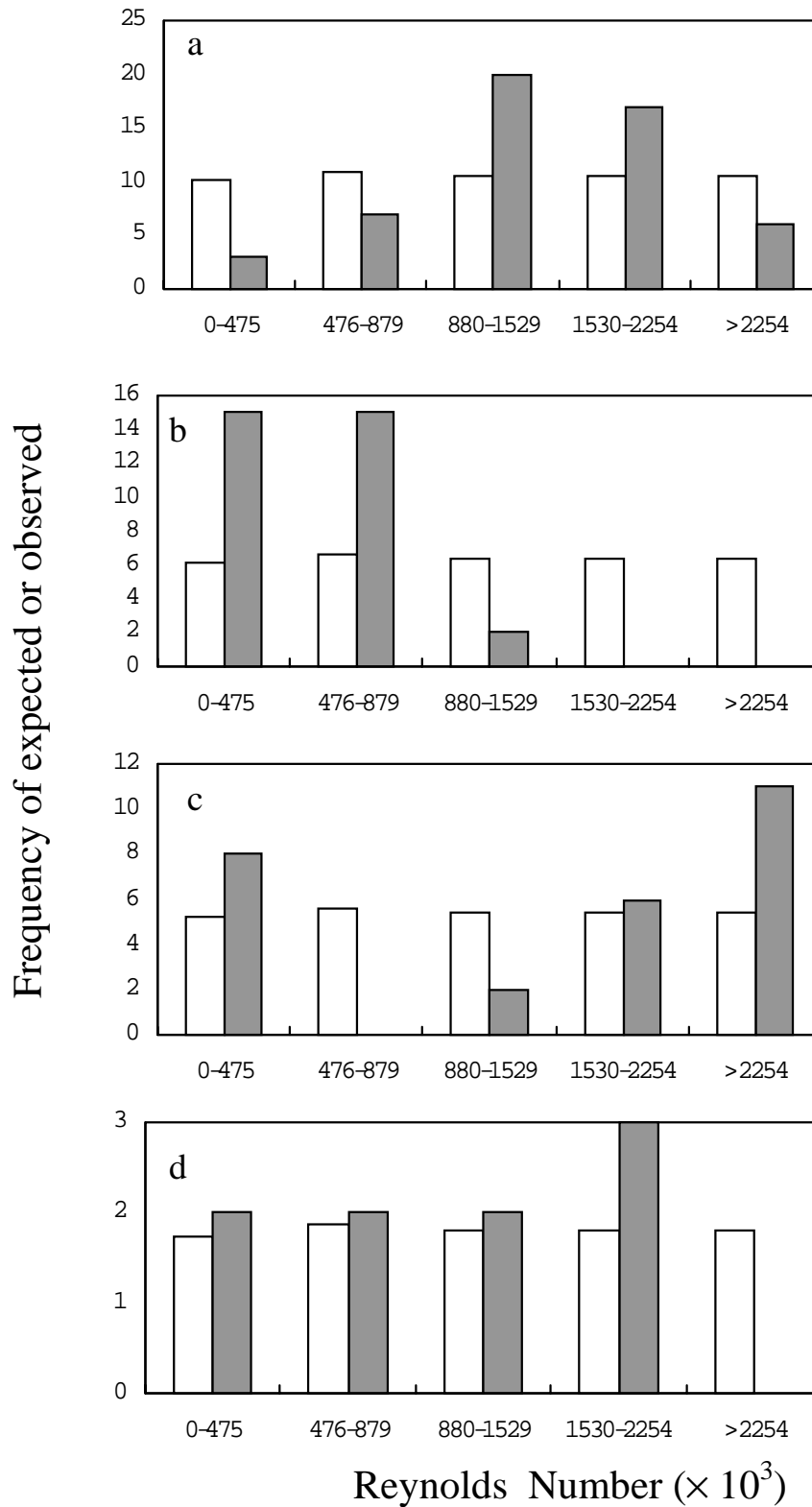
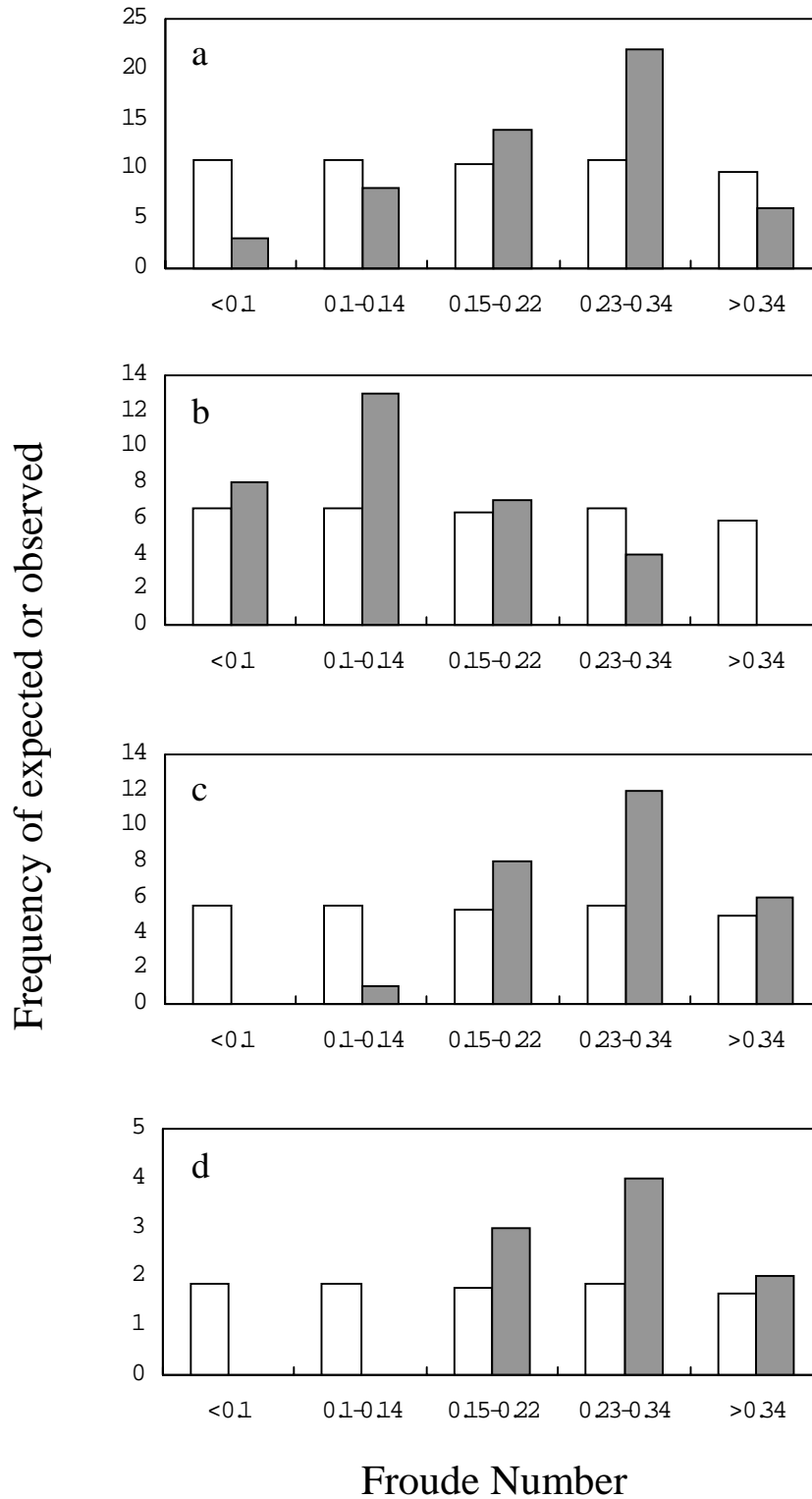


Figure 1.17. Frequency of expected and observed use of Froude Number (Fr) classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.



utilized the same microhabitats as the nest spawning darters, spawning in the sand among the cobbles on which nest spawners attached their eggs. Such areas had moderate depth and velocity (Table 1.6). These differences in preference are evident in the bimodal pattern of selection of depth, with peaks at low depths (0-20 cm) and moderate depths (31-50 cm) (Figure 1.14c). The interspecies variation in velocity use also resulted in a more uniform distribution than would be expected, though all species avoided very low velocities (<10 m/s) (Figure 1.15c). Egg-buriers were also the only guild to use high velocities (>0.70 m/s) in high proportions (Figure 1.15c). A bimodal pattern was evident in the pattern of use in Re, with peaks at very low levels (0-475) and high levels (>2254; Figure 1.16c). The low peak is due to the microhabitat use of golden redhorse (Table 1.6), which used relatively laminar flow in pools tails. The high peak is due to microhabitat use of the torrent sucker and the two *Percina* species (Table 1.6), which used very turbulent riffles. Egg-buriers used levels of Fr that were high relative to egg-clusterers but well below critical flow levels (Figure 1.17c).

Egg-buriers used the harshest near-bed hydraulic conditions of any guild, though they did display high levels of interspecific variation in all variables. Torrent sucker and the two *Percina* darters used the highest velocity at stream bottom observed (Table 1.6). However, the golden redhorse used low values (Table 1.6), causing a distribution that did not appear much different than the available (Figure 1.18c). This group used the highest shear stress of any of the four guilds (Figure 1.19c). Egg-buriers also used a wide range of rRe (Figure 1.20c) with golden redhorse using the lowest values and the two *Percina* darters the highest (Table 1.6). All species had low relative submergence and low δ relative to k (Table 1.6). This resulted in hydraulically rough flow near the streambed. In visual observations, the areas used by golden redhorse appeared to be more laminar than those used by the other species. The low shear stress ($1 \text{ Nm}^{-1}\text{s}^{-2}$) and rRe (23) and higher δ (0.5 mm) confirm these observations.

Central stoneroller was the only pit-building species whose reproductive microhabitats were located. I had a very low sample size ($N = 9$) of pit-builders despite the fact that central stoneroller was also seen as a nest associate of chub mounds. The only data reported for this species are from pits that they constructed themselves. Because of the low sample size, univariate and multivariate tests had little power, but

Figure 1.18. Frequency of expected and observed use of demersal velocity classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.

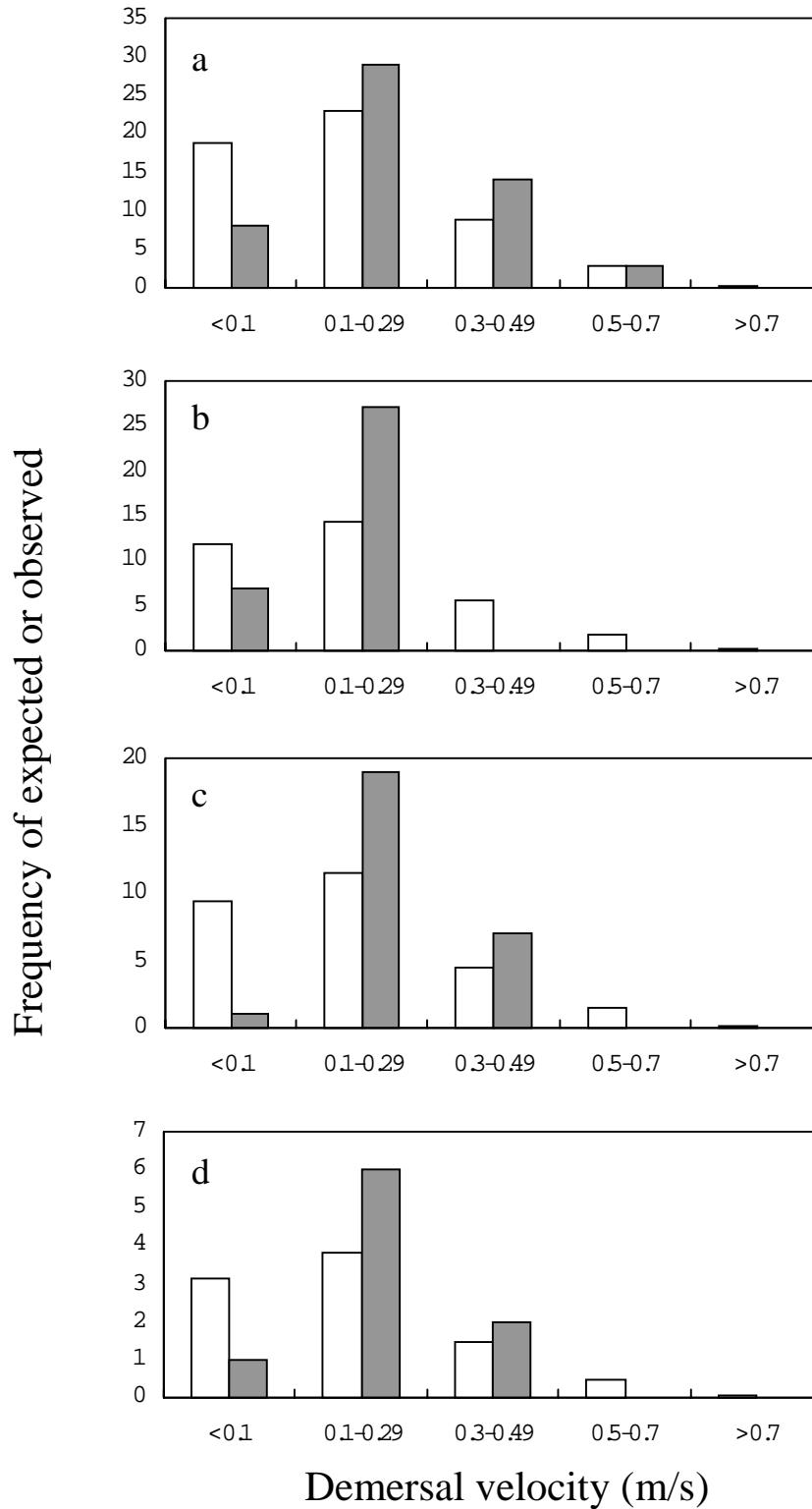


Figure 1.19. Frequency of expected and observed use of shear stress ($\text{Nm}^{-1}\text{s}^{-2}$) able to move each Wentworth substrate size class by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.

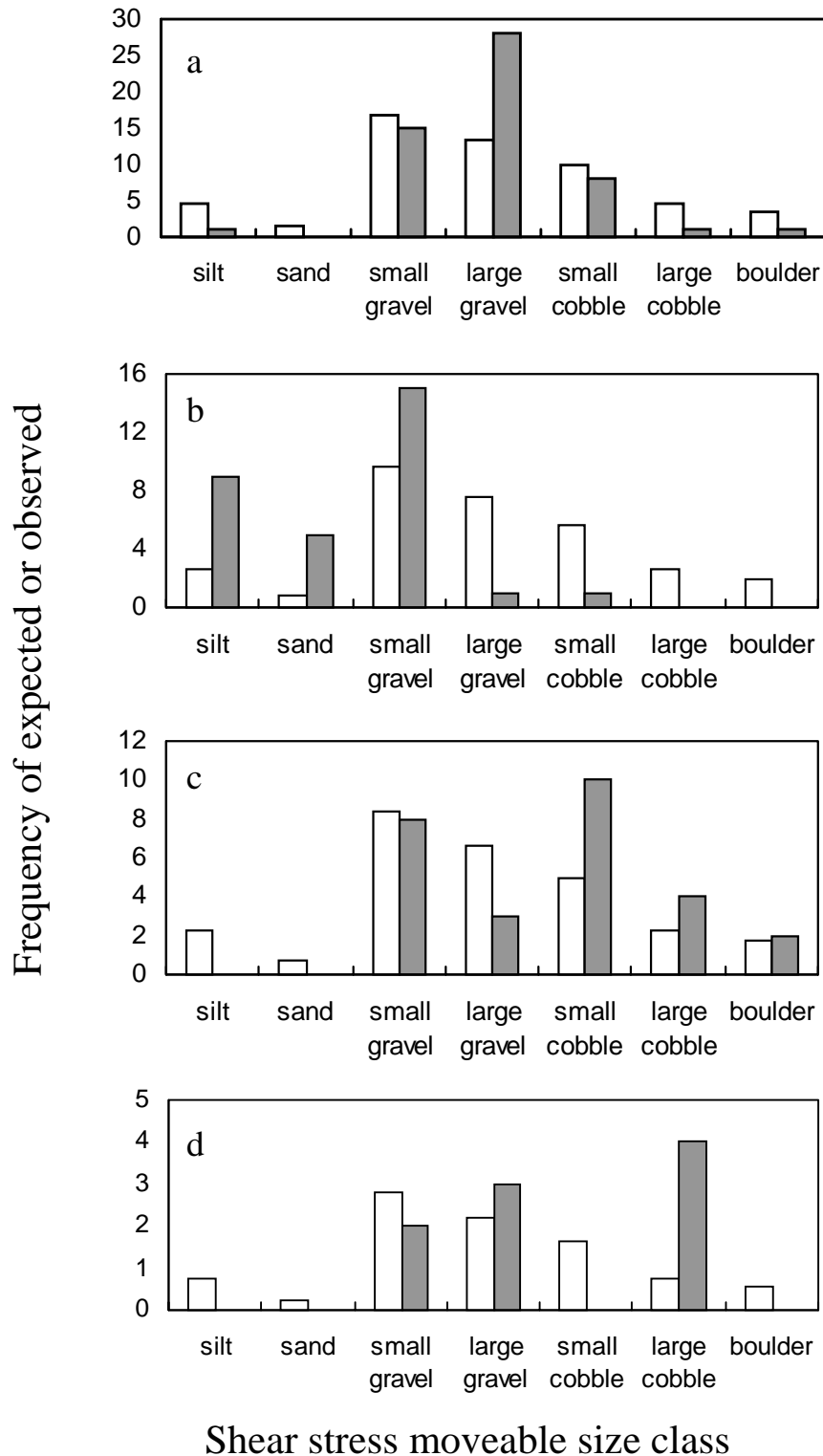


Figure 1.20. Frequency of expected and observed use of roughness Reynolds number classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.

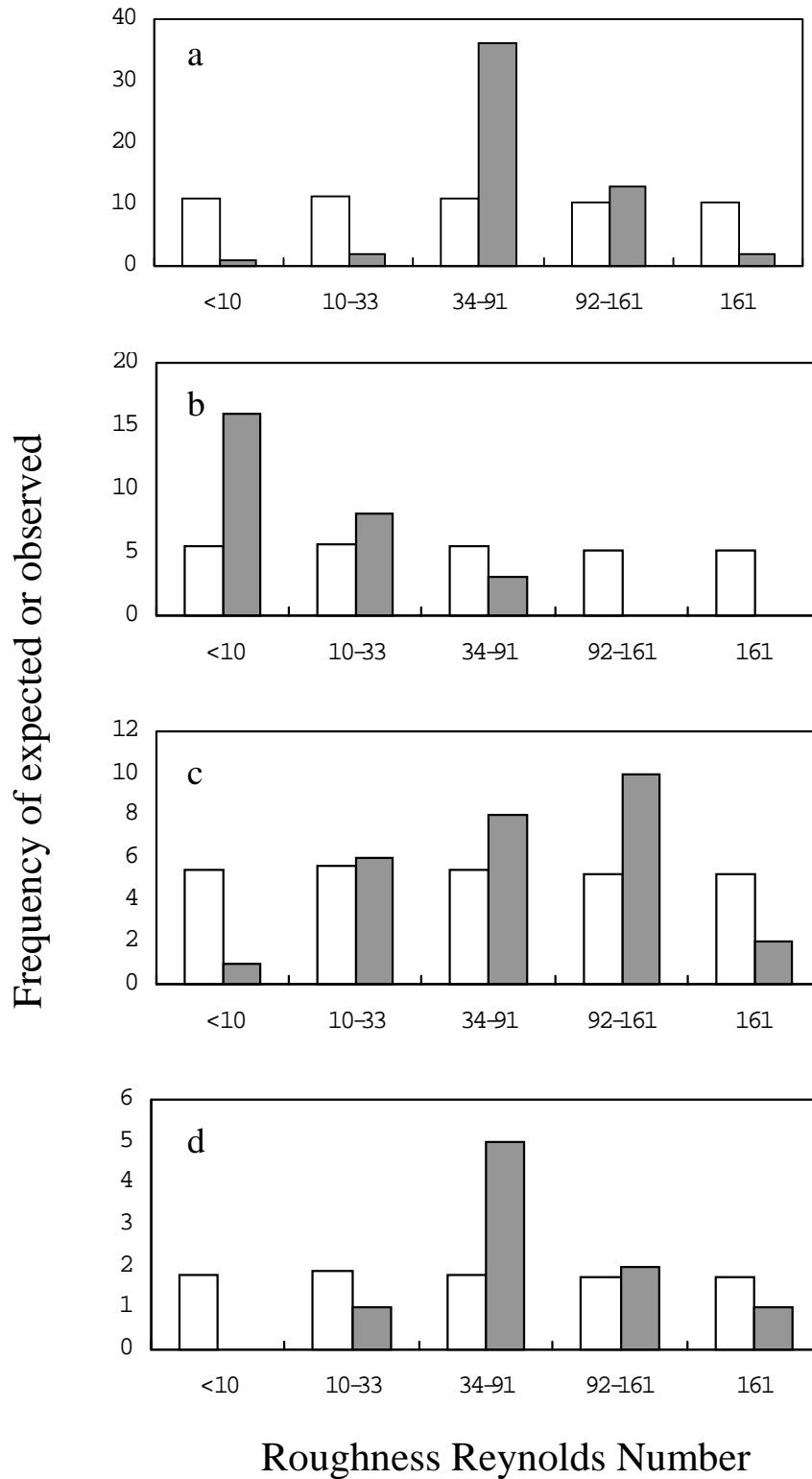


Figure 1.21. Frequency of expected and observed use of relative depth classes by a) egg-clusters, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.

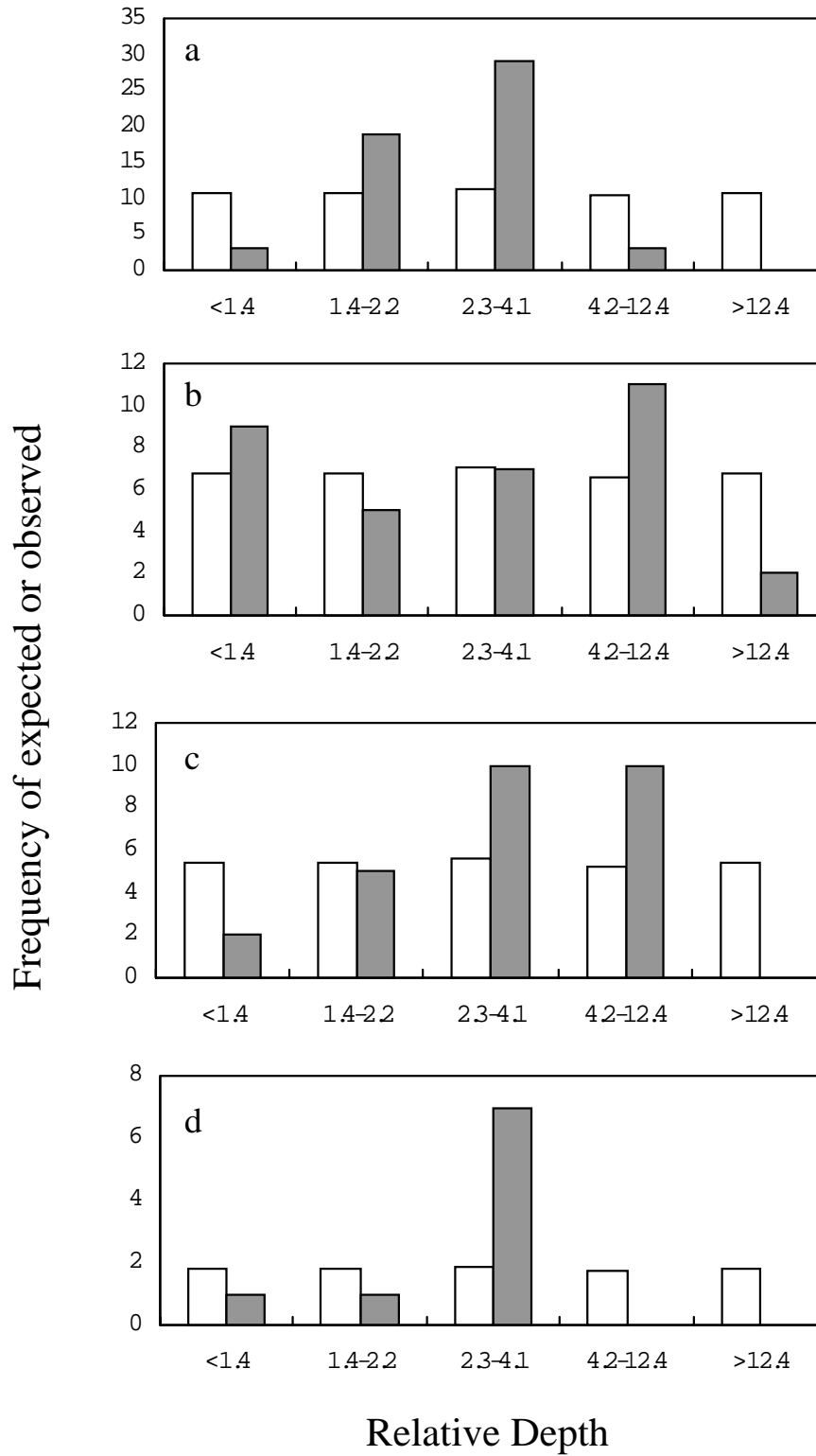
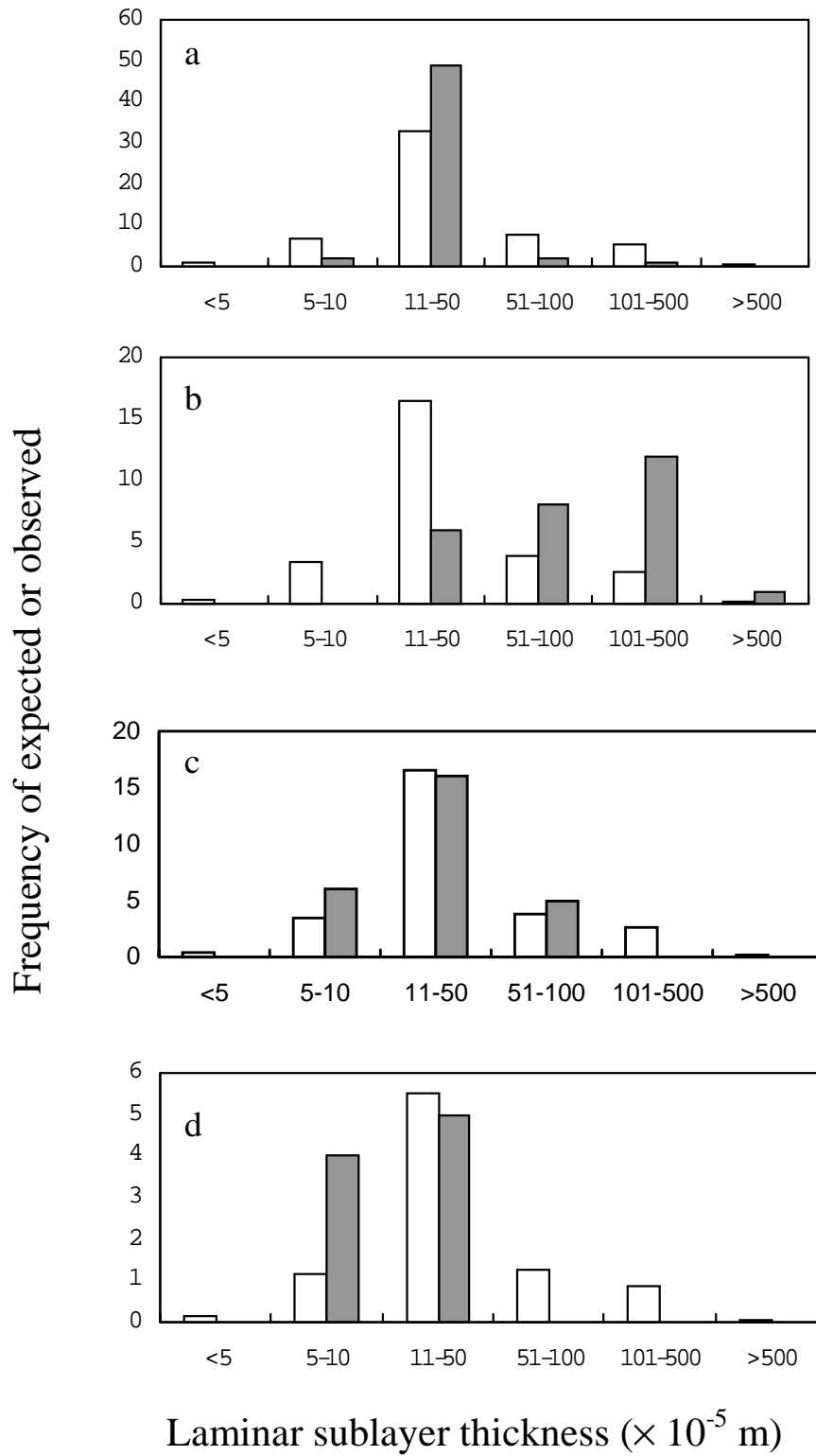


Figure 1.22. Frequency of expected and observed use of laminar sublayer thickness (δ) classes by a) egg-clusterers, b) mound-builders, c) egg-buriers, and d) pit-builders at NF1 and SF1 in 1998. Open bars are expected use frequencies and shaded bars are observed use frequencies.



variance was low relative to the available habitats for many variables (Table 1.5). Fewer K-S tests were significant for this guild (Table 1.4), and the multivariate microhabitat distribution was not significantly different than the available (MRPP; $p = 0.2564$).

Central stonerollers spawned in a pool tail/riffle head exchange area where there was a large amount of small gravel suitable for building pits. As a result, D50 and Dcen were in the gravel range (Tables 1.4, 1.6; Figures 1.5d, 1.6d). The flow in their microhabitat was dominated by frequent cobbles that determined flow patterns, as shown in the high k (Tables 1.4, 1.6; Figure 1.7d).

Central stonerollers were most characterized by their use of low siltation and embeddedness levels (Figures 1.11d-1.13d). The pool tail on which they spawned was mobile in high floods and had clean gravel deposited during a flood two months prior. This microhabitat also likely had good intergravel flow.

Central stonerollers used mean flow with characteristics similar to torrent sucker. Their reproductive microhabitats had relatively low depth and high depth-averaged velocity (Table 1.6; Figures 1.14d, 1.15d). Their spawning microhabitats were also turbulent and somewhat near critical flow, as shown by selection for high Re and Fr relative to the available (Figures 1.16d, 1.17d).

Patterns in selection of near-bed flow variables are difficult to identify for central stonerollers because of the low sample size. Their use of demersal velocities and shear stress are similar and indistinguishable from the available (Figures 1.18d, 1.19d). However, demersal velocity may have been depressed in the bottom of the spawning pit, where it was measured. Their use of relatively high rRe (Figure 1.20d), low relative depth (Figure 1.21d), and low δ (Figure 1.22d) indicate their use of microhabitats with turbulent and rough near-bed flow.

Though not included as a factor in the analysis, yearly differences in those species that were observed in both years were investigated. Microhabitat selection for most variables by all guilds was consistent across the two years of observations. However, there were some differences between the two years data, both within and across species. Low sample sizes in both years, though inevitable, resulted in poor sampling and may have resulted in some differences. However, some are likely not artifacts of sampling.

The Roanoke darter differed in its substrate preferences across years using lower D50 and k and higher Dcen in 1998. This was partly due to more extensive sampling in 1998. However, it is also partly due to alteration of the pool tail in which they were observed spawning in 1997. In 1998 they were only observed in downstream runs with lower k and D50. The higher Dcen was due to the presence of an outlier.

In other species, there were slight differences, some of them significant (2-sample Kolmogorov-Smirnov distribution tests and Mann-Whitney tests of equality of medians), but in no other case did preferences switch among substrate classes. Roanoke darter also used deeper microhabitats in 1998, a trend also explained by the alteration of the prior year's microhabitat. Roanoke darter, fantail darter, and bluehead chub exhibited a change in depth-averaged velocity and velocity at stream bottom across years (Table 1.6). This may be due to the fact that velocity varies highly and that they were likely selecting microhabitats by some other variable. Also, observations were made at different discharges across the two years. This would translate into differences in velocity as well. Most hydraulic variables remained very close within the species and interspecies differences were consistent across years.

Principal Components Analysis

Principal components analysis of use and availability grid data based on all 15 microhabitat variables was useful in elucidating patterns in microhabitat use in multivariate space and corresponded well with the univariate patterns. Three principal components accounted for approximately 60% of the total variation in all microhabitat observations. Principal component (PC) 1 was highly correlated with mean and near-bed hydraulic variables, PC2 was highly correlated substrate roughness and siltation variables, and PC3 was highly correlated with depth and Fr (Table 1.7).

Bivariate scatter-plots of standardized factor scores on the PC's showed wide scatter in microhabitat usage of egg-clusterers, but consistent selection for microhabitats in the mid to high range of the available microhabitats on the hydraulic axis, high levels of the substrate/embeddedness axis (Figure 1.23a) and mid range on the depth axis (Figure 1.24a). Mound-builders showed more consistent habitat selection and used areas low on the hydraulic axis, the mid-range of the substrate roughness axis (Figure 1.23a),

Table 1.7. Principal components for microhabitat use and available data from NF1 and NF2. Correlations of three principle components with all 16 microhabitat variables, eigenvalues and proportion of variability explained by the first three principal components are shown. Dashes indicate correlations less than 0.30. Variable abbreviations follow Table 1.2.

Variable or Statistic	PC1	PC2	PC3
D50	-	0.387345	-
Dcen	-	0.352396	-
<i>K</i>	-	0.433615	-
Em	-	0.314712	-
Emcen	-	-	-
Silt	-	-	-
D	-	-	0.660256
V	0.379553	-	-
Vbot	-	-	-0.30374
Shear	-	-	-
Re	0.333170	-	0.315034
Fr	-	-	-0.40309
Rre	0.396537	-	-
Rel	-	-	-
δ	-	-	-
Eigenvalue	4.72098	2.89748	1.69555
Proportion Variation	0.295	0.181	0.106

and the mid-range of the depth axis (Figure 24a). Egg-burier microhabitat selection also was widely scattered on both axes, but showed selection of areas low on the substrate axis, mid to high range of the hydraulic axis (Figure 23a) and the mid range of the depth

axis (Figure 24a). Pit-builder microhabitat use was the least variable and was enclosed in the range of egg-buriers and used areas low on the substrate range, the mid-high range of turbulence axis (Figure 23a) and the lower mid range of the depth axis (Figure 24a).

In accordance with field observations of two or more species of more than one guild spawning in the same areas (e.g., Roanoke darters and riverweed darters spawning in the same run), principal components plots revealed much overlap among the four guilds in multivariate space. On the plot of the hydraulic and substrate axes, egg-buriers overlapped with the ranges of all three other guilds, egg-clusterers overlapped slightly with mound-builders, and pit-builders were within the range of egg-buriers (Figure 23a). The plot of the depth and hydraulic axes revealed overlap of all guilds on the depth axis, with all guilds showing selection against the shallowest and deepest microhabitats (Figure 24a). Overlap of egg-buriers with mound-builders was due primarily to golden redhorse and bluehead chub overlap in use of low ranges of the hydraulic axis and mid ranges of the substrate roughness axis (Figure 23b) and low ranges of the depth axis (Figure 24b). Egg-burier overlap with egg-clusterers was due to overlap of Roanoke darter with fantail darter and riverweed darter in areas high on the velocity axis and high of the substrate roughness axis (Figure 23b) and high on the depth axis (Figure 24b). Pit-builders overlapped primarily with Roanoke darter high on the velocity axis and low on the substrate roughness (Figure 23b) and depth axes (Figure 24b). Egg-clusterers had a slight range of overlap with mound-builders due primarily to overlap with one johnny darter and near overlap of bluehead chub with riverweed darter in areas low on the velocity axis and high on the substrate roughness (Figure 23b) depth axes (Figure 24b).

Figure 1.23. Plot of standardized factor loadings of reproductive guild microhabitat use and available grid measurements on principal component 1 versus principal component 2 for a) guilds and b) species. Availability points are included in a) but excluded from b) to more clearly illustrate which species account for guild overlap. Variable abbreviations follow Table 1.2.

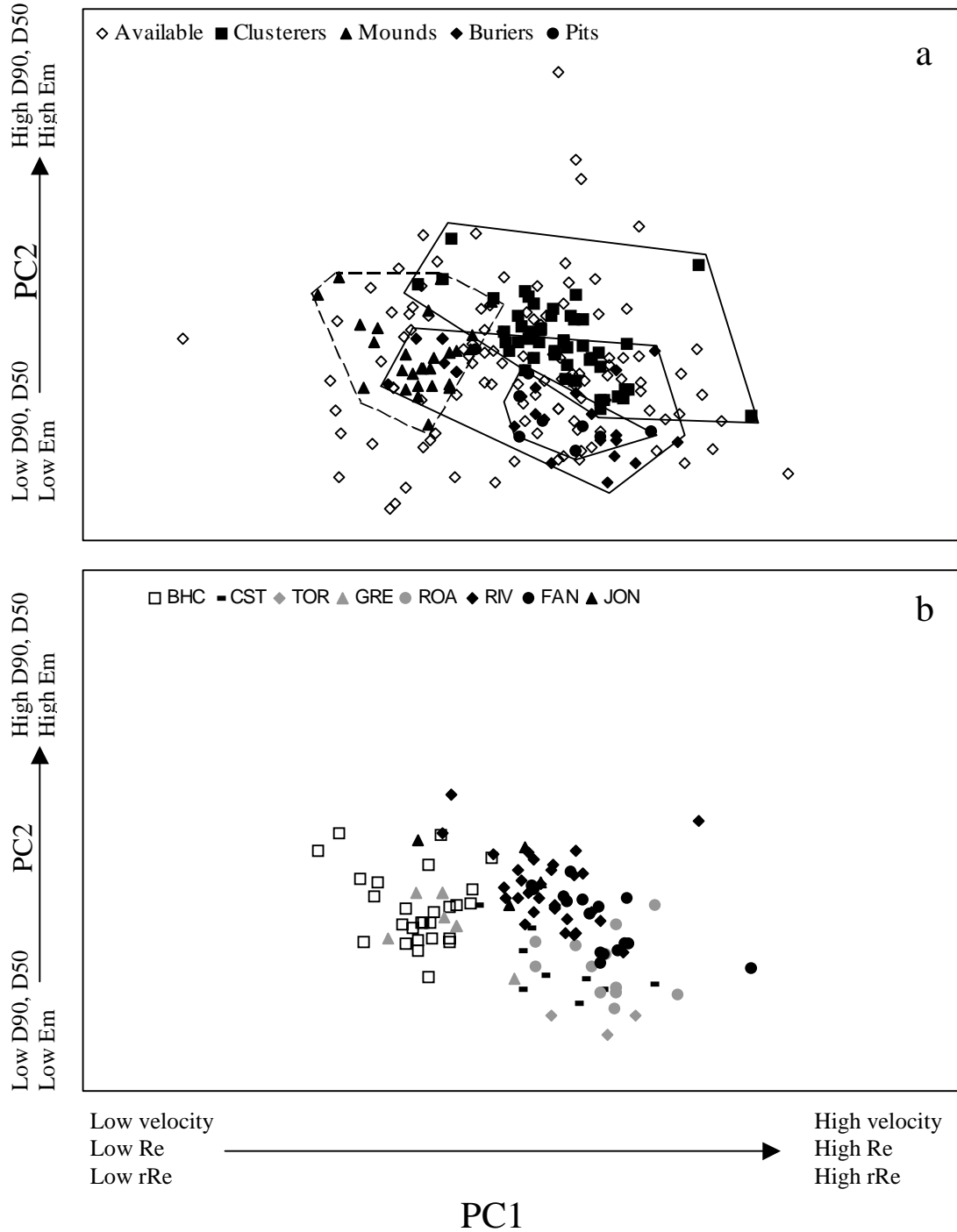
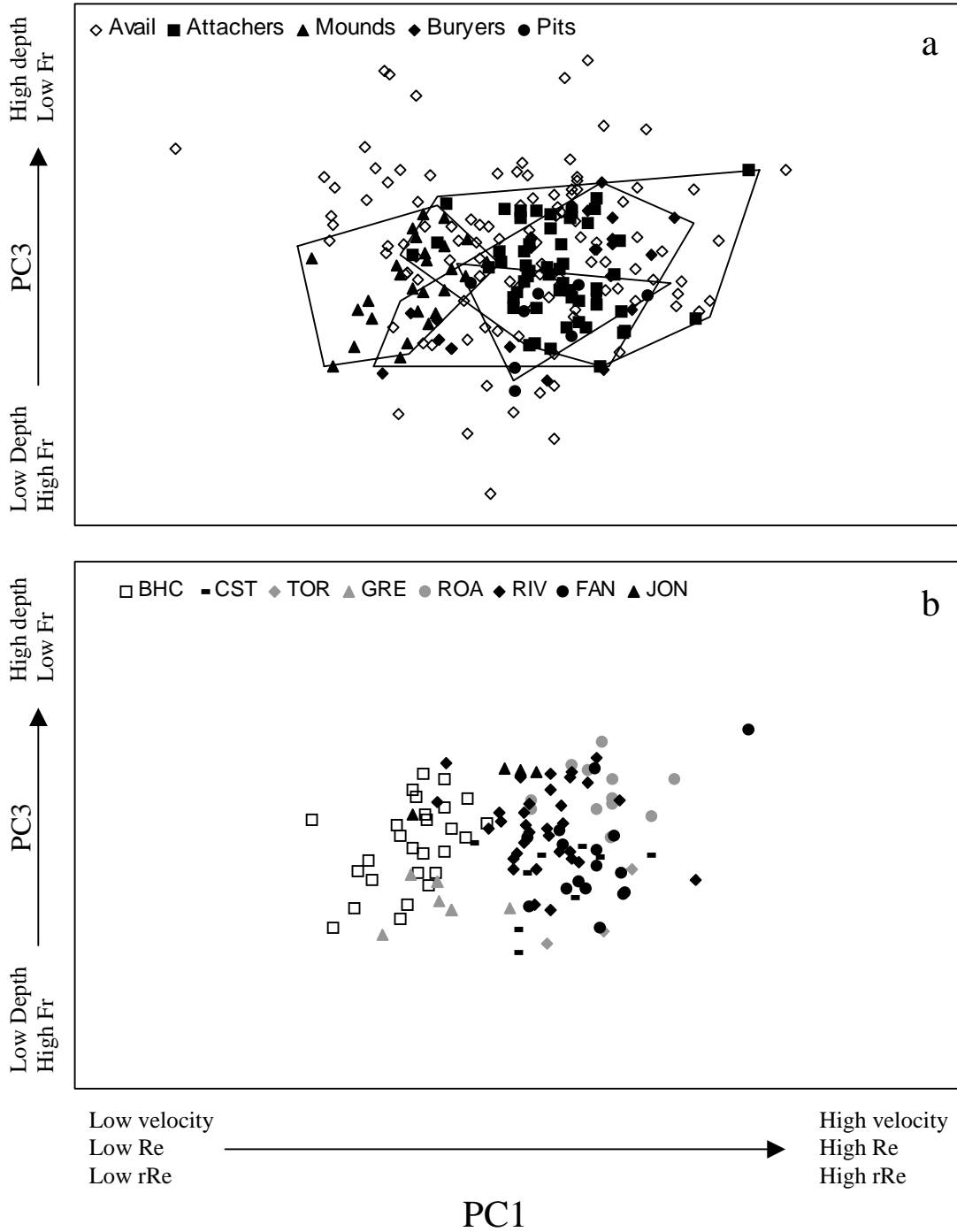


Figure 1.24. Plot of standardized factor loadings of reproductive guild microhabitat use and available grid measurements on principal component 1 versus principal component 3 for a) guilds and b) species. Availability points are included in a) but excluded from b) to more clearly illustrate which species account for guild overlap. Variable abbreviations follow Table 1.2.



Discussion

Spawning Microhabitat Characterization

Before this study, no detailed quantitative characterization of spawning microhabitat substrate and hydraulic conditions existed for most of the species and guilds studied. Thus, the first objective of this research was to locate and characterize the spawning microhabitats selected by these reproductive guilds and species in the Roanoke River, Virginia. To reach this objective, I attempted to identify consistent patterns in selection of a distinct portion of the available microhabitat conditions. I wanted to determine if fishes select spawning microhabitat based on a preferred range of a single variable (e.g., low velocity) or combination of two or more variables (e.g., shallow, fast water). I investigated the potential for selection for such distinct ranges of the available microhabitat conditions and tested the hypothesis that spawning microhabitat is uniformly distributed among all available microhabitats.

I reject the hypothesis of uniform distribution of spawning microhabitats among all available microhabitats for all guilds. This study demonstrates the selection of a distinct range of at least several individual variables for all guilds and of variable combinations for egg-clusterers and mound-builders. Furthermore, the study demonstrates the utility of a variety of hydraulic and substrate characteristics for predicting habitat use.

Egg-clusterers were distinct in their selection of many variables, spawning on cobble substrate particles in runs and riffles with moderate depth and velocity, high turbulence and hydraulically rough streambeds. However, as expected from literature descriptions of basic reproductive life history requirements (Page 1989, Jenkins and Burkhead 1993), egg-clusterers selected their spawning microhabitats primarily by the presence of large cobble with at least one flat side that was oriented to the bottom. This pattern was consistent across the three egg-clustering species, though the johnny darter used some edge microhabitats with a higher level of siltation and slightly smaller cobbles for nesting rocks.

Patterns of selection of other variables by egg-clusterers were artifacts of their selection of large particle sizes. Because of the correlation of presence of large substrate particles with high velocity and turbulence (Gordon et al.1992), selection of cobble

particles of primarily low embeddedness biased egg-clusterers toward the observed use of moderate to high velocity and shear stress. Lower velocity and shear stress would have resulted in deposition of transported sediment and smothering of the large rocks needed for spawning (Lisle 1989). Further, the larger roughness elements that egg-clusterers selected were flow obstructions that deflected flow and contributed to creation of the high turbulence (Carling 1992). The influence of the large roughness elements as flow obstructions also likely disrupted the smooth boundary layer, accounting for the low δ used by egg-clusterers. However, the high streambed hydraulic roughness that results from presence of large substrate particles creates patchy microhabitats with potential for low velocity along the stream bottom behind large substrate particles (Davis and Barmuta 1989). In addition, there is often a zone of laminar flow accompanying these dead zones behind roughness elements (Statzner et al. 1988).

The consistent and predictable selection of spawning habitats with cobbles by egg-clusterers shows clear association with reproductive behavior. Egg clusterers were expected to use large, flat rocks with a suitable crevice under them so they can invert and attach eggs (Page 1989). Further, their habit of attaching a large, single-layer cluster of eggs requires a large area of flat surface oriented downward.

Mound-builders were distinct in their usage of several microhabitat parameters, using small particle sizes, low velocity, low turbulence, high relative depth, and thick laminar sublayer. Chubs were most distinct in their selection of substrate particles in the small to large gravel range (D50 and Dcen 8-25 cm). Because mounds are constructed of particles picked up and placed by male chubs, maximum particle size of mound particles was limited to this range by size of the male chubs that likely cannot move any bigger particles.

Despite the clear selection for gravel substrate on mounds, it is not likely that chubs select mound-building sites based on substrate variables. My data are only from the mounds themselves and hence do not address the average and center particle sizes of the areas that chubs selected for mound building. However, measurements of k do quantify the roughness conditions of surrounding areas. These measurements indicated that they did not select a distinct range of roughness conditions; they utilized habitats ranging from stream edges with small gravel to deep pools with large boulders.

All measures of siltation were low for bluehead chub mounds. However, they likely create the area of low siltation themselves via mound building rather than selecting silt free areas. Visual observations suggest that areas in which they place their mounds usually had a higher level of siltation than the mound itself.

I hypothesize that the essential feature for selection of mound-building location was benign hydraulics, both mean and near-bed flows. Relative to other guilds and overall available conditions, they selected strongly for areas with low depth-averaged velocities (less than 0.30m/s), shear stress (very few grids $> 1.6 \text{ Nm}^{-1}\text{s}^{-2}$) and turbulence (Re less than 880, rRe less than 9100). In addition, mound-builders used the thickest laminar sublayers and among the highest relative depths of all guilds. The occurrence of some high relative depths resulted from their occasional use of deep microhabitats (> 50 cm) and their strong selection for roughness elements in the large gravel range. As a result, mound-builders used microhabitats that were as hydraulically smooth as any microhabitat used by any guild and that were among the most benign of any available microhabitats.

I hypothesize that bluehead chubs select mound-building habitats based upon the presence of benign hydraulics because it allows avoidance of mound destruction at low flows. Shear stress in mound-building microhabitats was low and was not sufficient to entrain the gravel mound particles in flow levels at which mounds were built (personal observations of stationary mound particles). Though mounds were often destroyed in higher flows (personal observation), bluehead chubs may have the ability to renest following these disturbances. There was some evidence of this in that I frequently observed mounds near stream edges that were abandoned as discharge dropped in early summer. It is likely that the builders of these mounds renested and built other mounds on which I observed spawning because mound-building adult bluehead chub males were not abundant in the study reach. Other nest-building species (e.g., smallmouth bass) have the ability to renest following destruction of nests in high flows (Lukas and Orth 1995). Further research should address this potential aspect of bluehead chub life history.

Another habitat factor that may be important in mound-building site selection that this study did not explicitly address was presence of cover from predators. Bluehead chubs (and nest associates) are brilliantly colored while spawning which may make them

vulnerable to predation. Further, they often build mounds in deep areas which may make them vulnerable to piscine predators and shallow areas which may make them vulnerable to avian predators (Power 1984). Thus, it is important that chubs build mounds in the immediate vicinity of escapement cover. Many mounds I observed were built near deep pools or tree roots that may have afforded such cover. Male mounds often retreated to these areas when approached by snorkelers.

Microhabitats used by bluehead chubs in this study were similar to those of other *Nocomis* chubs (Miller 1964, Lobb and Orth 1988, Vives 1990). Use of a narrow range of gravel substrates in low to moderate velocities agrees with observations for bigmouth chub (*Nocomis platyrhynchus*; Lobb and Orth 1988) and river chub (*N. miropogon*; Miller 1964). Other investigators have also observed occasional use of moderate to high depth at which mounds are built (Lobb and Orth 1988). These similarities suggest that microhabitat requirements of mound-building *Nocomis* chubs are consistent within the genus and may be transferable to other locations.

Though the life history and spawning behavior of the species classified as egg buriers are very similar, their microhabitat use differed among species. As a result, egg-buriers were bimodal in selection of many of their variables, not showing distinct ranges of selection as a group. Instead, they showed many species specific patterns that increased the overall guild variation and made characterization more difficult. However, they were consistent in their selection of spawning microhabitats based primarily on the presence of substrate particle sizes small enough in which to bury eggs. In accordance with previous spawning habitat descriptions (Page 1989, Jenkins and Burkhead 1993), most species only used areas where these smaller particles occurred in combination with low depth, high velocity, and high turbulence.

Despite their uniformity in selection of turbulent areas with substrate small enough for egg burying, egg-buriers were variable in their usage of specific size ranges of substrate roughness. This variation corresponded to two taxonomic divisions of the species classified as egg-buriers. The two *Percina* darters used riffles and runs with flow patterns dominated by larger cobble sized particles that provided velocity refugia for the spawning darters. However, suckers most often spawned in pool tails and shallow riffles with roughness elements in the gravel range that were more exposed to flow.

Spawning microhabitat of pit-building central stonerollers was well characterized, despite the low number of observations. They are very similar to egg-buriers in their reproductive behavior, differing only in their slight nest site preparation, and this showed in the similarity of their microhabitat variable use. Like egg-buriers, they used particle sizes small enough in which to bury their eggs. Roughness was very consistently low, in the large gravel to small cobble range. They were also frequently seen spawning on chub mounds that often share these characteristics though no measurements were made at such sites. This indicates that the presence of clean gravel sized substrate is the key factor to selection of their reproductive microhabitat. Also similar to egg-buriers, this group used very harsh hydraulics and turbulence conditions with high velocity and shear stress and high Re , low relative depth and laminar sublayer thickness indicating hydraulically rough streambed conditions.

I hypothesize that microhabitat selection by both egg-buriers and pit-builders represents use of microhabitats that have the small, clean substrate required for successful incubation of buried eggs and flow that is shallow, fast, and turbulent enough to allow predator avoidance. Predation is likely a strong selective force during the nuptial periods due to increased risk of predation of the brightly colored males. Presence of predators is known to restrict foraging behavior (Werner et al. 1993, Mittelbach 1984, Power 1984) and may also limit spawning habitat use. Golden redhorse is the one species that diverge in that they use slower microhabitats that may make them more vulnerable to predation. However, their large size precludes their predation by all stream dwelling predators.

The reproductive guild approach is a useful way to generalize habitat management issues when component species are similar in body size, taxonomy and reproductive life history. In this study, the egg-clusterer guild was an ecologically valid grouping that appears to be consistent in microhabitat use. Related species of the *Etheostoma* subgenera *Catonotus* and *Boleosoma* are very similar to these species in their life history and recorded spawning microhabitat preferences (Page 1989). Thus, characterizations and predictions made for egg-clusterers would be most useful across regions. Further, these spawning microhabitats were also very similar to habitat observations by other authors outside of the reproductive period (e.g., Matthews et al. 1982, Schlosser and Toth 1984, Hlohowskyj and Wissing 1986, Stauffer et al. 1996)

suggesting that these species remain in the same habitats during and after the reproductive period. This suggests that habitat suitability criteria developed for normal habitat use may be applied to the reproductive habitats as well. Such habitat suitability criteria and predictive models developed for other darters have transferred well across streams (Freeman et al. 1997, Leftwich et al. 1997).

In contrast to the egg-clusterers, the egg-burier species in the Roanoke River were not uniform with respect to their microhabitat selection and may not have been a valid grouping. Differences in microhabitat use correlated with taxonomic differences with catostomids using different microhabitats than percids. This agrees with the work of other researchers (e.g., Angermeier and Winston 1999) who have found that many life history traits diverge at the family level. Thus, taxonomic relations should be considered when developing guild assignments for the purpose of habitat prediction.

Chapter 2. Stability of spawning microhabitats

Objective

The second objective of this research was to evaluate the stability of the microhabitats selected by various guilds. I assessed stability of spawning microhabitats by comparing bed movement rates in areas used to the available microhabitats and by developing models to predict movement of surface substrate particles based on microhabitat characteristics and particle size. I then used these models to predict stability of microhabitats selected by fishes. The procedures tested the following hypothesis:

H₀: Movement rate of surface substrate particles will be equal among all available microhabitats, including those used by the various guilds and species.

However, I expected differences in stability among spawning habitats of the four guilds. Some specific alternate hypotheses were:

H_a: Bed movement rate will be low in egg-clusterer spawning habitats.

H_a: Bed movement rate will be high in the spawning microhabitats of the other three guilds.

Methods

Microhabitat stability

Bed stability of spawning habitats was first assessed by comparing levels of shear stress to D50, D_{cen} and *k* used by each guild and species (see Chapter 1). When shear stress is high relative to substrate size, the spawning habitats or portions of the spawning habitats will be unstable (Lane 1955). Two methods were then employed to empirically verify assessment of stability based on shear stress relative to particle size. I placed painted rocks (i.e., tracer particles) along the availability transects (see Chapter 1: Methods for description of availability transects) and repeatedly surveyed a subsection of the transects to determine movement of substrate particles and coarse changes in cross-sectional shape. I was primarily interested in detecting movement of surface particles not depth of scour. Thus, tracer particles were used because they detect finer movements that may not have been detected by scour chains.

At each of the two sites (NF1 and SF1; Figure 1.1), a subset of three availability transects was selected for surveying and placement of tracers. These transects were selected to represent a range of mesohabitat conditions and to include areas of spawning aggregations. A total of six to eight transects per site, representing a wider range of conditions, were eventually monitored due to repeated experiments (see below). Transects were surveyed relative to two benchmarks at each site, using a surveyor's transit and stadia rod. This method allowed production of a detailed picture of the cross-sectional stream shape with elevation measurements made with a resolution of about 10 mm. Thus, I was able to detect small amounts of aggradation or degradation. This method was successful in following changes in salmonid habitat and for evaluating effects of instream improvement structures in the Pacific Northwest (Lisle 1989).

Tracer particles were placed every meter across the channel along the same transects. Tracer particles were rocks taken from the stream, taken to the lab, washed, bleached, dried, and painted with fluorescent highway marking spray-paints. To place particles along the transects, I picked up a particle every meter along the transect and replaced it with a tracer of the same size and shape. The three longest axes of each tracer particle were recorded and a different color was used for each transect so I could determine from which transect and location along the transect rocks had moved. Tracer particle sizes ranged from 20 to 200mm. Areas of sand received as small a particle as possible, though some larger particles were placed in these areas to determine the relative vulnerability in these areas.

Because tracers were located on the transects along which the availability grid measurements were made, microhabitats receiving tracer particles were characterized with the same grid measurements made to characterize microhabitat availability. The availability grid measurements made at two to four locations on each transect were used to measure microhabitats into which tracers were placed. For analysis, the microhabitat characteristics measured at these grid sites were then assigned to tracer particles within 1 m of the grid. This allowed analysis of the frequency of tracer particle movement in all ranges of microhabitats, including those used by the four guilds. This allowed me to estimate the vulnerability to movement of all ranges of the various microhabitat variables

and to evaluate the relative stability of the microhabitat conditions that fishes selected for spawning.

To detect bed movement in both baseflow and bankfull flow conditions, the transects were resurveyed and all tracer particles were relocated following floods and/or every month. Tracers were noted as present or absent from their previous location. If moved, the location from which they had moved was determined based on color and the three diameter measurements. To assure correct assessment of movement, I conducted extensive searching at the point of origin, digging down as far as 0.25 meter for assurance that particles had moved. Data are reported as proportion of movement of tracer particle size classes (modified Wentworth scale) and proportion of movement that occurred in various microhabitat characteristics.

Tracer particles were replaced (i.e., a new experiment began) every time that a flood resulted in a loss of a majority of the tracer particles. This occurred three times during the duration of the study, so there were a total of four experiments. Following two of these four experiments, tracer particles were placed along different transects, allowing estimation of movement rates in a greater sample of the habitat in the reach. This resulted in a total of six transects being monitored at SF and eight transects being monitored at NF. Experiment 2 was only conducted on the North Fork site. Results of tracer experiments will be analyzed (see below) for both baseflow conditions (i.e., monitoring of tracer movement before a flood occurred) and above bankfull conditions (i.e., after a flood occurred).

Tracer particle analysis

Logistic regression was used to test the hypothesis that tracer particle movement rate is equal for all microhabitat characteristics present in the two study sites. Individual and multiple variable models were developed to determine the microhabitat variables that best predicted movement of tracer particles individually and in combination. Models were constructed for both sites (NF1 and SF1) individually and together for baseflow and above bankfull flow conditions (resulting in a total of six models). Variables included in this analysis were all grid microhabitat variables and the three tracer particle diameter

axis measurements (c, b, and a). Models were evaluated using the same parameters as for the microhabitat use models.

The generalized logistic function is $P = e^u / (1 + e^u)$ where P is the probability of spawning occurrence and u is the linear model utilizing all predictor variables: $u = c + b_1X_1 + b_2X_2 + \dots + b_nX_n$ where c is a regression constant, b_n are regression coefficients, and X_n are independent variables selected for the model. Coefficients were calculated using the maximum likelihood method (SAS Proc Logistic, SAS Institute 1985).

The ability of each microhabitat variable to predict tracer movement was tested by developing single variable logistic models. Individual model performance was evaluated using the chi-square for covariates, which indicated the likelihood of fitting a model with an intercept and the independent variables (Harrell 1985). Variables for which models were likely to predict tracer movement (p-value of Chi-square for covariates < 0.05) were then lumped together in multiple logistic regression models to find the combination of variables which best predicted microhabitat use.

For the purpose of determining prediction of movement or non-movement from the probability output of the model, I selected a probability level of 0.50. Thus, if the predicted probability of use is less than 0.50 the areas was classified as non-moved and if it is greater than or equal to 0.50 it was classified as moved. Model performance was evaluated using classification tables with five summary descriptors: proportion correct (overall observations correctly classified), sensitivity (proportion of movement observations correctly classified), specificity (proportion of non-moved tracers correctly classified), false positive rate (proportion of moved tracers incorrectly classified), and false negative rate (proportion of non-moved tracers incorrectly classified).

The logistic regression models developed to predict movement of tracer particles were then used to predict movement of substrate particles in used and available microhabitats. All models included tracer particle diameter as a variable that predicted movement of tracers. When these models were used to predict movement in areas used by the various guilds, I substituted the grid values of each roughness variable (separately) for tracer particle b-axis in calculations. This allowed me to predict movement (and thereby assess stability) of a substrate particle with diameter corresponding to the average particle size in the spawning area (D50), the particle on which the species spawn (Dcen),

and the roughness elements of the spawning area (k). In performing this substitution, I assumed that a tracer particle of a given diameter would have an equal probability of movement as a natural (unpainted) particle of the same size. I considered a predicted movement probability of 0.50 as the level at which a particle was predicted to move. Data were expressed as the proportion of spawning or available microhabitats predicted to experience movement of D50, Dcen, or k .

Results

Tracer particle analysis

Four size classes of tracer particles were placed in the stream; small gravel, large gravel, small cobble and large cobble (up to 253mm diameter). Tracer particles moved more frequently at SF1 in both baseflows and bankfull flows (Table 2.1). In addition, all four size classes of tracer particles moved more frequently in bankfull flows (Table 2.1,

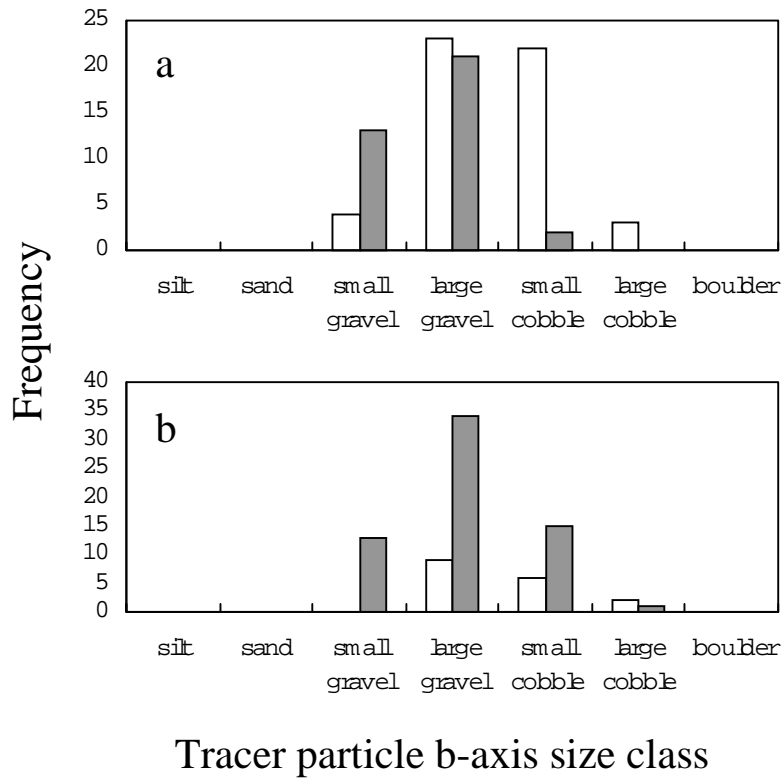
Table 2.1. Proportion of movement and sample size (in parentheses) of four size classes of tracer particles in baseflow and bankfull flows at NF1 and SF1 (see Figure 1.1 for site locations) during 1997-1998.

Tracer Particle Size Class	Proportion moved in flood		Proportion moved in baseflow	
	NF1	SF1	NF1	SF1
Small gravel (2-16mm)	1.0 (7)	1.0 (6)	0.86 (7)	0.70 (10)
Large gravel (17-64mm)	0.76 (29)	0.86 (14)	0.40 (25)	0.58 (19)
Small cobble (65-164mm)	0.33 (6)	0.87 (15)	0 (5)	0.11 (19)
Large cobble (165-256mm)	0 (2)	1.0 (1)	0 (1)	0 (2)

Figure 2.1a). At both flow levels, small gravel sized particles moved in the highest proportion, followed in order by large gravel, small cobble and large cobble (Table 2.1, Figure 2.1a,b).

There were differences in movement rate of tracer particles in different levels of k , mean embeddedness, and rRe. At flows below bankfull tracer particle movement was

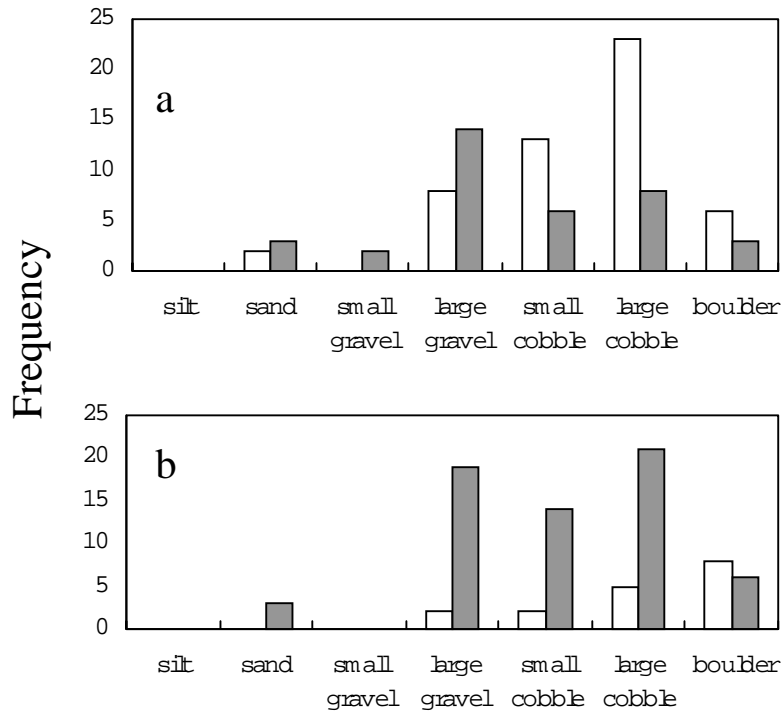
Figure 2.1. Frequency of tracer particles of modified Wentworth size classes moved and not moved in a) below bankfull flow conditions and b) above bankfull flow. Open bars are frequency not moved and shaded bars are frequency moved.



frequent in microhabitats with k in the sand to large gravel range and less frequent in areas with k in small cobble, large cobble, or boulder ranges (Figure 2.2a). At higher flows almost all microhabitats experienced much bed movement, though there was lower frequency of movement in areas with k in the boulder range (Figure 2.2b). At baseflow, areas with mean embeddedness above a threshold of 10% experienced relatively low rates of tracer particle movement (Figure 2.3a), whereas at bankfull flows this threshold was at 40% embeddedness (Figure 2.3b). Tracer particle movement was less frequent in microhabitats with very low or very high rRe at baseflow (Figure 2.4a). However, at higher flows all ranges of rRe experienced high movement rates, with only areas with $rRe > 1.6 \times 10^5$ experiencing lower frequency of movement (Figure 2.4b).

Movement of tracer particles was predictable from microhabitat and tracer particle characteristics. Logistic regression results for individual variables indicate that movement of tracer particles can be predicted best from tracer particle diameter, substrate variables (particularly k), mean embeddedness, and rRe (χ^2 test for covariates; $P < 0.05$).

Figure 2.2. Frequency of tracer particles moved and not moved in substrate roughness classes a) below bankfull flow conditions and b) above bankfull flow. Open bars are frequency not moved and shaded bars are frequency moved.



Substrate roughness Wentworth class

Despite significance of the chi-square test for covariates for these individual variables, the individual models did not perform well as evaluated by sensitivity and false positive rate. When multiple variables were considered in the same model, predictive ability was increased in some cases. Composite models were then developed from combinations of these variables.

Movement at baseflow conditions was not predictable with a high level of accuracy for either site. Movement at above bankfull conditions at NF1 and when both sites were considered together was best predicted by composite models including substrate axis b , k , mean embeddedness, and rRe , though the model coefficients diverge slightly (Table 2.2, 2.3). Models could not be developed for SF1 at bankfull flow because of the very low number of tracer particles that did not move.

Figure 2.3. Frequency of tracer particles moved and not moved in mean grid embeddedness classes a) below bankfull flow conditions and b) above bankfull flow. Open bars are frequency not moved and shaded bars are frequency moved.

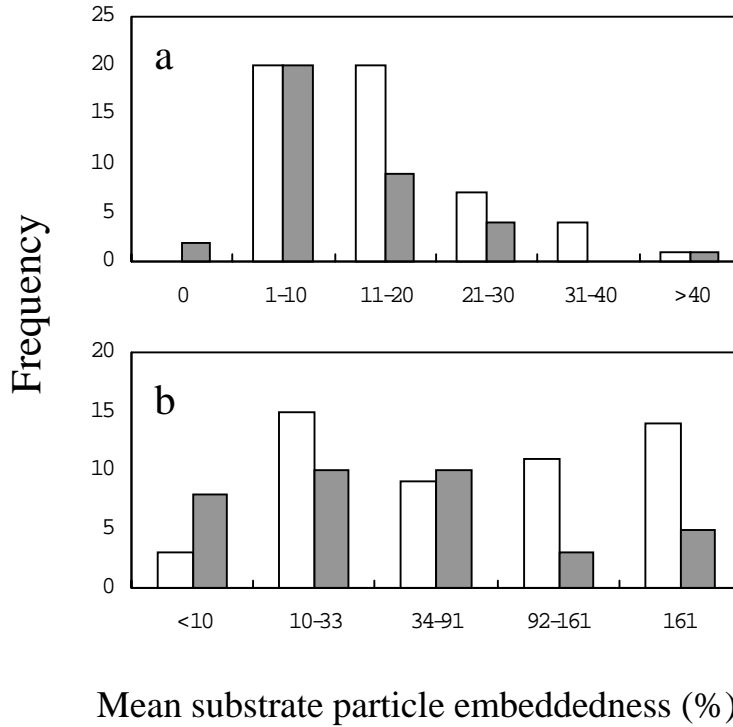


Figure 2.4. Frequency of observed tracer particles movement and non-movement in roughness Reynolds number classes a) below bankfull flow conditions and b) above bankfull flow. Open bars are frequency not moved and shaded bars are frequency moved.

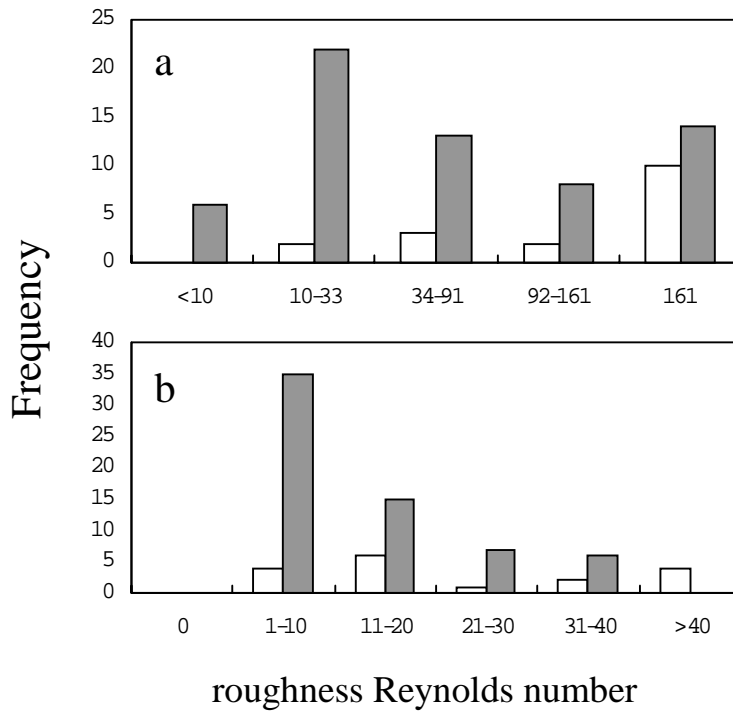
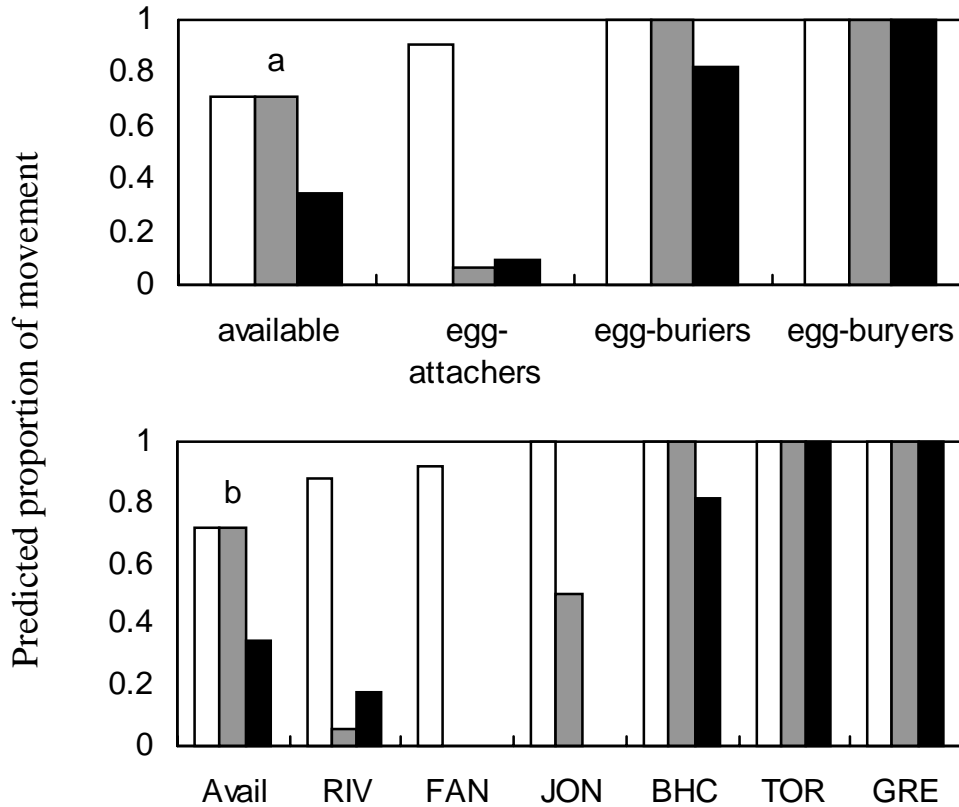
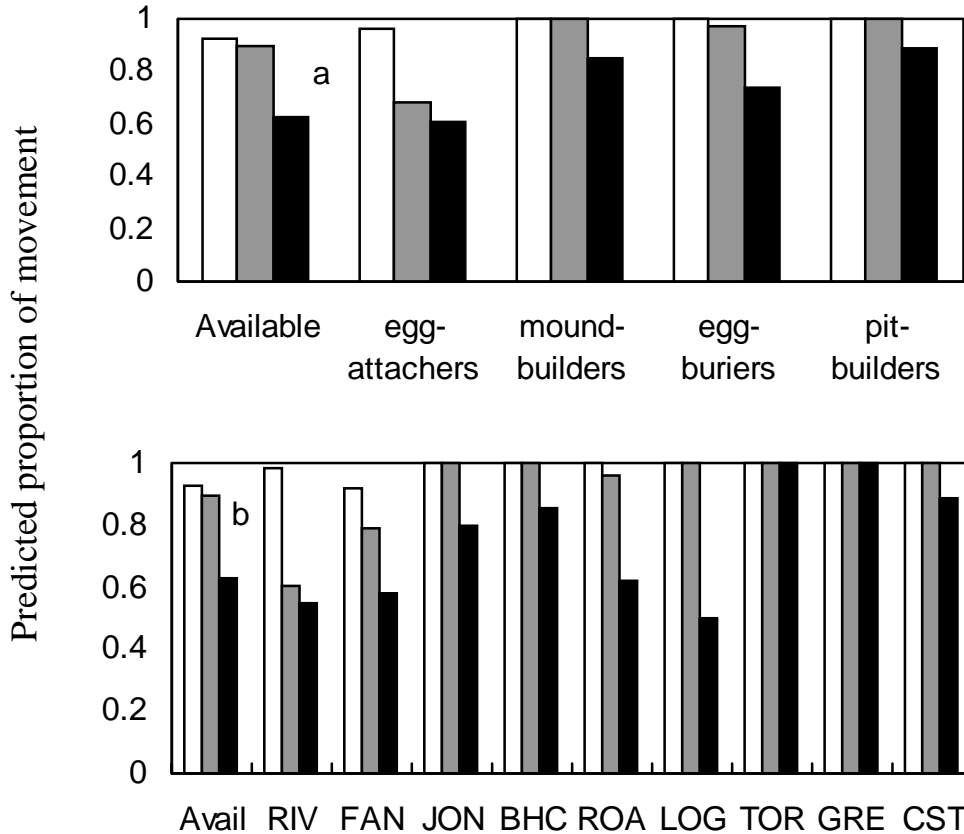


Figure 2.5. Proportion of particles corresponding to grid D50 (open bars), Dcen (shaded bars) and k (closed bars) predicted by logistic regression from tracer particle movement results at NF1 for a) reproductive guilds and b) individual species. Species abbreviations are the same as for Table 4.



Egg-clusterers did not use a high shear stress at time of spawning relative to the diameter of nesting rocks (Dcen) or substrate roughness (k ; Figures 2.7). This trend held equally true for all three egg-clustering species (Figure 2.8). Thus, I would predict that Dcen and k of egg-clusterer sites would be stable. Logistic regression model predictions corroborate this, with both models predicting that microhabitats used by egg-clusterers would experience low frequency of movement of all three substrate variables, but with frequency of movement of D50 the highest (Figures 2.5, 2.6). Probability of movement of the average particle size (D50) was the highest in both models (approximately 0.88 and 0.96 at NF1 and at both sites, respectively; Figures 2.5, 2.6). There was a lower frequency of predicted movement for roughness elements (0.10 and 0.61) and Dcen (0.08 and 0.69; Figures 2.5, 2.6). At NF1, few egg-clusterer spawning particles or roughness

Figure 2.6. Proportion of particles corresponding to grid D50 (open bars), Dcen (shaded bars) and k (closed bars) predicted by logistic regression from tracer particle movement results at SF1 and NF1 for a) reproductive guilds and b) individual species. Species abbreviations are the same as for Table 4.



elements would be expected to move. In the case of individual species, these percentages held true for riverweed darter, but were lower for fantail darter and higher for johnny darter (Figures 2.5, 2.6).

Mound builders used very low particle sizes. Despite this, their habitats are not predicted to be unstable at the flows at which they spawned because of their use of very low shear stress (Figures 2.7, 2.8). However, models of bed movement at bankfull flow predicted that mound-builder microhabitats are very vulnerable to bed movement at higher flows. All three substrate variables would experience very high movement at both sites (Figures 2.5, 2.6). Only in about 20% of the cases would roughness elements be predicted to be stable at NF1, 10% overall; the other two variables would be expected to move in all cases.

Figure 2.7. Substrate roughness variables (D_{cen} , k) and shear stress in microhabitats used by four reproductive guilds in the upper Roanoke River drainage in 1997 and 1998. Bars and points are means and error bars are one standard error.

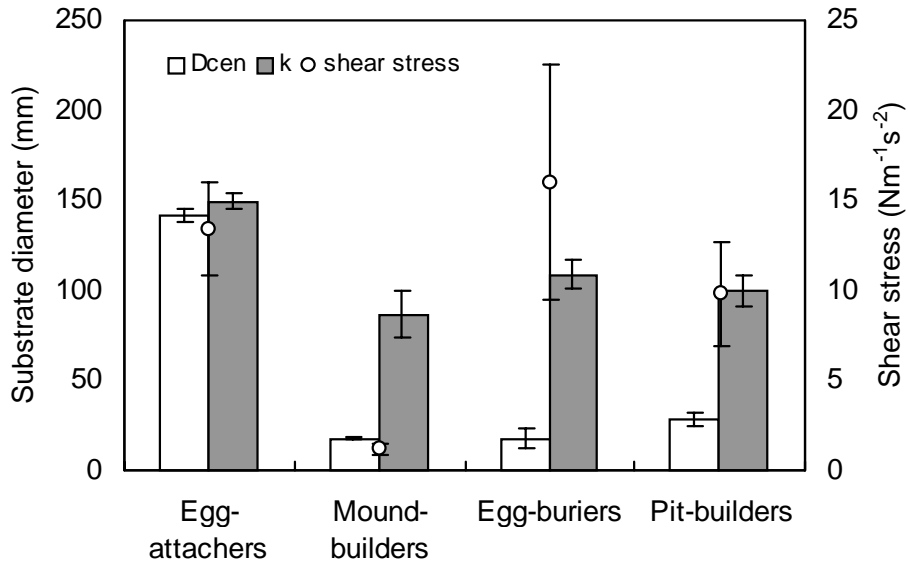
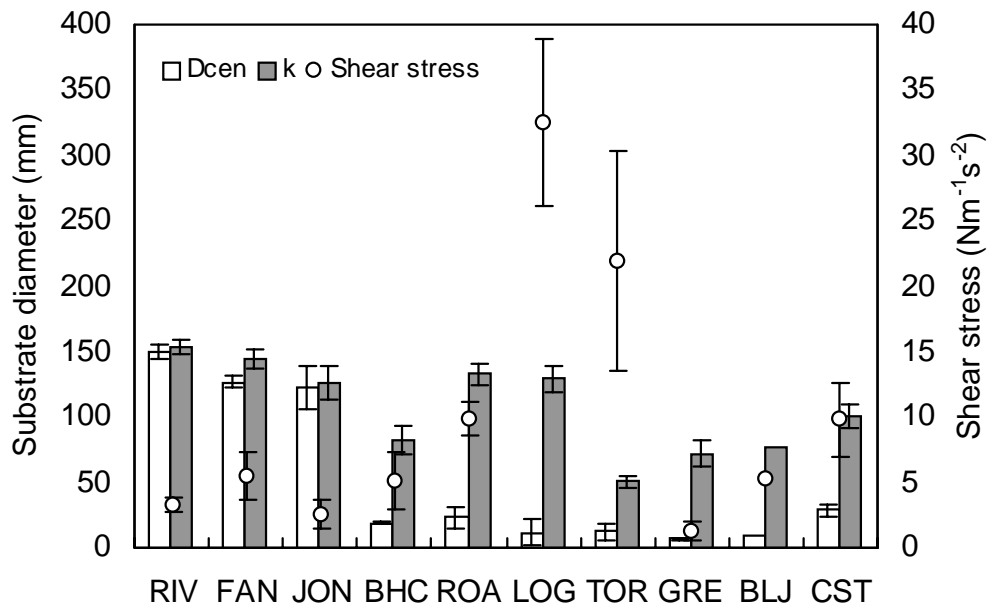


Figure 2.8. Substrate roughness variables (D_{cen} , k) and shear stress in microhabitats used by ten species in the upper Roanoke River drainage in 1997 and 1998. Histograms and points are means and error bars are one standard error. Species abbreviations are the same as in Table 4.



Egg-buriers used a high shear stress relative to both D_{cen} and k , resulting in predicted instability of spawning habitats even at low flows (Figure 2.7). However, comparisons for individual species show that, though shear stress is sufficient to move D_{cen} of all species, it is sufficient only to move k of torrent sucker and Roanoke logperch microhabitats (Figure 2.8). All other egg-buriers have stable k at low flows. However, logistic regression models predicted high levels of movement of all variables at bankfull flows. Predicted movement rate of D_{cen} and D_{50} was 100% for both models for the guild and all species (Figures 2.5, 2.6). Predicted movement rate was also high for k (Figures 2.5, 2.6), though it was somewhat lower for the model incorporating all sites (Figure 2.6). This pattern was especially true for the *Percina* darters (Figure 2.6b). The differences in predicted movement by the two models were likely due to the occurrence of few egg-buriers at NF1.

Pit-builders showed a pattern similar to egg-buriers in that they used below bankfull flow shear stress that was higher than D_{cen} but not higher than k (Figures 2.7, 2.8). They also had a high predicted probability of movement of all substrate roughness variables, but with k slightly lower (Figure 2.6).

Transect monitoring

Most monitoring transects saw no major changes during base flow conditions. However, one noteworthy exception was one NF1 transect crossing a highly mobile gravel bar that had taken over the width of the channel and formed a riffle (Figure 2.9a). This gravel bar area experienced as much as 10 cm of gravel and sand deposition from July 1997 to October 1997 under baseflow conditions (Figure 2.9a). In a subsequent flood in January 1998 up to 40 cm of gravel was scoured from certain portions on this same transect (Figure 2.9a). This transect was 6 m downstream of the riffle location where torrent suckers were observed spawning. Nearly all tracer particles placed on this transect were moved or covered. There was also a small amount of erosion of a point bar portion of an upstream riffle transect (Figure 2.9b). All tracer particles under 100mm placed in this area moved. At NF1, there was very little gross change in a run dominated by cobbles (Figure 2.9c), the areas in which egg-clusterers spawn. Under baseflow conditions, no SF1 transects experienced much bed elevation change.

Figure 2.9. Repeated transverse stream cross-sections along three tracer particle transects at NF1 in a) a gravel bar near which torrent suckers spawned, b) a riffle and gravel bar edge and c) and run dominated by cobbles.

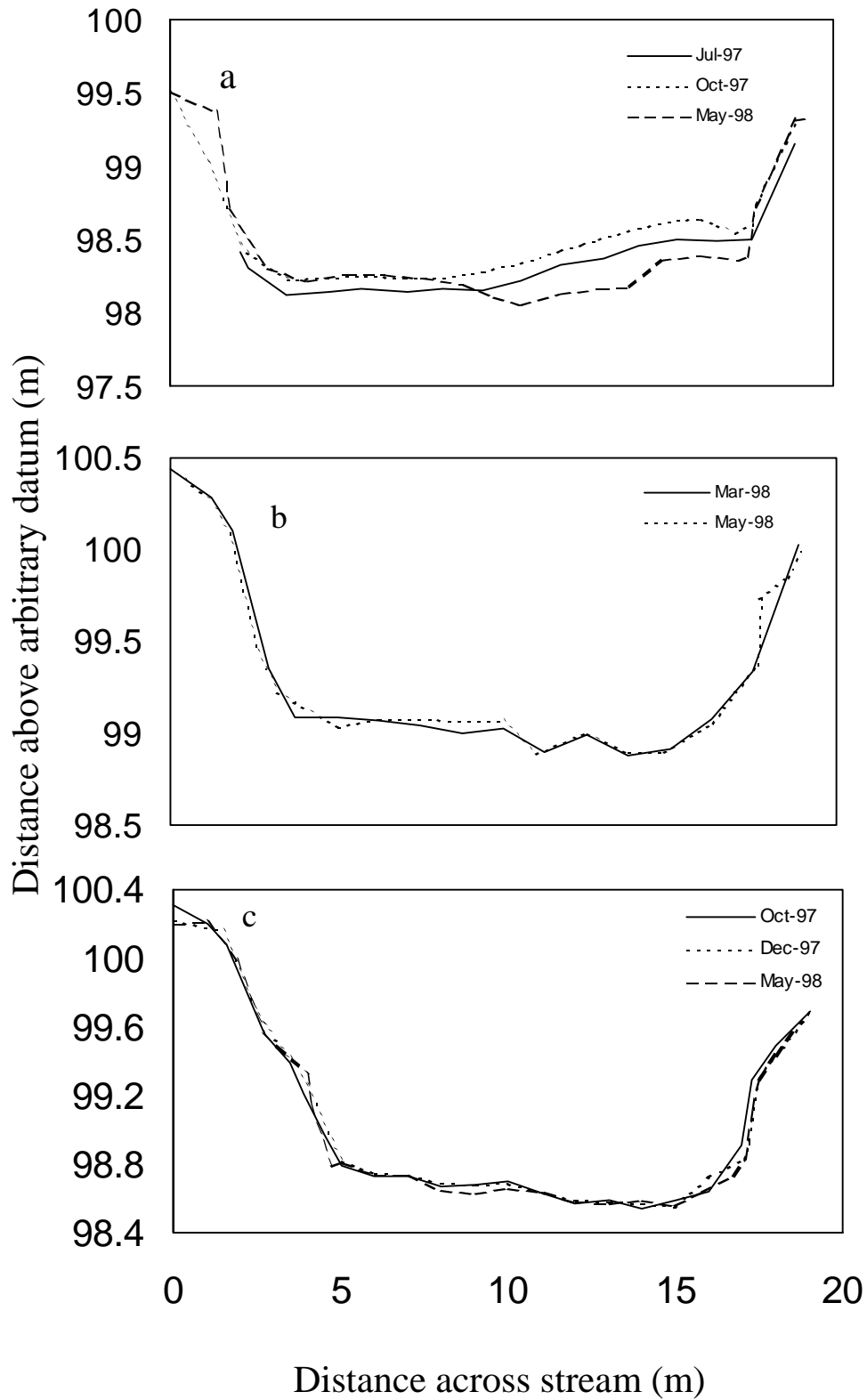
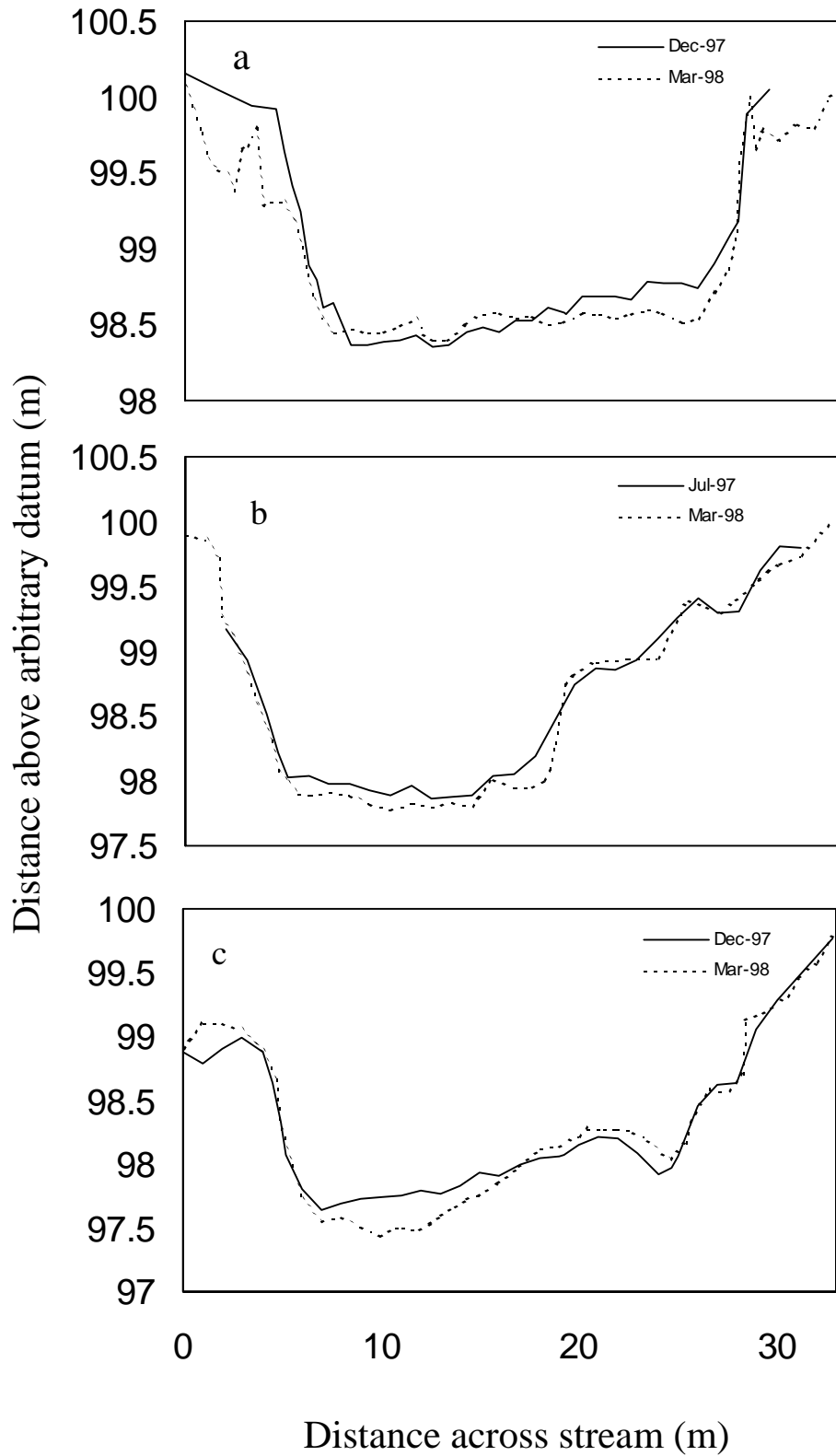


Figure 2.10. Repeated transverse stream cross-sections along three tracer particle transects at SF1 in a) a pool tail where Roanoke darter spawned in 1997, b) a riffle and c) and run dominated by cobbles.



Transects at both sites experienced more changes following bankfull flow events. During a large flood in late January 1998 most transects experienced major changes in bed elevation in some portion of the bed profile. There was much scour in several portions of the NF1 gravel bar (Figure 2.9a). However, riffle and pool transects at NF1 did not experience much change (Figure 2.9b,c).

More prevalent and severe bed elevation changes were experienced at SF1. A pool tail transect experienced more than 1m of scour on one bank and up to 10cm of scour at various portions of a gravel area below a culvert (Figure 2.10a). There was also as much as 8cm of gravel deposition in another area in which Roanoke darter spawned in 1997 but not in 1998 (Figure 2.10a). There was also some deposition on a transect in a riffle where egg-clustering darters spawned (Figure 2.10b). However, the mid-channel cobble areas in which the species spawned were not as affected. In a downstream run, there was as much as 10cm of deposition in one gravel bar area of the transect and as much as 30cm of scour on the opposite side in the thalweg (Figure 2.10c).

Discussion

The second objective of this research was to determine if any guilds or species use areas with less bed movement than surrounding areas, with the eventual goal being to evaluate use of stable spawning microhabitats. I tested the hypothesis that there is an equal probability of surface streambed particle movement in all used and available microhabitat. To test this hypothesis I developed models predicting movement of surface bed particles based on microhabitat characteristics. These models were then applied to the spawning microhabitats used by each reproductive guild to predict the probability of movement of three relevant substrate parameters: roughness elements, average particle sizes, and particles on or under which eggs were placed. If a guild or species uses areas with the characteristics of a refuge, then I would predict a low level of bed movement in their spawning habitats.

I observed a lower rate of movement in areas of high substrate roughness, of high embeddedness, high substrate particle size, and high near-bed flow turbulence. These trends allowed identification of patterns of relative stability between the microhabitat used by each guild and the overall available microhabitat.

Particle movement is not predictable with logistic regression models at low flows because the prevailing sizes of tracer particles were not moveable at low flows. I did not use any tracer particles smaller than 15 mm in diameter because they immediately moved downstream when placed on the streambed. Thus, any fish that use particles sizes in the low end of the small gravel range or smaller (e.g., diameter at center of grid for Roanoke darter) is likely subject to movement of their microhabitat even at relatively low flows.

There were apparent differences between used and available microhabitats in the movement rate of surface tracer particles at high flows for most reproductive groups. I conclude that egg-clustering darters do appear to be using areas that experience less bed movement and reject the hypothesis of equal vulnerability to bed movement in all microhabitats for this guild. Egg-clusterers displayed a strong preference for D_{cen} and k in the cobble size range and did not use areas with shear stress reaching the critical level for these particle sizes. In addition to these substrate preferences, they prefer areas with higher embeddedness and roughness Reynolds number. These microhabitats are among the microhabitats that are the most stable of any available.

Logistic regression models predicted an 0.80 probability that rocks receiving egg-clusters will be stable (i.e., rocks will not move) in high flows, that the surrounding average particle size will experience a low frequency of movement, and that the roughness elements around the microhabitat are unlikely to move. This suggests that the hydraulic refugia created by these particles will remain stable during higher flows.

Mound-builders use microhabitats that experience a higher frequency of movement than the available conditions, so the hypothesis is rejected for this guild. They are obligate users of very small particle sizes with low embeddedness that are easily moved at high flows, though shear stress at low flows was not sufficient to entrain these particles. Further, logistic regression models predict that D_{50} , D_{cen} and k of mound microhabitats all experience high rates of movement relative to available microhabitats at both sites.

Egg-buriers select microhabitats with higher frequencies of surface bed movement than the available, so the hypothesis can be rejected. Their strong preference for gravel particle sizes makes their spawning microhabitats vulnerable to entrainment even at low flows, as shown by the high shear stress relative to particle size. Thus, the

average particle size in the area and the egg depositional gravel would be very unstable. This was evident even during the time of sampling. It was common to see these particles bouncing at the point of incipient motion. They would move when a diver's hand changed the flow pattern slightly and when the fishes displaced them during spawning. Despite this, shear stress was not high enough to entrain roughness elements in *Percina* spawning microhabitats.

Tracer particle results also indicate that the spawning microhabitats of egg-buriers, which are characterized by low particle sizes and embeddedness, do experience a high level of surface bed movement. The rates of movement of egg-burier D_{cen} and D₅₀ are very high, as are the roughness elements (k) of the suckers. However, the frequency of movement of k used by the *Percina* darters are lower.

Results of transect surveying also corroborate these results. Egg-buriers were the only guild that spawned in areas that experienced major changes in bed elevation in transect resurveying. There was severe erosion in a riffle at NF1 in which torrent suckers spawned. There was also as much as 20 cm of change in bed elevation in areas that Roanoke darters used and they had to shift to a more downstream site in which they did not spawn the year before.

Logistic regression models predicted that the particles egg-buriers spawn in, the average size, and most of the roughness elements around them will be mobile in large floods. The only exception is the Roanoke darter and Roanoke logperch that use the same roughness areas as the egg-clusterers that do not experience bed movement. However, the high level of surface movement did not always translate to large changes in bed elevation. Thus, scour may not always reach depths sufficient to erode away eggs that these species bury down in the substrate.

Pit-builders select microhabitats with higher frequencies of surface bed movement than the available, so the hypothesis can be rejected for this guild as well. Their strong preference for gravel particle sizes in pits makes their spawning microhabitats vulnerable to entrainment even at low flows, as shown by the high shear stress relative to particle size. Thus, the average particle size and the egg depositional gravel would be very unstable. Logistic regression also predicted high movement rates of all three substrate components for this guild as well.

Logistic regression models consistently predicted higher rates of movement of all substrate variables for all guilds and species at the South Fork. This was because the SF1 site experienced very high rates of movement of all particle sizes at very high flows and because floods seemed to be more severe at that site during this study. Movement rates in floods at the South Fork were likely higher because the channel is larger there which results in higher shear forces and stream power, so would expect higher rates of movement (Thorne et al. 1984).

Summary and Conclusions

Townsend (1997) found that insect communities in streams with high frequency of streambed disturbance had the highest incidence of species with traits allowing use of stable microhabitats (e.g., small size, clingers and habitat generalists). I conclude that there is evidence of occurrence of such traits in the reproductive life history of fishes. This study found that some fishes do possess preferences for certain spawning microhabitats that have the characteristics of areas with low bed movement (Lancaster and Hildrew 1993, Hildrew and Giller 1994). Egg-clusterers use large cobble nesting rocks in microhabitats dominated by cobbles and boulders that are more stable than the surrounding habitats. It is likely that eggs attached to these large cobbles are not destroyed in high flow disturbances. Thus, egg-clustering, which necessitates use of large particles, confers fitness to these species in the face of the bed movement selective force. Furthermore, egg-clusterer spawning habitat is similar to their habitat the rest of the year. This suggests that egg-clusterers may utilize stable habitats throughout the year, a trait necessitated by their benthic lifestyle that makes them vulnerable to bed movement year round.

Mound-builders and nest associates use the least stable microhabitats. Though mounds are stable at low flows, it is likely that bankfull shear stress reaches levels in excess of the critical shear required to entrain mound particles. This likely accounts for the destruction of mounds observed every spring following high flows (personal observations).

However, mound destruction may not result in reproductive failure for bluehead chubs or its associates. These species do have some life history trait that allows them to persist in high flows naturally and under anthropogenic impacts because they are often one of the most abundant species in heavily impacted areas of the upper Roanoke drainage. The strong selection of low velocity and shear stress microhabitats certainly constitutes selection of stable habitats at low flows if completion of the early life cycle occurs before bankfull flow events. In addition, chubs and associates may be fractional spawners (Heins and Rabito 1986, Johnston and Page 1992) a trait that would allow them to compensate temporally for destroyed mounds. Some stream fishes have been documented to re-nest when their microhabitats are destroyed (Lukas and Orth 1995).

New chub mounds are frequently observed in the same locations as mounds that were destroyed in floods (personal observation), and I speculate that these mounds may be built by the same individuals.

Egg-buriers do not use stable microhabitats. Thus they are not likely using the same type of refugium as the egg-clusterers. However, despite the strong evidence of surface bed instability in the microhabitats of the egg-buriers, there is potential for use of hyporheic refuges from bed movement (Townsend 1997).

Salmonids, which are similar to egg-buriers in their microhabitat preferences and egg-burying behavior, bury eggs in areas with high porosity that allow eggs sufficient intergravel flow to sustain respiration (Kondolf and Wolman 1993). As a result, eggs are sheltered from surface bed movement in the hyporheos (Montgomery et al 1996). These areas must have well sorted gravel and little natural fine sedimentation (Kondolf and Wolman 1993). The gravel D50 and Dcen and low embeddedness in areas used by egg-buriers suggests that they may be using these types of hyporheic shelters. Future research should focus on investigation of use of hyporheic shelter through volumetric sediment sampling that would allow measurement of porosity and volumetric fine sediment content (e.g., Lotspeich and Everett 1981). It is likely that microhabitats supporting successful egg-burier reproduction must have high porosity and have little fine sediment in gravel interstices. Volumetric sediment sampling also would allow assessment of egg burial depths and subsequent evaluation of vulnerability of buried eggs to deep scour among the variety of egg-buriers (*sensu* Montgomery et al. 1996).

Pit-builders also do not select stable spawning microhabitats. However, central stonerollers must possess life history adaptations to bed movement because it is one of the most ubiquitous species in the upper Roanoke River drainage. Stonerollers may also may use the hyporheic shelters that protect their buried eggs from surface bed movement and small amount of scour.

Implications

This work shows that there is evidence of differential adaptation among reproductive guilds to naturally occurring streambed movement. These behavioral traits and microhabitat requirements have provided reproductive fitness of these groups and

allowed them to persist. However, channel processes have been altered in most streams and rivers worldwide (Petts 1984), changing the selective forces upon which fish reproductive life history traits have been forged. Alterations in channel shape at the hands of hydropower operation or channelization have resulted in disruption of the equilibrium between channel and imparted sediment (Petts 1984, Leopold et al.1964). When the equilibrium is disrupted, there are changes in the location and amount of streambed that experiences bed movement and erosion (Petts 1984, Gregory et al. 1994). Increases in frequency or intensity of high flows causing bed movement results in increases in bed movement in mobile portions of the streambed. Some of these areas are gravel bars and erosional riffles that have little protection from armored layers of cobbles and boulders. Hydropower peaking flows have scoured stream channels below dams down to bedrock, completely removing areas of small gravel substrates (Petts 1984). Egg-buriers, which have a strong affinity for these types of habitats for spawning, may be declining because of such practices.

It is apparent that stream managers must maintain the reproductive habitats of fishes if populations are to remain stable. This work has shown that maintenance of quasi-stable habitats during the reproductive period may be a requisite of management of these fishes. The ‘rule of thumb’ of proportionality of substrate size and critical shear stress may be employed for this purpose and is already in use in design of artificial channels and restoration of natural channels (Lane 1955, Newbury and Gaboury 1993). This study shows that there is evidence of the validity of this ‘rule’ for prediction of spawning microhabitat stability, at least at baseflow. This ‘rule’ could be applied to peaking flows below hydropower facilities by adjusting flows levels that would not create shear stress in exceedence of the critical level. Such management would require information on the microhabitats used by fishes and the ability to predict the distribution of shear stresses below dams. This study has provided information on the habitats used by various fishes in the Roanoke drainage, some of which may be transferable to other areas. It has also demonstrated that it is possible to characterize spawning habitats by variables relevant to prediction of bed stability. Current methods of modeling flows below hydropower facilities are not adequate for characterization of shear stress conditions. However, two-dimensional models are in development which have the

potential to model patchiness in shear stresses at various discharges (Leclerc et al. 1995). These models used in conjunction with information on microhabitat use and stability will allow management of bed movement frequency and intensity in spawning areas below dams.

This study shows that fishes have some microhabitat affinities for stable microhabitats similar to those observed for macroinvertebrates (Lancaster and Hildrew 1994, Townsend 1997). Refugia are of the utmost importance for invertebrates because their small body size limits their ability to disperse quickly and avoid the effects of bed movement (Townsend and Hildrew 1994). For invertebrates, floods constitute a significant disturbance event, necessitating recolonization of altered habitats for persistence of populations. In contrast, destruction of a single clutch of eggs may not have serious population ramifications for fishes. The reproductive period for most fishes is extended and adequate opportunity exists for multiple spawning events in a single season. However, flow alteration may decrease time between bed movement events being less than incubation time resulting in complete reproductive failure. In these cases, the management priority must be to ensure that time between flood events is held to a natural level. In peaking flow management, daily flows could also be prescribed that do not result in a frequency of bed movement that would be shorter than the incubation time of vulnerable fishes such as chubs and egg buriers.

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Appendix A. Microhabitat use prediction with logistic regression

Objective

In addition to characterizing spawning habitat, I wanted to develop predictive models of spawning microhabitat use. Such models could be used by managers to calculate the probability that a fish will spawn in certain microhabitats based on several easily measured microhabitat variables. I used logistic regression to predict microhabitat use. However, this analysis should be considered highly speculative because of a potential assumption violation (see below). None the less, this analysis is included because results warrant further testing.

Methods

Microhabitat Use Models

Logistic regression models were used to predict spawning microhabitat use because of the binary nature of the response (use or available) and because it provides a probabilistic prediction of microhabitat use. However, my data potentially violate the assumption of mutual exclusivity of the binary responses. In other words, it is certainly true that a given spawning microhabitat patch belongs to both the available microhabitat distribution and to the distribution of used microhabitats. Yet, for the purposes of model construction I considered my data to be binary because none of my grid measurements fell into both classes (used and available). In other words, on the occasion of sampling, the locations I measured as available microhabitats were not used by any guild for spawning. Thus, for model development I consider my data to constitute microhabitat use and non-use (i.e., truly binary).

I constructed models for guilds to make the models generalizable and transferable to other streams. However, where sample sizes were adequate, models were developed for individual species as well. The ability of each microhabitat variable to predict microhabitat use was tested by developing single variable logistic models. Individual model performance was evaluated using the chi-square for covariates, which indicated the likelihood of fitting a model with an intercept and the independent variables (Harrell

1985). Variables for which models were likely to predict microhabitat use (p-value of Chi-square for covariates < 0.05) were then lumped together in multiple logistic regression models to find the combination of variables which best predicted microhabitat use. All microhabitat variables from all four variable categories were tested for each guild. I wanted to develop models with as few a number of variables as possible. However, a tradeoff soon arose over the number of variables to be included and the added accuracy of the models as more variables were added. Thus, I tried to find the best model that could be developed with only three variables included.

For the purpose of determining prediction of use or non-use from the probability output of the model, I selected a probability level of 0.50. Thus, if the predicted probability of use is less than 0.50 the areas was classified as non-used and if it is greater than or equal to 0.50 it was classified as used. Model performance was evaluated using classification tables with five summary descriptors: proportion correct (overall observations correctly classified), sensitivity (proportion of use observations correctly classified), specificity (proportion of non-use observations correctly classified), false positive rate (proportion of use observations incorrectly classified), and false negative rate (proportion of non-use observations incorrectly classified). Logically, the best model would have high sensitivity and low false positive rate. Models were developed for use and non-use data pooled from NF1 and SF1.

Logistic regression models developed for each guild were then validated with data taken the prior year at three sites. These sites included the two for which the models were developed and one additional site on the South Fork (SF2). Data were available for use only, since no availability data were taken in 1997. Data are presented as the percentage of the use observations classified correctly (i.e., sensitivity).

Results

Microhabitat use prediction using logistic regression

Logistic regression models were developed for all four guilds and for riverweed darter, fantail darter and Roanoke darter, which had adequate sample sizes. In general, models classified a high proportion of observations correctly (Tables A.1-A.8). Models always classified non-use microhabitat observations with a high degree of accuracy

Table A.4). This model performed well internally, classifying 85% of the observations correctly (Table A.4). However, in the validation stage it only classified 47% correctly and only 50% (one out of two) at NF1. There were no observations of bluehead chub at SF1 with which to validate the model for that site.

The best model for egg-buriers included five variables (D50, k , Re, relative depth, and δ , Table A.5). This model performed poorly, with a sensitivity of 0.48 and false positive rate of 0.32 (Table A.5). It performed poorly when in the validation stage, only predicting 11% of use observations correctly (Table A.8). The Roanoke darter model performed even more poorly, correctly classifying none of the four observations (Table A.6, A.8).

The best model for pit-builders included silt, mean embeddedness and depth (Table A.7). This model performed well, with a sensitivity of 0.78, correctly classified eight out of nine pit-building sites (Table A.7). This model could not be validated because no pit-builders were observed spawning in 1997.

Table A.4. Parameters of a logistic regression model for prediction of mound-builder spawning habitat at NF1 and SF1 in 1998. Variable abbreviations are the same as in Table 4.

Variable	Parameter Estimate	Standard Error	P> Chi-Square
Intercept	14.872	4.353	0.0006
Dcen	-0.042	0.023	0.0662
D90	0.018	0.012	0.1340
Em	-1.404	0.572	0.0142
Silt	-0.708	0.317	0.0252
Vbot	16.82	6.974	0.0159
Fr	-25.43	8.098	0.0017
Rre	-0.118	0.038	0.0016
Relsub	-0.246	0.076	0.0013
Prop. Correct = 0.92	Sensitivity = 0.85	False Positive Rate = 0.15	
	Specificity = 0.94	False Negative Rate = 0.06	

Table A.5. Parameters of a logistic regression model for prediction of egg-burier spawning habitat at NF1 and SF1 in 1998. Variable abbreviations are the same as in Table 4.

Variable	Parameter Estimate	Standard Error	P> Chi-square
Intercept	8.686	2.858	0.0024
D50	-0.035	0.022	0.0.82
D90	-0.061	0.018	0.0005
Re	-0.016	0.005	0.0007
Fr	-1.156	0.331	0.0005
Relsub	-4.992	3.387	0.1405
Prop. Correct = 0.85	Sensitivity = 0.48	False positive rate = 0.32	
	Specificity = 0.96	False negative rate = 0.10	

Table A.6. Parameters of a logistic regression model for prediction of Roanoke darter spawning habitat at NF1 and SF1 in 1998. Variable abbreviations are the same as in Table 4.

Variable	Parameter Estimate	Standard Error	P> Chi-Square
Intercept	17.23	6.721	0.0104
D90	-0.091	0.035	0.0087
Vbot	14.70	6.188	0.0175
Re	0.034	0.010	0.0006
Fr	-36.37	11.56	0.0016
Rre	-0.013	0.043	0.7610
Rel	-2.096	0.666	0.0017
Lam	-18680	12768	0.1435
Prop. Correct = 0.93	Sensitivity = 0.53	False positive rate = 0.33	
	Specificity = 0.97	False negative rate = 0.05	

Table A.7. Parameters of a logistic regression model for prediction of pit-builder spawning habitat at NF1 and SF1 in 1998. Variable abbreviations are the same as in Table 4.

Variable	Parameter Estimate	Standard Error	P> Chi-square
Intercept	5.494	2.299	0.0169
Em	-1.671	0.503	0.0009
Silt	-0.994	0.544	0.0679
Depth	-0.076	0.062	0.2208
Prop. Correct = 0.95	Sensitivity = 0.78	False positive rate = 0.22	
	Specificity = 0.98	False negative rate = 0.02	

Table A.8. Proportion of microhabitat use observations correctly classified by logistic regression models for several guilds and species at SF1, SF2 and NF1 in 1997. Sample size is in parentheses.

Guild or Species	SF1	SF2	NF1	Overall
Egg-clusterers	0.90 (10)	0.94 (17)	1.00 (3)	0.94 (33)
Riverweed	0.13 (8)	0.31 (13)	0.25 (4)	0.24 (25)
Fantail	0.50 (2)	0 (4)	0 (2)	0.13 (8)
Mound-builders	-	0.46 (13)	0.50 (2)	0.47 (15)
Egg-buriers	0.50 (2)	0 (7)	-	0.11 (9)
Roanoke	0 (1)	0 (3)	-	0 (4)

Discussion

Microhabitat use of egg-clusterers was predicted well by the logistic regression models, with a high percentage of use observations classified correctly both internally

and with the independent data set from the prior year. This high level of model accuracy held equally true for all individual species as predicted by the guild model, though the species-specific models did not correctly classify a high percentage of the previous year's spawning microhabitats.

These models likely performed better than those for the other groups because sample size was greatest for egg-clusters and because they were uniform in their microhabitat use. They consistently selected for particle sizes at the upper end of the distribution, not using particles at the center of the grid less than 100 mm in diameter. The model picked up on this lower threshold of Dcen for microhabitat use. Thus, as Dcen enters into the cobble range, there is a high probability that they will spawn in that area.

Logistic regression models also successfully predicted microhabitat use of mound-builders, with 85% of nests being correctly classified and with a high average probability of occurrence calculated by the model. This is likely because they used narrow ranges of most variables and because of the upper thresholds of several variables (see above) above which no spawning occurred. However, the model for this group was overspecified and was not validated in applications to an independent data set.

Microhabitat use was difficult to predict for egg-buriers because of the high intragroup variation. Modeling individual species microhabitat use would have produced better results, but low sample sizes precluded doing much work with individual species. For example, torrent sucker used a very unique microhabitat, the availability of which is very low. If I had a larger sample size of this species, we likely would see a very strong predictive ability for this species.

Several factors may have also limited the usefulness of logistic regression models for these microhabitat use and availability data. The primary value of logistic regression is identification of thresholds above or below which microhabitat use occurs. Selection for microhabitat variables by most guilds did not occur in this type of threshold. Instead, most guilds selected of a distinct range of at least one variable, often in the center of the non-use distribution. As a result, these variables, though important to microhabitat selection by fishes, do not contribute to prediction of habitat use by the models. Low number of microhabitat use observations and measurements for some guilds and all

species also limited the usefulness of the models. In many cases, there were more than 10 times as many non-use microhabitat observations as use observations. This resulted in more weight being given to the non-use microhabitats in model development. As a result, the best prediction occurred for egg-clusterers which had the highest number of use observations.

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SAS Institute. 1985. Sas User's Guide: statistics. Statistical Analysis System Institute, Cary, North Carolina.

Vita

Ryan Kennerly Smith was born on 18 October 1973 in Mission Viejo, California. He lived in Mission Viejo until 1992 when he graduated from Mission Viejo High School. In the fall of 1992 he moved to Fort Collins, Colorado where he lived for the next four years while attending Colorado State University. While obtaining a Bachelor of Science degree in fishery biology he was able to conduct research into the vegetation preference and thermal tolerance of the imperiled Arkansas darter. In 1996 he obtained his degree and moved on to Virginia Polytechnic Institute and State University for his Masters work.