EFFECTS OF EXCESSIVE SEDIMENT ON STRESS, GROWTH AND REPRODUCTION OF TWO SOUTHERN APPALACHIAN MINNOWS, *Erimonax monachus* AND *Cyprinella galactura*

by

ANDREW B. SUTHERLAND

(Under the direction of Judith L. Meyer)

ABSTRACT

North America's rich freshwater fish fauna continues to decline, in part due to excessive sedimentation in streams and rivers. The objective of this dissertation was to investigate the effects of elevated suspended sediment concentration (SSC) on the spotfin chub (*Erimonax monachus*), an imperiled southern Appalachian minnow, and on a surrogate species, the whitetail shiner (*Cyprinella galactura*). Using a multi-tank sediment-suspension apparatus, a whole-body cortisol assay and spectral confocal microscopy, I investigated the effects of SSC (0, 25, 50, 100, and 500 mg/L) on the stress response (cortisol concentration), specific growth rate (percent change in mass per day), and gill condition (i.e., lamellar thickness and interlamellar area) of young-of-year (YOY) spotfin chubs and whitetail shiners. I also examined the effects of SSC on the spawning success of whitetail shiners. In the upper Little Tennessee River (LTR), I determined spotfin chub spawning habitat character and extent and tested the feasibility of supplementing spawning through the creation of artificial spawning sites. In the laboratory the sediment-suspension apparatus maintained SSCs within 95% of target values, thus providing controlled conditions for these studies, while not producing excessive turbulence. Exposure of

YOY to elevated SSC caused a significant increase in cortisol levels, in both species and a significant decrease in growth rate at three life stages (2, 4, and 8 months of age). Increased SSC elicited a stress response in spotfin chubs 3-fold higher than controls; this response was similar to previous accounts of rainbow trout exposed to acute handling stress. For spotfin chubs, a 15-fold decrease in specific growth rate occurred at the highest SSC (500 mg/L). Gill damage observed by quantitative confocal microscopy was minimal at 0, 25, and 50 mg/L, moderate at 100 mg/L, and severe at 500 mg/L. Specific growth rate was significantly and inversely related to increasing gill lamellar thickness. Whitetail shiner spawning effort decreased from 88% in control tanks to 50% in 500 mg/L tanks. Total spawning output at 500 mg/L SSC was only 10% of that in controls, and fish delayed reproduction until SSCs were lower. The number of propagules spawned decreased significantly with increasing mean SSC above 25 mg/L. In the upper LTR, discharge is sufficient to increase SSCs above 100 mg/L > 50% of the year, and above 500 mg/L > 10% of the year. Therefore, SSC treatments that elicited negative effects on stress, growth, and spawning success are likely to be experienced by spotfin chub populations in the field. In the upper LTR, most spotfin chub spawning was located in swift, moderately deep bedrock riffles that were devoid of fine sediment. However, spotfin chub nests were also located in slow, shallow habitats with 25-50% fine sediment (< 2mm). Spotfin chub spawning was limited to ~ 4.4% of the riverbed; this is twice previous estimates. The distance between spawning habitat patches was ~ 10 – 100 m. The distance between localized groups of habitat patches ranged from 194 – 1840 m. Of 50 supplemented spawning rocks, one was used for a nest and two more were guarded by nuptial males. Spawning habitat enhancement may be an inexpensive means of increasing reproductive success among imperiled native fishes. Increased SSC was shown to negatively affect the stress response, growth rate, gill condition and spawning

success of spotfin chubs. SSCs used in these experiments are similar to those frequently encountered by spotfin chubs and other species. The sublethal effects documented here support the hypothesis that elevated suspended sediment contributes to the imperilment of southeastern native fishes.

INDEX WORDS: Upland minnow, Appalachian stream, Southeastern US, Turbidity, Cortisol production, ELISA, Gill histology, Spawning success, Reproductive habitat, Habitat enhancement, Imperiled fishes

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CHAPTER 1

INTRODUCTION

The decline of freshwater fauna continues to accelerate (Folkerts 1997, Richter et al. 1997). This 'quiet crisis' is especially acute for North America's freshwater fish fauna, with ~ 40% of species at risk (Master et al. 1998). Fish imperilment in North America increased by 31% in the 1980's (Williams et al. 1989) and has increased by 125% in the southeastern US in the past 2 decades (Warren et al. 2000). The southeastern US is particularly vulnerable because it is a hotspot of aquatic biodiversity and is experiencing rapid population growth (Burkhead et al. 1997, Master et al. 1998). Rapid urbanization, combined with poor land management and poor planning for the protection of species at risk, is causing an acceleration in the number of southeastern fishes vulnerable to extirpation or extinction (Neves and Angermeier 1990, Walsh et al. 1995, Warren et al. 2000). Over 35% of at-risk fish and mussel species in the US are located within two southeastern river systems, the Tennessee-Cumberland River basins and the Mobile River basin (Master et al. 1998).

Declining abundance and range of southeastern fish populations is inextricably linked to widespread lotic habitat degradation, fragmentation and loss (Walsh et al. 1995, Master et al. 1998, Warren et al. 2000, Burkhead and Jelks 2001). Habitat destruction is caused by excessive erosion and sedimentation, widespread reservoir construction, channelization, urbanization and other forms of pollution (Neves and Angermeier 1990, Warren and Burr 1994, Burkhead et al. 1997, Richter et al. 1997, Allan 2004). Of the many human activities that fragment and degrade aquatic habitat, excessive sedimentation is one of the most pervasive; over 40% of US river miles (USEPA 1990), and over 45% of river miles in the southern Appalachians (SAMAB 1996) are impaired by excess sediment. The destructive consequences of excessive sedimentation for fishes are well documented for salmonids and centrarchids (see reviews by Bruton 1985, Waters 1995, Newcombe and MacDonald 1991, Newcombe and Jensen 1996, Henley et al. 2000). However, the threat of excessive sedimentation to native non-game fishes has remained largely ignored by the general public and policy makers, and remains relatively unexplored by researchers (Burkhead et al. 1997, Burkhead and Jelks 2001). In particular, research on the effects of elevated suspended sediment concentration (SSC) on upland non-game fishes is lacking.

One of the objectives of this dissertation was to investigate the effects of elevated SSC on two species of southern Appalachian upland minnows, the spotfin chub (*Erimonax monachus*) and the whitetail shiner (*Cyprinella galactura*). To do this, I developed an experimental apparatus capable of maintaining SSCs up to 500 mg/L ($\leq 45\ \mu m$ diameter) in suspension for up to one week (Chapter 2). With this apparatus I tested the effects of increased SSC (0, 25, 50, 100, and 500 mg/L) on the stress response (whole-body cortisol concentration) (Chapter 3), and specific growth rate (percent change in mass per day) of young-of-year (YOY) spotfin chubs and whitetail shiners (Chapter 4). I also determined the effects of elevated SSC on the gill condition (i.e. lamellar thickness and interlamellar area) of YOY spotfin chubs (Chapter 4), and on the spawning success of adult whitetail shiners (Chapter 5). Spawning success was measured as spawning effort (the number of replicates where spawning occurred per treatment) and spawning output (number of propagules [clear eggs, eyed eggs and larvae] spawned).

In addition to elevated SSC, another pervasive and destructive impact of excessive sedimentation is the homogenization of stream substrate through the deposition of fine sediment (Walsh et al. 1995, Burkhead et al. 1997). Sediment deposition reduces endemic fish species, increases tolerant species and homogenizes fish assemblages on a regional scale (Scott and

Helfman 2001, Sutherland et al. 2002, Walters et al. 2003). Conversely, substrate heterogeneity is positively correlated with increased habitat quality and availability for all aquatic fauna (Lemly 1982, Berkman and Rabeni 1987, Lenat and Crawford 1994, Waters 1995), and with increased fish diversity (Gorman and Karr 1978).

Excessive sediment deposition and habitat homogenization is a primary cause of imperilment for ~ 40% of southeastern fishes (Etnier 1997). Imperilment within the southeast is linked to siltation of habitat because many fishes within this region are benthic feeders and spawners (Neves and Angermeier 1990, USFWS 1996, Burkhead et al. 1997; Johnston 1999). Benthic specialization, and benthic spawning in particular, is common for the majority of the 188 vulnerable, threatened or endangered fish species in the southeastern US (Warren et al. 2000, Burkhead and Jelks 2001). The spotfin chub is typical of imperiled, benthic-specialized, upland fishes of the southeast, species which rely on unembedded substrate for reproduction (Jenkins and Burkhead 1984, Jenkins and Burkhead 1994). Previous observations suggest that spotfin chubs spend a limited amount of time over sand-covered habitats and may completely avoid areas covered by sediment finer than sand (i.e. silt and clay; Jenkins and Burkhead 1994). Evidence suggests that this is especially true when spotfin chubs spawn; they seem to prefer silt-free crevices for breeding (McLarney 1989, McLarney 1990).

The USFWS spotfin chub recovery plan includes determining the impact of sediment deposition on habitat for all life stages (USFWS 1983). Before sediment-related impacts can be assessed, we must know what types of habitat the spotfin chub requires throughout its life history. Therefore, another objective of this dissertation was to determine the character and extent of suitable spawning habitat for spotfin chubs inhabiting the upper Little Tennessee River (Chapter 6), which is one of only five river systems still harboring the spotfin chub. In addition, artificial spawning sites were created to determine if this inexpensive method could be a useful way of mitigating the effects of excessive sedimentation on spawning habitat of small riverine fishes (Chapter 6). This research was designed to improve understanding of the mechanisms causing observed sediment-related declines in native fishes of the southeastern U.S.

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CHAPTER 2

A SIMPLE RECIPROCATING APPARATUS FOR MAINTAINING LONG-TERM TURBIDITY IN BIOLOGICAL EXPERIMENTS[1]

[1]A.B. Sutherland, Submitted to *Limnology and Oceanography: Methods* 3/25/05

Abstract

Elevated turbidity adversely affects the behavior, physiology, and distribution of marine and freshwater organisms. Although previous laboratory turbidity studies have varied in topic, scope, and design, they all require an experimental apparatus with the ability to maintain constant sediment concentrations (or turbidities) for extended periods of time. Some researchers have devised methods that work well but offer low replication at high cost. Many others rely on inexpensive means that perform poorly, except for short duration experiments. A reciprocating apparatus is described here which uses motor-driven paddles and compressed air to keep fine sediment in suspension for extended periods of time in numerous tanks simultaneously. This apparatus is a low-cost alternative to more complex systems. Also, this sediment suspension system does not produce excessive turbulence, which can be detrimental to small test organisms. With this apparatus, suspended sediment levels ranging from 25 – 500 mg/L were maintained within 95% of initial values for 7 days.

Introduction

Turbidity is a well-documented correlate of impairment in aquatic ecosystems (see Waters 1995, Newcombe and Jensen 1996). A growing body of research illustrates that increased turbidity can have adverse effects on both marine and freshwater organisms (Lemly 1982, Bruton 1985, Cyrus and Blaber 1987, Newcombe and MacDonald 1991, Lenat and Crawford 1994). Excessive sedimentation in aquatic systems negatively affects resident biotic communities directly and indirectly at multiple spatial scales (Roth et al. 1996, Allan et al. 1997, Jones et al. 1999). Many studies on the effects of sediment have focused on large-scale linkages between excessive sedimentation and the abundance, diversity, and structure of fish and macroinvertebrate assemblages (Berkman and Rabeni 1987, Richards et al. 1996, Wang et al. 1997, Lammert and Allan 1999, Sutherland et al. 2002). Many of these field and laboratory studies suggest that population- and assemblage-level effects of elevated sediment loading are in part caused by increased suspended sediment concentration (SSC). To determine the mechanisms behind these impacts, some researchers have investigated the effects of turbidity on growth, survival, stress response, foraging behavior and reproduction (Swenson and Matson 1976, Gradall and Swenson 1982, Sigler et al. 1984, Berg and Northcote 1985, Redding et al. 1987, Barrett et al. 1992, Gregory 1994, Burkhead and Jelks 2001). The majority of this research has been conducted using salmonids and other game fishes (see reviews in Waters 1995, and Newcombe and Jensen 1996).

Although the literature regarding game fishes is extensive, many unanswered questions remain regarding the effects to invertebrates, non-game fishes, and other vertebrates. Research on effects of turbidity on the physiology of aquatic organisms is limited. With the exception of research on commercial and game fishes, little information exists regarding the mechanisms regulating biological impacts of sedimentation.

Understanding how increased turbidity affects aquatic organisms (e.g., through physical abrasion, visual impairment, disruption of spawning cues, physiological stress, reduced growth) is necessary for development of science-based turbidity standards and wise land-use planning. Previous turbidity studies have varied in scope and design, yet each has required an apparatus that is able to maintain constant sediment concentrations (or turbidities). Here I describe an apparatus designed to keep fine sediment (< 45 μm) in suspension for extended periods of time in numerous experimental tanks simultaneously. This sediment suspension system is a low-cost alternative to large artificial stream systems. This apparatus allows for high replication and extended periods of sediment exposure at near constant turbidities.

Materials and Procedures

General Description

The design of this apparatus is not complex. It consists of a motor-powered drive mechanism that slowly moves a paddle within each of a variable number of experimental tanks (Figures 1 & 2). While two baffles on each paddle slowly sweep the floor of a given tank, the paddle also delivers a column of compressed air that resuspends settled particles. The number of experimental tanks that are possible with this design is a function of length of drive shaft, size of tanks and power of the motor.

The inspiration for the design of this apparatus came from a system commonly used to hatch and rear game fishes (pers. comm. E. Henderson; E & K Fisheries, Dearing, GA, USA). The basic premise behind the design is the use of a single motor to power a reciprocating drive shaft, to which multiple paddles are connected. In the fish-hatching prototype, these paddles are used to fan clutches of eggs of nest-guarding game fishes (e.g. channel catfish, *Ictalurus punctatus*). This fanning motion mimics parental behavior and provides the same vital function (i.e., oxygenation and removal of metabolic waste and sediment).

The apparatus described below is based on the same principle: the transfer of power from one source to many experimental units. In the fisheries prototype, single-baffle paddles move back and forth several inches above clutches of eggs in the bottom of a raceway. In the apparatus described here, paddles have been re-designed with two baffles that move along the bottom of individual experimental tanks, while delivering a slow-moving curtain of air (Figures 1 & 2). Similar to the fisheries hatching machine, paddles are connected to a central drive shaft that is powered by a variable-speed gear motor. Each time the paddle travels along the bottom, any sediment that has settled is resuspended. To aid in resuspension, compressed air is introduced into each paddle, emanating from the bottom through a series of small holes, thus creating a slow moving screen of air bubbles. This moving wall of diffuse air bubbles creates upward water movement, helping to resuspend larger particles and increase water oxygenation. This combination of air bubbles and slow sweeping action is sufficient to maintain a suspended sediment concentration of approximately 500 mg/L for extended periods, but is not so vigorous that it creates excessive turbulence.

The design of this apparatus is intrinsically flexible and lends itself to specific adaptation. The dimensions and materials for each component can be changed to suit the needs and resources of the investigator. Design details and construction specifications given below are for an apparatus used by the author in several turbidity experiments.

Detailed Description and Construction

Motor

The drive mechanism power source used in experimental trials of this apparatus is a Dayton® ½ HP, 5.8 amp, 90 volt DC variable-speed gear motor (Model 6Z413A; Dayton Electric Mfg. Co., Niles, IL, 60714, USA; Figure 1). This type of gear motor is fan cooled, which is preferable because high workloads for long durations can cause increased heating of the motor. In experimental trials with this apparatus, this motor easily powered 20 paddles in 38 L tanks. The motor was capable of powering all 20 paddles at very high speeds, indicating that it could have powered many more paddles at the low speed required for experimental trials. A variable-speed gear motor of this type is recommended, as it allows the investigator the ability to determine optimal paddle speed for maintaining a given turbidity without stressing test organisms. A speed controller, which can easily be connected to most gear motors, allows precise and replicable velocity. A Dart® speed controller was used in experimental trials of this apparatus (Model 253G-200E; Dart Controls Inc., Zionsville, IN, USA).

Because of high rotational force, the motor must be securely mounted with bolts to an immovable bench or table. If the motor is allowed to move, even slightly, the whole drive mechanism can become misaligned and unstable. This can cause sudden and destructive movement of drive shaft and breakage of drive mechanism, paddles, or tanks.

Connecting Arms

Two connecting arms transfer the circular motion of the gear motor to the reciprocating motion of the drive shaft and paddles (Figure 3). The distance traveled by the drive shaft and paddles is equal to twice the effective rotation radius (R) of the shorter connecting arm (arm A).

The effective radius of arm A is slightly smaller than its length and equals the distance from the center of the motor shaft to the center of the carriage bolt attaching connecting arm A to arm B. The effective radius of arm A can be determined by measuring the distance a paddle needs to travel to cover the bottom of a given tank, and then dividing this distance by two. This system therefore can be adapted to any length (or width) tank. This apparatus can also be adapted to a series of different sized tanks by simply determining the drive shaft travel distance based on the smallest tank (i.e., effective radius of arm A equals ½ length of smallest tank). To cover the distance of the longest tank(s) (and intermediate-length tanks) within this series of variable-length tanks, one must increase the width of each paddle frame (see Figure 4 and description below) to fit each individual tank. The length of arm B is not as precise, though it must be longer than arm A, so that the drive shaft does not hit the motor shaft upon rotation of arm A. If arm B is too long, there may be excessive flexing, which creates drive shaft instability. A length for arm B that has proven successful is approximately 1.5 to 2 times length of arm A.

Connecting arms are constructed of 0.64 cm steel to insure a minimal amount of flexing during operation. Flexing of the connecting arms can result in a sideways motion of the drive shaft, which increases friction and potential for apparatus breakage. Connecting arms are attached to motor, drive shaft, and each other using steel carriage bolts (1.27 cm x 10.16 cm). Arm A is attached securely to and rotates with the motor shaft (Figure 3). To attach arm A to the motor, a rigid steel coupling must first be attached to the motor shaft. To the other end of the rigid coupling is attached the smooth end of a carriage bolt, with the head removed. Arm A is then threaded onto the other end of the bolt and secured with a Teflon® coated nut. Carriage bolts 1 and 2 (see Figure 3) are threaded only on the last 2.5 cm of their length, allowing them to smoothly rotate within the two flange-mount ball bearings attached to connecting arm B. Teflon coated nuts are used to secure bolts 1 and 2. Teflon bearings are used wherever spacers are needed to separate or secure parts.

Drive Shaft

The purpose of the drive shaft is to simultaneously transport a variable number of paddles within the experimental tanks. The drive shaft is made of 2.54 cm schedule-80 PVC pipe. Small sections of pipe are connected together with 2.54 cm cross couplings to which paddles are attached (Figure 1). Using appropriate bushings, a 1.27 cm piece of pipe is attached to the 2.54 cm cross coupling creating a drive shaft arm. Paddles are then attached to this arm by using a metal screw (see Figure 4). Drive shaft arms are plugged with silicone sealant to prevent compressed air from escaping. The end of the drive shaft is capped with a standard rounded PVC cap. The drive shaft is connected to the connecting arms by using a 0.64 cm eyebolt (Figure 3). After drilling a hole in the cap, the threaded end of the eyebolt is secured within the cap with two Teflon coated nuts, one each on the inside and outside of the cap. The eye of the bolt is then held onto carriage bolt 1 by using Teflon bearings as spacers. This allows for smooth movement of the carriage bolt within the eye of the eyebolt, which in turn allows connecting arm B to move freely up and down.

The most important factor in the efficient performance of the drive shaft is precise alignment, which reduces friction and prevents apparatus breakage. Alignment of the drive shaft is achieved using several metal sleeves (Figure 1). Accurate alignment of each sleeve in all three planes is essential. Even a slight misalignment could result in undue friction, misalignment of paddles, or the breakage of one or more parts.

Also important to precise alignment is insuring that the drive shaft is not flexible. Because the drive shaft is made of PVC, it tends to bend vertically and horizontally under stress. To minimize flexibility, a steel rod is placed inside the entire length of the drive shaft. The diameter of the steel rod is slightly smaller than the inner diameter of the PVC shaft, creating a tight fit. The steel rod is kept in place using several metal screws that are screwed through the PVC shaft, serving as set-screws. A final way to minimize flexing is to increase the number of metal sleeves, through which the drive shaft passes. As well as providing reliable performance of paddles, precise drive shaft alignment can reduce the workload of the motor. By reducing friction, a given motor can operate more paddles. Conversely, if friction is reduced, a given number of paddles can be powered by a smaller motor.

As well as alignment, and reducing flexibility, a further way to reduce friction is by lubricating the path of the drive shaft. An effective lubricant is standard high-temperature, lithium, machine grease. Grease can be applied liberally to any surface encountered by the drive shaft. To prevent contaminating tank water, care must be taken to insure that no grease comes into contact with paddles or experimental tanks.

Paddles

The purpose of the motor, connecting arms and drive shaft are the efficient and reliable transport of the paddles. The paddles are the most crucial component of the apparatus and are responsible for maintaining turbidity levels in the experimental tanks. Whereas the motion of the baffles can suspend a large percentage of the finest particles, the energy used to suspend larger particles comes from the compressed air introduced into each paddle. The motor is essentially being used to power the movement of this air source. Compressed air is introduced through the top of the shaft of each paddle (Figure 4). The air travels down the PVC shaft and into the square paddle frame and emanates through small holes (diameter ~ 1.5 mm) drilled into the bottom of the frame. A vortex is created by these air bubbles, starting near the floor of the tank and traveling upwards. This slow-moving vortex creates an eddy, into which particles are drawn and resuspended.

Paddle dimensions are determined by the shape of experimental tank (Figure 4). The length (i.e., axis perpendicular to drive shaft) of each paddle is slightly smaller than the tank, so that it can move freely without making contact. The width (i.e., axis parallel to the drive shaft) is dependant on the distance traveled by the drive shaft per reciprocation. If, for example, laboratory space necessitates that the drive shaft moves only a short distance, the paddle width can be increased so that it travels the entire width of the tank. The height of the paddle shaft is dependant on the depth of the tank. To minimize friction, the paddle baffles should just barely make contact with the bottom of the tank. Ensuring that the paddle frame is aligned precisely parallel to the tank bottom to reduce friction at one or more points along the baffle is important. As with the drive shaft, ensuring that the paddle is precisely aligned in all three planes to prevent friction with tank and undue stress on drive shaft and motor is imperative.

The paddle frame is constructed of 1.27 cm schedule-40 PVC pipe and couplings. On top of the shaft is a PVC coupling that accepts a threaded tubing adapter. Air is introduced into each paddle through Nalgene® tubing that is connected to a compressed air source. The paddle is connected to the drive shaft arm by using a standard ‘T’ coupling. Easy removal of the paddle mandates that it should not be cemented to the drive shaft arm. Easy removal is necessary if tanks are to be cleaned, or if paddles need repair. To attach the paddle to the drive shaft arm, a small hole is drilled through the 'T' coupling and drive shaft, while they are aligned, and then they are secured with a metal screw.

Paddle baffles are made from standard weather stripping rubber. Testing paddle materials to pre-determine if they are inert is important; therefore several fish were reared for four months in tanks containing rubber weather stripping material. Cortisol levels were measured in these fish, and the weather stripping was found to have no affect on stress response (A. Sutherland, unpublished data). Stainless steel screws are used to attach baffles to paddles. Screw holes are sealed with silicone.

Tanks

Tanks used in experimental trials are standard rectangular 38-liter glass tanks. Deeper tanks require longer paddles shafts; longer and wider tanks require different shaped paddle frames. A requirement of all tanks, regardless of their dimensions, is that they remain immovable. To insure stability of the tanks, metal brackets can be secured to the lab bench and around the base of the tanks. This arrangement also allows precise realignment of tanks in the event that they need to be moved temporarily.

Assessment

Turbidity maintenance was determined during a 21-day growth trial of post-larval spotfin chubs (*Erimonax monachus*), a federally listed species. The apparatus was set up with 20 experimental tanks, with 4 replicates of 5 sediment concentrations ranging from 0 to 500 mg/L of silt and clay particles (< 45 μm). Turbidity (NTU) was measured daily with a portable Hach® Model 2100P turbidimeter and converted to suspended sediment concentration (SSC) by using a sediment rating curve determined for test sediment (Figure 5). Because of the inability to use bio-filtration during a suspended sediment experiment, metabolic wastes were removed by changing water weekly.

Suspended sediment concentrations were maintained within 90 - 95% of initial values for 7 days (Figures 6 & 7). The mean SSC for the highest treatment level (500 mg/L) remained within 94.3% of the initial concentration. The mean SSC for the 100 mg/L treatment ranged from 106.5 to 91.1 mg/L (91.1% of initial). The estimated mean SSC for the 100 mg/L treatment is slightly higher than 100 mg/L because of the variance associated with the rating curve (i.e., when creating rating curve, the turbidity values for 100 mg/L samples were less than 100 NTU; average = 92.3 NTU). The mean SSC for the 50 and 25 mg/L treatments remained within 95.2, and 89.6% of initial concentrations, respectively. The estimated mean SSC for the 0 mg/L treatment reached a high of 4.7 mg/L, despite the absence of sediment in these tanks. This increase in turbidity may be related to a combination of factors including addition of fish food, growth of bacteria, and presence of fish waste products in the water. Artifacts such as these will vary with experiment design and do not reflect the sediment suspension performance of the apparatus.

One of the initial concerns with this apparatus was that the moving paddle and curtain of air would stress experimental organisms. A series of stress trials were conducted on juvenile and post-larval whitetail shiners (*Cyprinella galactura*) housed in tanks containing this apparatus (Sutherland et al., unpublished data). In each test, the speed of the paddle was very slow (~ 5 mm/sec), yet sufficient to maintain the highest suspended sediment treatment level (~ 500 mg/L). Data suggest that there was not a significant stress response due to the apparatus being in the tanks. Stress hormone levels were not significantly different between fish reared in apparatus tanks (turbidity control tanks) and those not reared in apparatus tanks. Cortisol levels were < 5 ng/g for both groups of fish (Chapter 3). Shiners quickly adjusted to the presence of the slow moving wall of air. Many individuals repeatedly swam through the bubbles, riding the upward current. No adverse effect of the paddle movement was detected.

The apparatus described here is a closed system. Because the control and maintenance of turbidity precludes water column filtration, the buildup of metabolic wastes must be factored into experimental design (i.e., size and number of organisms per tank, volume of water/tank, duration of experiment, water temperature, and feeding rates). In addition, the constant screen of air bubbles, which creates the water movement necessary for sediment suspension, increases the concentration of dissolved oxygen in the water. Therefore this design may not be suitable if moderate or low oxygen concentrations are required.

Although this apparatus maintains suspended sediment (< 45 μm clay and silt) concentrations of near 500 mg/L for extended periods, upper limits of performance have not been established. One could reasonably assume that higher concentrations of particles could be held in suspension if they were of smaller size and/or different structure and composition.

Discussion

Field and laboratory turbidity studies have varied in topic, scope, and experimental design. However, all have required an experimental apparatus with the ability to maintain constant sediment concentrations for extended periods of time. Difficulty maintaining a constant turbidity for long duration is partially a function of sediment particle size. Consequently, many studies are conducted with very fine clay, even though the particle size distribution of near-bed suspended load in many rivers and streams is dominated by larger clay, silt and even sand

(Gordon et al. 1995). Studies that use natural, larger, locally available sediment are often of short duration (e.g. few hours to days; Redding et al. 1987, Barrett et al. 1992). Maintaining sediment in suspension is also influenced by sediment mineral composition. Some naturally occurring clays (e.g. kaolinite) are very cohesive and readily form larger particles, which makes maintaining constant turbidity difficult. Because of their structural properties and availability, some researchers use commercial-grade volcanic clays (e.g. montmorillonite-based bentonite), although in many areas it may not be representative sediment. Using larger clay and silt-sized particles is necessary to more closely replicate the conditions of near-bed suspended load or to test the effects of aspects of suspended sediment other than turbidity (e.g. particle scour of mica-based silt on gill tissue).

Efficient techniques have been developed that are able to maintain near-constant turbidity levels indefinitely. One apparatus uses a computer-controlled beam transmissometer to continually measure turbidity and add turbid water from a source tank as needed (Grecay 1989). Though elegant, complex methods such as this may be too costly if numerous tanks and high replication are needed. Other researchers have conducted turbidity tests in large artificial stream environments or in situ with channels constructed in or next to streams (Sigler et al. 1984, Berg and Northcote 1985, Barrett et al. 1992). Some of these researchers have had success maintaining near constant SSC (e.g. $\leq$ 3 g/L for 2 – 3 weeks; Sigler et al. 1984). However, because of logistical and cost concerns, these studies are usually limited to one or two channels. This approach limits the researcher to only a small number of treatment replications per trial.

Some researchers have developed less complex, less costly and easily replicable turbidity maintenance techniques where sediment is kept in suspension with water column agitation (Redding et al. 1987, Gregory 1994, Burkhead and Jelks 2001; see Grecay 1989). One problem with these methods is that the turbulence necessary to maintain high constant turbidity can create an inhospitable environment for test organisms resulting in artificial behavioral responses or increased stress in sensitive organisms. In addition, eliminating all non-turbulent areas (i.e., eddies where sediment settling occurs) is difficult; therefore, maintaining a near-constant turbidity for extended periods is also difficult. Some researchers are able to partially compensate for this difficulty by conducting short duration experiments (Berg and Northcote 1985, Breitburg 1988, Gregory 1994). However, if research goals require a longer-term exposure to turbidity (e.g. studying the effects of turbidity on growth or spawning behavior), then short-term methods are not sufficient.

The device described here is designed to keep fine sediment (< 45 μm) in suspension for extended periods of time in numerous experimental tanks simultaneously. This sediment suspension system allows for high replication and is an alternative to complex costly laboratory techniques and large flow-through systems.

Comments and Recommendations

The objective of this paper was to present a simple, flexible device that will enable efficient turbidity-related biological research. The design presented here is an adaptable model that is functional and cost effective. The total cost for the 20-tank system described above was $895 (Table 1), excluding the cost of the compressed air source. The most expensive piece of equipment is the variable-speed gear motor, whose size and cost will vary with the number of paddles being transported. Because additional drive shafts and tanks can be easily connected to the connecting arms, expanding the design to include more experimental tanks does not markedly increase the cost.

This design is offered as a framework upon which to make improvements. One modification that may improve its performance is replacement of the metal sleeves with linear ball bearings and replacement of the drive shaft with a high precision linear bearing shaft. These changes would markedly reduce friction, thereby increasing the number of paddles (and thus replicates) that a given motor could operate. Multiple drive shafts could also be powered from one motor, increasing the number of treatments and replicates, without markedly increasing costs.

Although aquatic scientists agree that excessive sedimentation negatively affects aquatic communities, quantifiable relationships between sediment concentration and effect remain elusive. However, understanding these relationships is vital when developing scientifically based turbidity standards. The apparatus described here offers a cost-effective approach for quantifying the response of aquatic organisms to suspended sediment.

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Table 2.1: Cost of 20-tank sediment suspension apparatus used in growth and stress trials with juvenile and post-larval fishes. Cost of compressed air source not included.

Apparatus Sub-System	Part	Quantity	Cost per each	Total Cost
Power Source	motor	1	$300	$300
	speed controller	1	$50	$50
	hardware	-	$5	$5
Connecting Arms	steel arms	2	$10	$20
	ball bearings	3	$25	$75
	hardware	-	-	$10
Drive Shaft	PVC pipe and couplings	-	$50	$50
	metal sleeves	5	$2	$10
	lithium grease	1	$10	$10
	hardware	-	-	$5
Paddles	PVC pipe and couplings	-	-	$100
	rubber for baffles	40 ft	$0.50/ft	$20
	flexible tubing	40 ft	$0.25/ft	$10
	hardware	-	-	$10
Tanks	30 liter glass tanks	20	$10	$200
	brackets	80	$0.25	$20
Total Cost				$895

Figure 2.1: Diagram of sediment suspension apparatus including enlargement of drive mechanism, drive shaft cross coupling, and arm. See text for detailed description.

Figure 2.2: Photograph of sediment suspension apparatus in use.

Figure 2.3: Diagram of drive mechanism as seen from above, showing how motor, connecting arms and drive shaft are attached to each other. See text for detailed description.

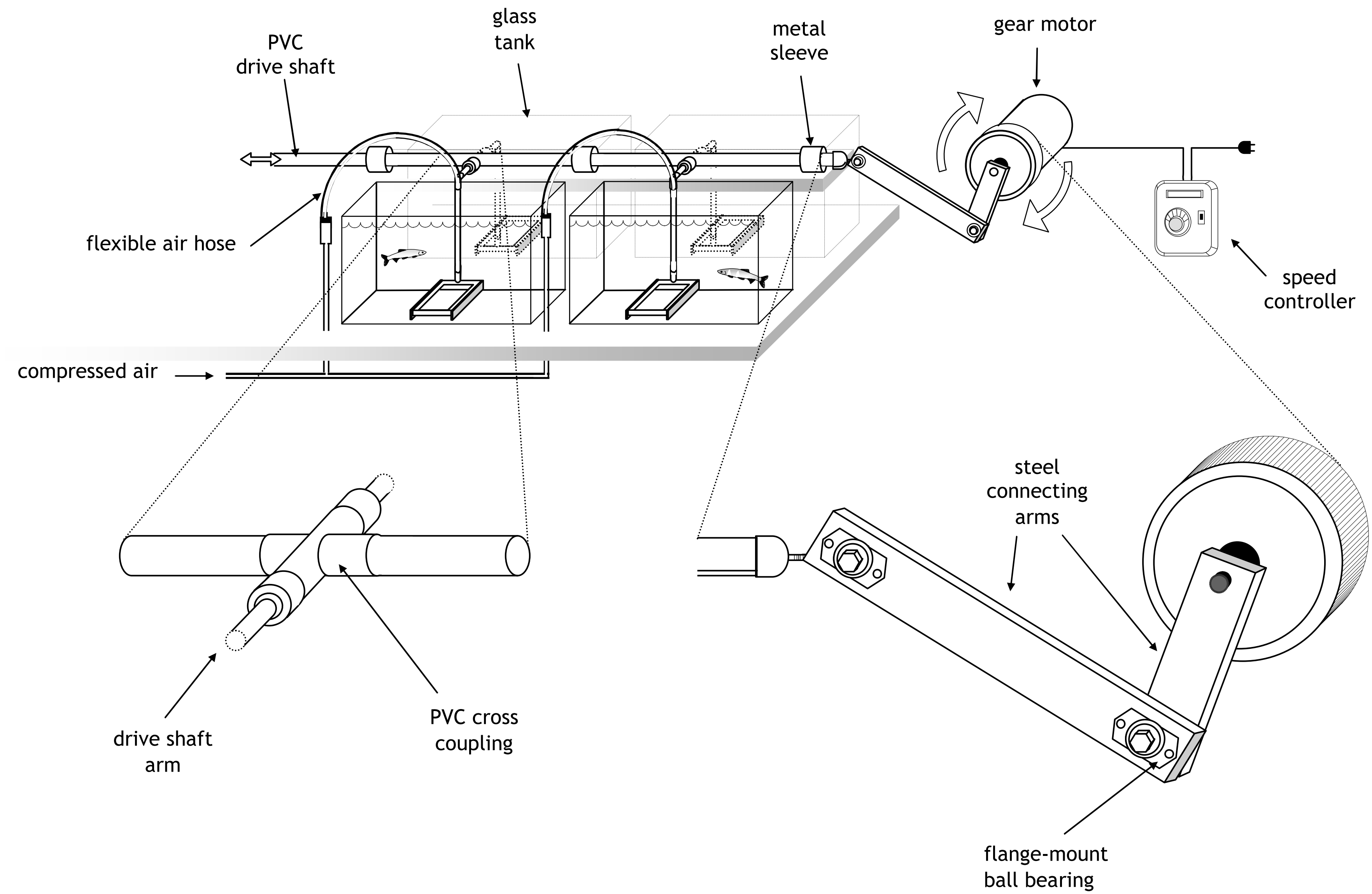
Figure 2.4: Diagram of paddle assembly, showing paddle frame, baffles, air line and attachment to drive shaft arm. See text for detailed description.

Figure 2.5: Sediment rating curve describing the relationship between turbidity (NTU) and suspended sediment concentration (SSC; mg/L) for sediment used to test apparatus performance. Turbidity for the rating curve was measured for twenty samples each of 7 SSC treatment levels (0, 10, 25, 50, 100, 250, and 500 mg/L). The relationship between NTU and SSC is described by the following equation: SSC = 1.2316(NTU) – 6.8426; r^2 = 0.99. Error bars represent standard error (n = 20).

Figure 2.6: Mean suspended sediment concentrations (SSC) for 25 and 50 mg/L sediment treatments, measured during 21 day spotfin chub growth trial. SSC (mg/L) were calculated from measured turbidity (NTU) using a sediment rating curve (Figure 4). Dotted lines indicate weekly water and sediment changes. Scale differs in each panel.

Figure 2.7: Mean suspended sediment concentrations (SSC) for 100 and 500 mg/L sediment treatments, measured during 21 day spotfin chub growth trial. SSC (mg/L) were calculated from measured turbidity (NTU) using a sediment rating curve (Figure 4). Dotted lines indicate weekly water and sediment changes. Scale differs in each panel.

Figure 2.1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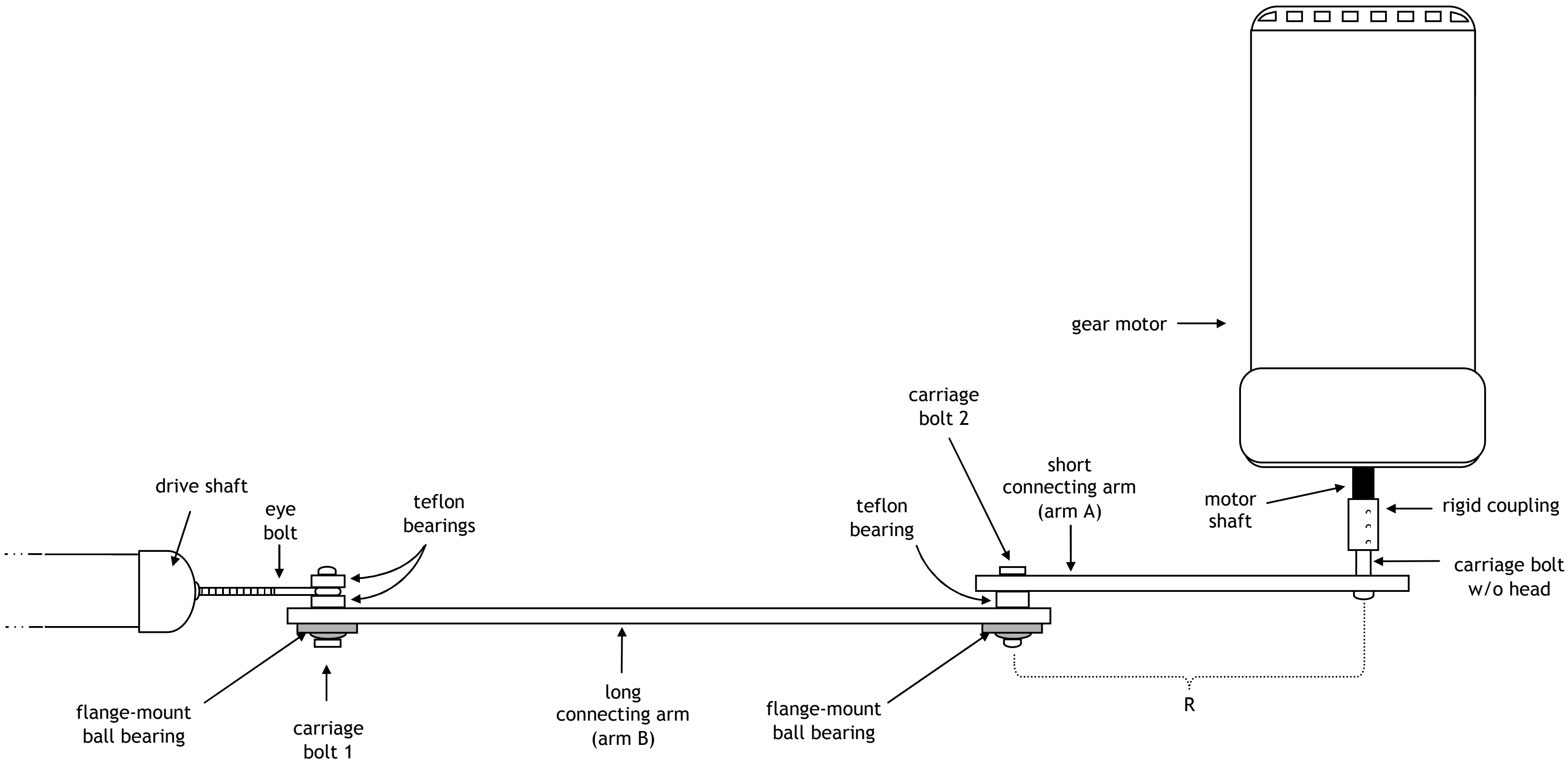
tank
PVC
drive shaft
metal
sleeve
gear motor
flexible air hose
speed
controller
compressed air
steel
connecting
arms
drive shaft
arm
PVC cross
coupling
flange-mount
ball bearing

Figure 2.2

Figure 2.3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carriage
bolt 2
short
connecting arm
(arm A)
motor
shaft
rigid coupling
drive shaft
eye
bolt
teflon
bearings
teflon
bearing
carriage bolt
w/o head
flange-mount
ball bearing
carriage
bolt 1
long
connecting arm
(arm B)
flange-mount
ball bearing
R

Figure 2.4

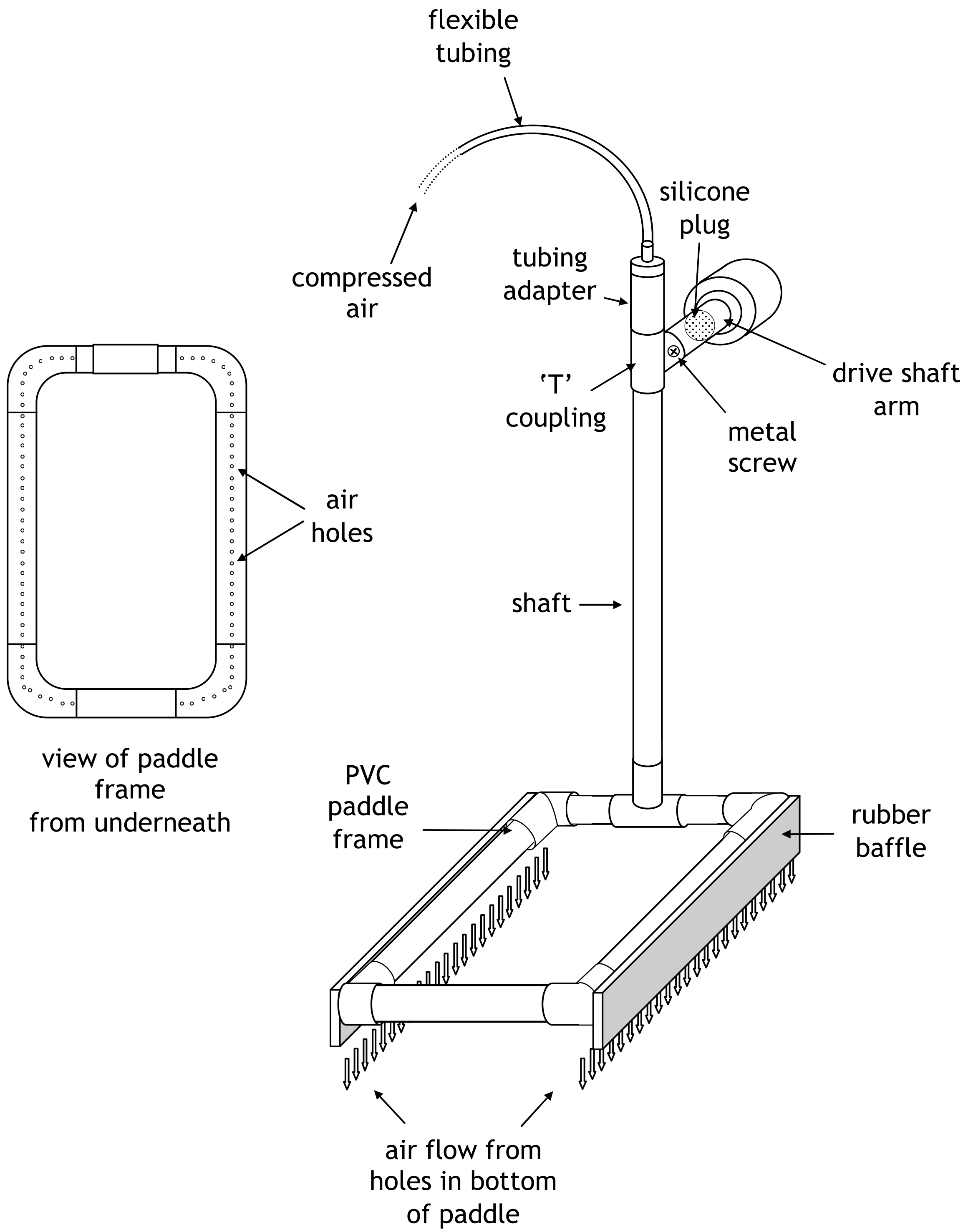
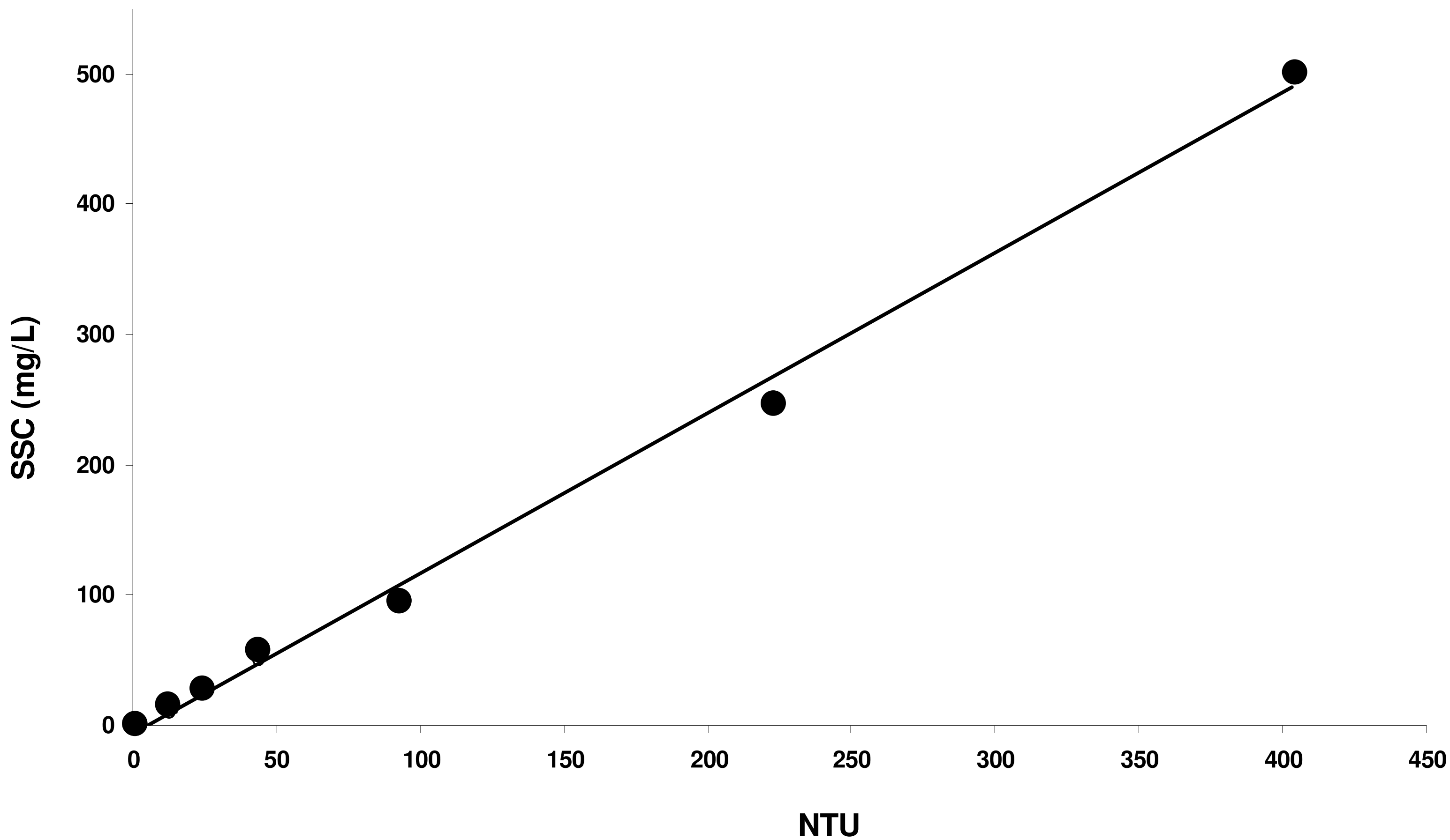
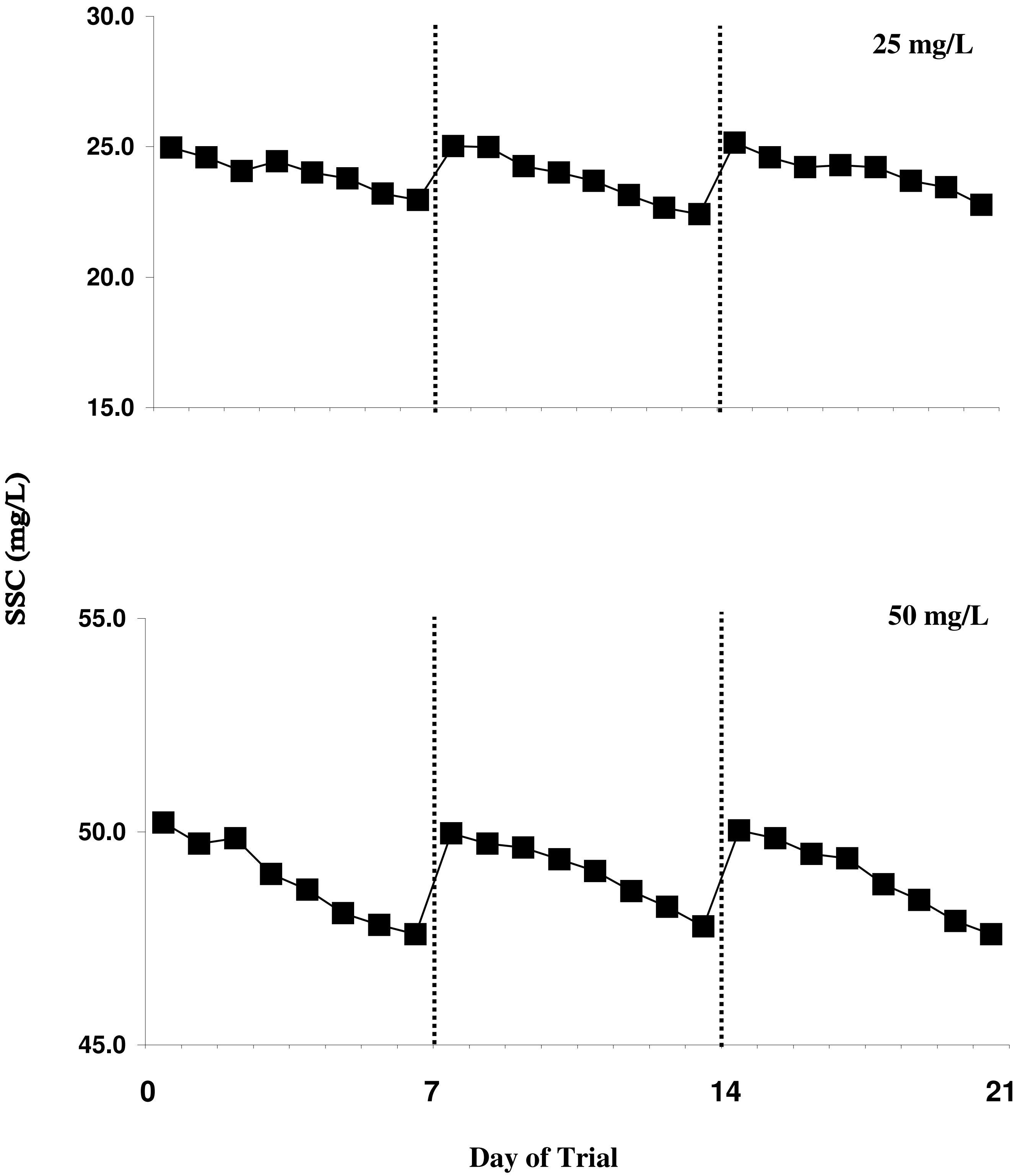

flexible
tubing
compressed
air
silicone
plug
tubing
adapter
'T'
coupling
drive shaft
arm
metal
screw
air
holes
shaft
view of paddle
frame
from underneath
PVC
paddle
frame
rubber
baffle
air flow from
holes in bottom
of paddle

Figure 2.5



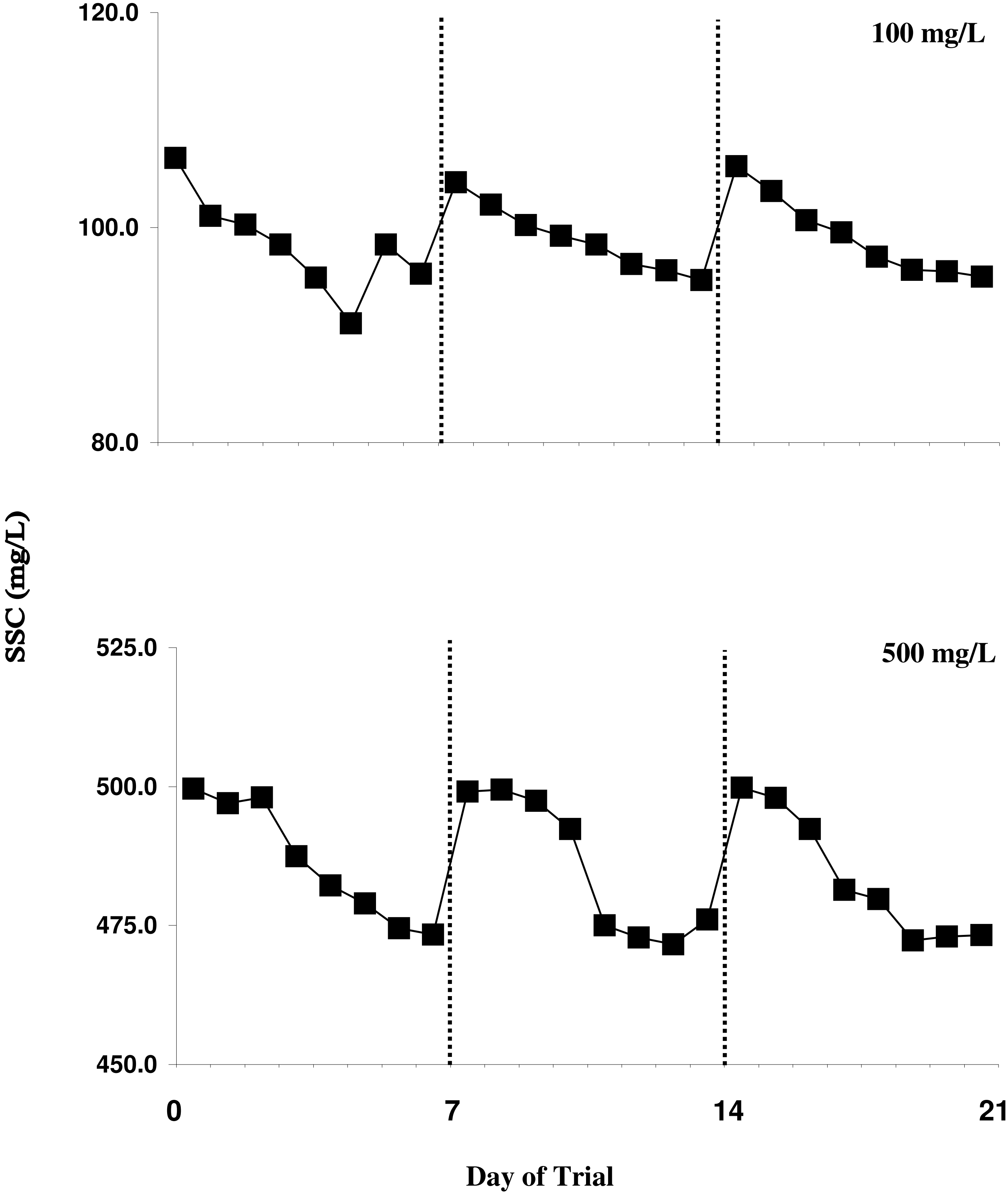
SSC (mg/L)
500
400
300
200
100
0
0
50
100
150
200
250
300
350
400
450
NTU

Figure 2.6



30.0
25.0
20.0
15.0
25 mg/L
SSC (mg/L)
55.0
50.0
45.0
50 mg/L
0
7
14
21
Day of Trial

Figure 2.7


120.0
100.0
80.0
100 mg/L
SSC (mg/L)
525.0
500.0
475.0
450.0
500 mg/L
0
7
14
21
Day of Trial

CHAPTER 3

EFFECTS OF INCREASED SUSPENDED SEDIMENT CONCENTRATION ON THE STRESS RESPONSE OF TWO SOUTHERN APPALACHIAN MINNOWS, *Erimonax monachus* AND *Cyprinella galactura*[2]

[2]A.B. Sutherland, J. Maki, and V. Vaughan. To be submitted *Canadian Journal of Fisheries and Aquatic Sciences*

Abstract

A primary stress response (i.e., relative increase in whole-body cortisol concentration adjusted for fish mass; ng/g) was measured in 2- and 8-months old whitetail shiners (*Cyprinella galactura*) and 4-months old federally threatened spotfin chubs (*Erimonax monachus*) exposed for 48 hours to increased suspended sediment concentrations (SSC; 0, 25, 50, 100, and 500 mg/L). Hydrophobic fractions were extracted from individual frozen fish after sonication and centrifugation of tissues. Extracts were resuspended in a buffer compatible with a commercially available enzyme-linked immunosorbent assay (ELISA) kit. Serially diluted concentrations of human cortisol and extracts collected from unstressed fish were used to standardize the assay. Two-months old whitetail shiners had the highest resting level of cortisol at 0 SSC and elicited the greatest response (3- to 4-fold increase) when exposed to SSCs > 25 mg/L. Resting cortisol levels were lowest in 8-months old whitetail shiners and levels remained similar to control fish at 25, 50, and 100 mg/L SSC. Four-months old spotfin chubs showed a non-linear response with a possible threshold effect between 50 and 100 mg/L. At SSC > 100 mg/L the spotfin chub demonstrated a 3-fold increase in cortisol levels over control fish. Exposure to SSC levels > 100 mg/L caused a significant increase in cortisol levels above baseline in both species and in all three life stages. This investigation shows that cortisol levels in young minnows increase dramatically upon exposure to SSCs > 25 mg/L. These data suggest that even moderate levels of suspended sediment (i.e. 100 mg/L) can severely stress young-of-year spotfin chubs. The imperilment of spotfin chubs may in part be due to stress imposed on young fish by elevated suspended sediment.

Introduction

Understanding the effects of excessive deposited and suspended sediment is critical to the maintenance and recovery of much of the threatened aquatic fauna in the southern Appalachians and throughout North America (Waters 1995, Burkhead et al. 1997, Warren et al. 2000). Siltation and turbidity negatively affect over 40% of impaired river miles in the US (USEPA 1990). In the southern Appalachians siltation affects over 45% of impaired stream miles (SAMAB 1996). Excessive sedimentation negatively impacts the community structure, diversity and abundance of stream fishes (Walsh et al. 1995, Waters 1995, Newcombe and Jenson 1996, Burkhead et al. 1997, Warren et al. 2000). An extensive body of literature focuses on the indirect impacts of sediment-induced habitat homogenization and fragmentation on fish populations and assemblages (Warren and Burr 1994, Walsh et al. 1995, Burkhead et al. 1997, Scott and Helfman 2001, Walters et al. 2003). Many studies also describe the direct effects of increased sediment on behavior, growth and mortality of fishes, primarily salmonids and other game fishes (Sigler et al. 1984, Redding et al. 1987, Newcombe and MacDonald 1991, Magee et al. 1996). However, research involving the direct effects of increased sediment on non-game fishes is limited (Gradall and Swenson 1982, Burkhead and Jelks 2001). This paucity of research does not reflect the need for sediment-related research on non-game fishes. The continual decline of North America's non-game fishes (Warren et al. 2000, Warren and Burr 1994), coupled with the increasing awareness of the primary role of sediment as pollutant (Waters 1995) argues for improved understanding of the effects of increased sediment on this diverse fauna.

Negative impacts of stress on fish have been well documented, and include abnormal behavior, immunosuppression, and reductions in growth rate, egg production, thermal tolerance, and swimming stamina (Wedemeyer 1984, Davis et al. 1985, Schreck 1990, Schreck et al. 1997). Stress-induced immunosuppression (e.g., reduction in antibody and macrophage production) has been associated with increased susceptibility to disease and increased mortality (Pickering and Duston 1983, Helfman et al. 1997). While much research has been conducted to determine the primary neuro-endocrine responses of fish to external stressors, the vast majority of these studies focus on stressors associated with intensive fish culture (e.g., artificial environment, artificial diet, and handling; Donaldson 1981, Schreck 1981, Wedemeyer et al. 1984, Barry et al. 1993, Barry et al. 1995). Some have also documented the effects of specific point source environmental pollutants (e.g., chemical spills, industry effluent) on stress in fish (McLeay and Gordon 1977). Much of the research on direct effects of suspended sediment on fishes has focused on mortality of various life stages of salmonids after chronic exposure (Newcombe and MacDonald 1991).

Little is known about sediment impacts on non-salmonids, or the direct effects of sediment as a sub-lethal environmental stressor of fish (see Redding et al. 1987, Servizi and Martens 1992). Of the few studies that look at direct physiological effects of excessive sediment, all focus on salmonid species. A positive correlation between suspended sediment concentration (SSC; mg/L) and stress has been observed at relatively high SSC (low treatment = 400 – 600 mg/L; high treatment = 2000 – 3000 mg/L; Redding et al. 1987). No research has evaluated the sediment-induced stress response using young-of-year (YOY) fish, non-game species, or low SSCs (10s – 100s mg/L).

Environmental stress activates the pituitary-interrenal axis in fish, causing the release of catecholamine and corticosteroid hormones (Mommsen et al. 1999). In fish, cortisol is the principal corticosteroid released during stress. Cortisol concentration in blood plasma rises dramatically and causes a cascade of metabolic changes within the stressed individual (Thomas 1990, Mommsen et al. 1999). Cortisol is a commonly used indicator of stress in fish because there is a direct positive relationship between exposure to environmental stressors and cortisol production (Barton and Iwama 1991, Mommsen et al. 1999). Furthermore, studies show that there is a strong relationship between high corticosteriod production, immunosuppression and susceptibility to disease (Pickering 1984, Thomas and Lewis 1987). Finally, cortisol can serve as a biochemical indicator of stress because it is relatively easy to measure (i.e., as opposed to measuring stress-related changes in metabolism).

While there are commercially available enzyme-linked immunosorbent assay (ELISA) kits available for human research, researchers measuring cortisol levels in fish have previously developed their own assays (Caldwell et al. 1990, de Jesus et al. 1991, and Barry et al. 1995). However, the usefulness of these commercial kits for measuring cortisol in non-human vertebrates such as fish has not been explored. This study tests the validity of using a commercial human-plasma ELISA kits for measuring cortisol levels in fish, and the usefulness of these kits for measuring cortisol in whole-body (i.e., homogenized) fish samples.

The spotfin chub (*Erimonax monachus*) is typical of imperiled fishes in the southern Appalachians and elsewhere. Once widespread throughout clear upland rivers in the upper and middle Tennessee River system, both their abundance and distribution have declined over the past century, due in large part to human-induced sedimentation (Jenkins and Burkhead 1984, USFWS 1996). The sporadic occurrence and declining population densities of the spotfin chub have resulted in their placement on the U.S. Fish and Wildlife Service (USFWS) list of threatened species. As part of the USFWS spotfin chub recovery and maintenance effort (USFWS 1983), we investigated the effects of excessive sedimentation on *E. monachus*. We examined the effect of exposure to increased suspended sediment concentration (0, 25, 50, 100 and 500 mg/L) for 2 days, on the primary stress response (i.e., whole-body cortisol concentration) of 4-months old spotfin chubs, as well as 2-months and 8-months old whitetail shiners (*Cyprinella galactura*), a phylogenetically similar surrogate for the spotfin chub.

Materials and Methods

Fish Propagation and Husbandry

Initial stress trials were conducted to test experimental methodology using the whitetail shiner as a surrogate for the spotfin chub. The whitetail shiner is phylogenetically similar to the federally threatened spotfin chub which was until recently placed within the satinfin shiner group (i.e., *Cyprinella spp*.; Jenkins and Burkhead 1994). Rationale for using the whitetail shiner as a surrogate is based on the fact that the only known hybridization of the spotfin chub was with a whitetail shiner (Burkhead and Bauer 1983). In addition spotfin chubs share scale morphology, osteology, spawning habits, and secondary sexual characteristics with the genus *Cyprinella* (Jenkins and Burkhead 1994). These similarities apply principally to members of the *whipplei* clade, which includes *C. galactura* (Mayden 1989). The physiological responses of these two closely related fishes are expected to be similar.

Young-of-year (YOY) whitetail shiners were propagated in the laboratory from adults collected in the upper Little Tennessee River (Swain Co. and Macon Co., NC). Captive breeding and propagation techniques employed were similar to those used by others for crevice spawning *Cyprinella* species (Gale and Gale 1977, Rakes et al. 1999). However, in addition to using stacks of unglazed ceramic tiles (as is common for spawning *Cyprinella*), whitetail shiners also spawned readily in standard pleated filter cartridges designed for aquatic ultraviolet sterilizers. Filter cartridges work well because they fit inside standard hatching jars, making it unnecessary to remove eggs from the spawning substrate, thereby reducing possibility of egg damage. Substrate type (tile versus cartridge) seemed less important than flow velocity to whitetail shiners when choosing spawning location in spawning tanks. To induce spawning, photoperiod and temperature were set to simulate late summer conditions (15 hours daylight; 26° - 28° C). A submersible pump (2850 liters/hour) was placed 30 cm from and directed towards spawning substrate. Eggs were hatched in standard hatching jars and each cohort of larvae was reared in a separate 30 liter flow-through tank. YOY fish were fed a diet of brine shrimp nauplii (*Artemia spp*.) and a high-protein micro-encapsulated commercial starter diet (< 100 µm; Zeigler® larval diet). Prior to each experiment the fish were allowed to acclimate to the apparatus for 96 hours. During this time period, 8-months old fish were fed a diet of dry pelleted Purina® AquaMax (D04; 1.5mm), and 2-months old fish were fed Zeigler® larval diet (< 400 µm) , at a daily rate of 1% initial body mass. Initial body weights, used to determine feeding rates, were determined by weighing 30 haphazardly chosen fish from the same cohort as the experimental fish.

Spotfin chub (*Erimonax monachus*) larvae were obtained from Conservation Fisheries Inc. (CFI; Knoxville, TN). Larvae were propagated at CFI from adults collected in the Buffalo River (Lewis Co., TN). Upon receipt, larvae were reared in 30-liter flow-through tanks. Young-of-year were fed a diet of brine shrimp nauplii and Zeigler® larval diet (< 100 µm). Before being used in experiments, spotfin YOY were reared until they were approximately four months old. This ensured their transition from benthic to pelagic habits, thereby minimizing potential stress caused by the experimental apparatus.

Suspended Sediment Experimental Apparatus

The experimental apparatus consisted of a motor-powered drive mechanism that slowly (~ 3 – 5 mm/sec) moved a paddle within each of 20 experimental tanks (Chapter 2). While two baffles on each paddle slowly sweep the floor of a given tank, the paddle also delivers a column of compressed air that resuspends settled particles. The combination of air and slow sweeping action is sufficient to maintain a suspended sediment concentration of approximately 500 mg/L for extended periods, but is not vigorous enough to create excessive turbulence. See Chapter 2 for a detailed description and performance analysis of the experimental apparatus.

Test Sediment

Sediment used in the stress experiments was collected from the Little Tennessee River basin (Macon Co., NC). Test sediment was determined to be free of organic pesticides and heavy metals (Appendix 1) by the Soil, Plant and Water Laboratory at the University of Georgia, College of Agricultural and Environmental Science (Athens, GA). The sediment was wet-sieved to obtain the < 45 μm fraction, because those are the largest particles that can be kept continually in suspension in the experimental apparatus (see Chapter 2). This sediment fraction is similar to the size of suspended sediment transported in the Little Tennessee River during baseflow (USGS 2001). Suspended sediment concentrations used in this study (0 – 500 mg/L) are within the range of conditions observed in the Little Tennessee River (turbidity range: 10 – 1500 mg/L, W. O. McLarney unpublished data).

Stress Trials

YOY whitetail shiners were exposed to one of five suspended sediment concentrations (0, 25, 50, 100 and 500 mg/L) for 48 hours and then whole-body cortisol concentration was measured. In each of two trials, 40 fish from the same cohort were randomly chosen from holding tanks and placed in the tanks (i.e., 2 fish per tank * 4 tanks per treatment level * 5 treatments * 2 trials = 80 fish). Before each stress trial began, the fish were allowed to acclimate to the apparatus for 96 hours. After acclimation, fish were exposed to sediment treatments for 48 hours. The stress trial duration was chosen after analysis of the 2001 summer hydrograph for the Little Tennessee River, which suggested that the majority of stormflow events (and thus suspended sediment pulses) last for approximately two days.

Fish were anesthetized with eugenol (i.e., clove oil) at the end of each stress trial. Ten ml of a 5:1 eugenol-ethanol mixture was added to each tank; this concentration achieved anesthetization within 2 minutes. This rate of induction was deemed acceptable, as other fish species have been shown not to experience a rise in cortisol within the first 3 to 5 minutes of exposure to a stressor (Dr. Terence Barry, University of Wisconsin-Madison, pers. comm.). Eugenol was used instead of Tricaine Methane Sulfonate (MS-222) because MS-222 took > 5 minutes to anesthetize fish and caused noticeable agitation and distress. Within 3 minutes of adding eugenol to the tanks, fish were placed into pre-weighed 20 ml glass scintillation vials (containing 1 ml distilled water), flash frozen in a dry ice/ethanol bath and stored at – 80°C. Prior to cortisol extraction, vials containing fish were weighed to determine fish weights, which were used to normalize cortisol levels.

For 8-month old whitetail shiners, cortisol was extracted from the largest of the two fish in each tank (i.e., 4 replicates * 5 SSC * 2 trials = 40 fish). Due to the small size of 2-month old whitetail shiners, cortisol was extracted from homogenates of both fish in each tank. Next, four trials were conducted using 4-month old spotfin chubs, using the same experimental procedures described for whitetail shiners. Cortisol was extracted from the largest of the two fish in each tank.

Cortisol Extraction

Due to the small size of the young fish used in these experiments, cortisol was extracted from whole-fish homogenates. Previous studies measuring cortisol in whole-fish extracts are limited (Hwang 1992, Barry et al. 1995), and have not been previously attempted using commercial ELISA kits. Frozen fish were homogenized by ultrasonication for 2 minutes (Heat Systems-Ultrasonics Inc. sonicator; setting 12) and immediately refrozen in a dry ice/ethanol bath. Diethyl ether (10 ml; Dr. Terence Barry, University of Wisconsin-Madison, pers. comm.) was added to each vial and contents thawed at room temperature and vortexed three times for 1 minute. The samples were centrifuged (1000g for 5 minutes) and the water phase frozen by placing samples in the freezer at – 80°C. The ether layer was decanted into 20 x 150 mm test tubes, which were placed into a 45°C water bath. The ether was evaporated under a stream of nitrogen gas, because cortisol breaks down when exposed to oxygen (Dr. Terence Barry, University of Wisconsin-Madison, pers. comm.). The resulting hydrophobic residue was dissolved in the extraction buffer (250 µl) provided in each ELISA cortisol kit (EA 65, Oxford Biomedical Research, Oxford, MI), and stored for less than one hour at 7°C.

ELISA

Cortisol was measured using a commercially available 96-well microtiter plate enzyme-linked immunosorbent assay developed for measuring cortisol levels in human blood plasma (ELISA kit EA 65; Oxford Biomedical Research, Oxford, MI). The test kit is a competitive assay, based on competition between the provided enzyme conjugate and sample cortisol, for a limited number of binding sites on an anti-cortisol rabbit antibody-coated plate. Fish cortisol levels were measured according to the standard protocol developed by the test kit manufacturer. The fish homogenates were resuspended in buffer and serially diluted in wells containing additional buffer. Following incubation, the wells were washed and the enzyme-linked reagent (cortisol horseradish peroxidase) was added. Following a second incubation and rinsing, the enzyme substrate (Tetramethylbenzidine; TMB) was added and color developed for 30 minutes. Plates were read on a spectrophotometer (650 nm) and absorbance values recorded.

The assay was validated for measuring cortisol in four whole-fish extracts by insuring that serial dilutions of samples inhibited the binding of cortisol in parallel with kit standards (i.e., binding of whole-fish extract cortisol was similar to that of test kit cortisol). The difference between slopes of fish extract serial dilutions and standard curves generated using human cortisol reference samples (i.e., standards made from kit cortisol) were compared using analysis of covariance (ANCOVA). Assay precision was evaluated by calculating the coefficient of variation (%) for four repeated measures of each sample extract for two whitetail shiner trials. Inter-assay variability was assessed by calculating the coefficient of variation for standard curves assayed on four different ELISA plates.

Data Analyses

Cortisol concentrations were compared using analysis of variance (ANOVA; JMP, SAS Institute, Cary, NC). Means of all pairs of treatments were compared using the Tukey-Kramer test ($\alpha = 0.05$). Differences among experimental trials were determined using ANOVA blocked by trial, after using Levene's test to assure that group variances were equal. Differences in magnitude of stress response (i.e., cortisol concentration) were determined between species (i.e., whitetail shiners versus spotfin chubs) and between life stages (i.e., 2-month old versus 8-month old whitetail shiners) using two-factor ANOVA. If two-factor ANOVA showed a significant effect, each treatment was compared (i.e., between species and between life stages) using the Student's t-test. The difference in the relationship between SSC and cortisol production (i.e., the difference in regression slopes) was also determined for life stages and species using ANCOVA. Linear regression was used to determine if there was a significant relationship between suspended sediment concentration and cortisol level.

Suspended sediment concentrations (SSC) were not held constant during stress trials. A small percentage of sediment settled during the course of each trial. Sediment settling was estimated during separate growth trials. See chapter 2 for sediment settling curve details. Sediment settling curve data were used to estimate the average SSC to which fish were exposed during the course of a growth trial. Linear regression analyses were conducting using estimated SSC instead of initial amount of sediment added.

Results

ELISA Validation

A commercially available human-cortisol ELISA kit was successfully employed to measure cortisol in whole-fish extracts. Slopes of serially diluted samples on four ELISA plates were not significantly different from standard curves (Figure 1). Intra-assay variability was fairly high for the first trial but decreased below 10% for most samples in the second trial as experimental techniques were perfected.   The reproducibility of the assay, calculated as inter-assay coefficient of variation (CV), was within acceptable limits: the average inter-plate CV of samples containing 0.1, 1.0, and 10.0 ng/ml of cortisol were 5.3, 7.9 and 7.1%, respectively. Coefficients of variation below 10% are considered acceptable (Dr. Terence Barry, University of Wisconsin-Madison, pers. comm.).

Cyprinella galactura (2-months old)

Whole-body cortisol concentration of 2-months old whitetail shiners did not differ significantly between experimental trials (ANOVA; $P = 0.411$; Table 1). Whole-body cortisol concentration was significantly different among suspended sediment treatments (ANOVA; $P < 0.0001$; Table 1). Cortisol level was not significantly different among the three lowest treatment levels (Figure 2), or among the three highest treatments (Tukey-Kramer multiple comparison; $\alpha = 0.05$). Although cortisol variability was high at each treatment level, there was a steady increase in mean cortisol concentration with increasing suspended sediment concentration (Figure 2). The relationship between 2-months old whitetail shiner whole-body cortisol concentration and increasing SSC was significant (Figure 3, $R^2 = 0.46$, $P < 0.0001$).

Cyprinella galactura (8-months old)

Whole-body cortisol concentration of 8-months old whitetail shiners did not differ significantly between experimental trials (ANOVA; $P = 0.275$; Table 1). Whole-body cortisol concentration was significantly different between suspended sediment treatments (ANOVA; $P < 0.0001$; Table 1). Variance in whole-body cortisol concentration (ng/g) was homogeneous among trials (ANOVA; $P = 0.111$; Table 1) and among treatments (ANOVA; $P = 0.292$; Table 1).

Cortisol level was not significantly different among the three lowest treatment levels (Figure 2), or between the 50 and 100 mg/L treatments (Tukey-Kramer multiple comparison; $\alpha = 0.05$). The highest treatment (500 mg/L) elicited significantly higher cortisol production than all other treatment levels; cortisol concentrations were approximately twice as high as those at 100 mg/L, and approximately 5-fold higher than the control. Whole-body cortisol concentrations increased with increasing SSC (Figure 3, $R^2 = 0.56$, $P < 0.0001$).

Erimonax monachus (4-months old)

Whole-body cortisol levels of 4-months old spotfin chubs did not differ significantly between trials (ANOVA; $P = 0.728$; Table 1). Whole-body cortisol concentration was significantly different among suspended sediment treatments (ANOVA; $P < 0.0001$; Table 1). Variance in whole-body cortisol concentration (ng/g) was homogeneous among trials (ANOVA; $P = 0.880$; Table 1) and treatments (ANOVA; $P = 0.094$; Table 1). Cortisol levels were not significantly different between the two lowest treatment levels (Figure 2), or between the control and the 50 mg/L treatment (Tukey-Kramer multiple comparison; $\alpha = 0.05$). Cortisol levels at the two highest treatments (100 and 500 mg/L) were significantly higher than the three lowest treatment levels. At these highest treatment levels cortisol concentrations were approximately twice those at the three lowest levels. The relationship between spotfin chub whole-body cortisol concentration and increasing SSC was significant (Figure 3, $R^2 = 0.40$, $P < 0.0001$).

Species and Life Stage Differences

Species

Stress response differed significantly between species (i.e., 2-months old whitetail shiners and 4-months old spotfin chubs; two-factor ANOVA; $P < 0.0001$; Table 2). Cortisol levels at the two lowest treatments (0 and 25 mg/L) were not significantly different between species, but were significantly different at higher SSC (Table 3). The general trend in stress response of spotfin chubs to increasing SSC was also different than for whitetail shiners. While cortisol increased steadily with increasing SSC in whitetail shiners, spotfin chub cortisol levels were lowest at 50 mg/L, and highest at 100 mg/L (Figure 2). The increase in cortisol was also significantly different between species; the slope of the regression of cortisol vs. SSC was approximately 3 times higher for whitetail shiners than for spotfin chubs (ANCOVA; $P < 0.0001$; Table 4).

Life Stage

The magnitude of stress response to sediment treatment differed significantly between whitetail shiner life stages (i.e., 2-months old versus 8-months old; two-factor ANOVA; $P = 0.0014$; Table 2). Cortisol concentrations were significantly higher for 2-months old fish at all treatment levels (Table 3). These differences in cortisol increased as SSC increased. The relationship between SSC and cortisol production was also significantly different between life stages (ANCOVA; $P < 0.0001$; Figure 3, Table 4); the slope of the regression of cortisol vs. SSC was approximately 4 - 6 times higher for 2-months old whitetail shiners than for 8-months old fish. Increase in stress response with increasing SSC decreased with age of fish; increases were greatest in 2-months old fish and least in 8-months old fish (Figure 3).

Discussion

<u>Stress response in minnows versus rainbow trout</u>

Exposure to elevated suspended sediment caused a significant increase in cortisol levels in both fish species and the three life stages evaluated. The experimental test duration of 48 hours, chosen to mimic stormflow conditions in the Little Tennessee River, was sufficient to cause significant stress in test fish. To understand the physiological significance of these results, they are compared to one of the few studies that has measured stress-induced whole-body cortisol production in YOY fish. Barry et al. (1995) studied stress response to handling in YOY rainbow trout (*Oncorhynchus mykiss*).

Eight-months old whitetail shiners exhibited the lowest stress response to elevated SSC. Whole-body cortisol concentrations of 8-months old whitetail shiners in control tanks (mean = 3.7 ng/g) were similar to resting (i.e., non-stressed) cortisol levels reported for 3 – 4-week old rainbow trout (5 – 6 ng/g; Barry et al. 1995). In fact, cortisol levels at all three of the lowest sediment treatment levels were similar to rainbow trout resting cortisol levels. However, the highest treatment (500 mg/L) elicited a stress response 3-fold higher (mean = 16.2 ng/g) and within the range reported for rainbow trout larvae exposed to handling and thermal stress (10 - 40 ng/g; Barry et al. 1995).

The next highest stress response was elicited from 4-months old spotfin chubs. Whole-body cortisol levels of spotfin chubs in control tanks (mean = 8.3 ng/g) were also similar to resting levels of 3 – 4-week old rainbow trout (Barry et al. 1995). Other than the 50 mg/L treatment, spotfin chubs exhibited an increase in stress response similar to what was observed in both life history stages of whitetail shiners, with cortisol levels at the highest treatment 3-4 times larger than the control. This magnitude of change in stress response is similar to the 2 – 4-fold increase documented in young-of-year rainbow trout subjected to intense handling and severe confinement (Barton et al. 1980).

Exposure of 2-months old whitetail shiners to elevated SSC elicited the greatest stress response. Mean whole-body cortisol levels of control fish were 3 – 4 times higher than the 8-months old whitetail shiner controls, spotfin chub controls, and rainbow trout resting levels (Barry et al. 1995). Stress response of 2-months old whitetail shiners at the highest treatment (60

– 80 ng/g at 500 mg/L) was 2 – 6 fold above that elicited from rainbow trout exposed to severe handling stress (10 – 40 ng/g; Barry et al. 1995).

Age-related changes in stress response

Not only were the youngest fish most stressed in the control tanks, the oldest fish were least stressed, suggesting that there may be some effect by the apparatus itself and these effects may be age related. Since cortisol levels at all treatment levels increased with decreasing age, it is possible that the stress response to both apparatus and SSC is inversely related to age. An inverse relationship between age and tolerance to suspended sediments, measured as LC50 during 96 hour bioassay, has also been suggested for YOY coho salmon (*Oncorhynchus kisutch*; Servizi and Martens 1991).

Stress response variability was also inversely related to age. Mean coefficient of variation of cortisol levels was greatest across all treatment levels for 2-months old whitetail shiners (54%) and least for 8-months old whitetail shiners (35%). In addition to an inverse relationship between fish age and cortisol mean and variance, age was also inversely related to rate of change of cortisol level with increasing SSC. This finding suggests that for a given incremental increase in SSC, more harm may be done to these young fish, than to older fish.

Age-related differences in stress response have been observed in other species. For all three life stages of both species studied here, exposure to the two high treatments (100 and 500 mg/L) caused a much higher stress response (2 – 7 fold increase) than reported for salmonid larvae due to acute handling stress (Barry et al. 1995). Both studies found a non-linear relationship between age and stress response. Rainbow trout stress responsiveness increases with developmental stage, but then decreases just before the onset of exogenous feeding (Barry et al. 1995). The current study suggests that some fish may experience a decline in responsiveness to external stressors well past the larval life stage.

Possible reasons for the difference between the stress responses of 2-months old whitetail shiners and 1-month old rainbow trout include stressor-specific (i.e., sediment versus handling) or species-specific differences in response. Supporting species-specific differences is a study that found species-specific differences in suspended sediment-induced stress response of yearling coho salmon and steelhead (*O. mykiss*) (Redding et al. 1987). The current study suggests that age and species may both influence the relative sensitivity of whitetail shiners and spotfin chubs to suspended sediment. Whitetail shiners are thought to be fairly sediment tolerant (Jenkins and Burkhead 1994). In contrast, the decline of spotfin chubs is partially attributed to excessive sedimentation, and they are thought to avoid areas with fine sediment (USFWS 1996, Jenkins and Burkhead 1994). These observations apply to adults but not necessarily to YOY. Although evidence exists for species-specific stress response in fish, age of YOY upland minnows may also be an important determinant of stress response.

Shape of stress response

Stress response of whitetail shiners (both life stages) increased linearly with increasing SSC. Similar linear relationships between SSC and physiological stress, measured as blood glucose level, have been documented for juvenile coho salmon (Servizi and Martens 1992). In contrast, spotfin chubs exhibited an increase above controls at 25 mg/L and a subsequent reduced response in tanks with 50 mg/L SSC, with cortisol levels similar to the control. The two highest treatments (100 and 500 mg/L) caused a large increase in stress, 5-fold above controls.

One explanation for the non-linear response exhibited by the spotfin chub may be that these fish were stressed by the presence of the moving experimental apparatus at the two lowest (i.e., least turbid) treatments, and stressed at the two highest treatments due to elevated SSC. At the mid-level treatment (50 mg/L) there may be decreased perception of the apparatus and the external environment, and therefore a lower stress response than 0 and 25 mg/L. Supporting this hypothesis is the finding that creek chubs (Cyprinidae: *Semotilus atromaculatus*) increase activity and rely less on cover in experimental tanks with moderate turbidity, relative to lower turbidity (Gradall and Swenson 1982). These authors suggest that creek chubs may become more active in moderately turbid water because they are visually isolated from predators. The decline in perceived risk of predation may also explain increased foraging rates of juvenile chinook salmon (*Oncorhynchus tshawytscha*) at moderate turbidity (Gregory 1994). Predator avoidance also improved for razorback sucker (*Xyrauchen texanus*) larvae at moderate turbidities (Johnson and Hines 1999). It seems plausible that in addition to increased activity, and lower reliance on cover, stress levels may decline in fish that perceive themselves to be visually isolated. This may also be the case for spotfin chubs at moderate SSC (i.e., 50 mg/L).

Another possible explanation for the non-linear stress response of spotfin chubs may be that the difference observed between the cortisol levels measured at the three lowest treatments, although statistically significant, is not biologically significant. If true, this hypothesis would suggest a threshold response, with low stress responses occurring at SSC ≤ 50 mg/L and a marked increase in response between 50 and 100 mg/L.

The relatively low levels of suspended sediment used in this study can be stressful for YOY fishes. Studying the effects of low SSC is important because many impacted streams within the native range of imperiled non-game fishes experience chronic baseflow sediment concentrations in this range (10 - 50 mg/L; Sutherland et al. 1998, Walters et al. 2003).

Imperiled fishes in the US are increasingly threatened by sediment from land disturbances such as second home development and suburban sprawl (Wear and Bolstad 1998). In addition, historic sediment inputs (i.e. from past land disturbances such as agriculture, logging, or mining) are stored in the streambed, tributary valleys and mainstem valley, and may be a sediment source for many decades (Harding et al. 1998, Trimble 1999). These current and historic land disturbances continue to provide new and increasing sources of fine sediment causing elevated baseflow turbidity in many streams throughout the southern Appalachians and throughout North America (Waters 1995, Burkhead et al. 1997, Sutherland 2002, Walters et al. 2003). Although baseflow sediment levels are low relative to stormflow conditions, they may cause a chronic, sub lethal stress response in fish (Redding et al. 1987). Even when fish are seemingly able to adapt to low level continuous stressors, their ability to perform routine tasks (e.g., obtaining food, mating, predator avoidance, growth and development) may be impaired (Redding et al. 1987, Schreck 2000). Additionally, there is evidence suggesting that chronic low-level stressors can have long term implications for fish populations, potentially reducing fitness, fecundity and spawning behavior (Billard et al. 1981). Therefore, sub lethal stress from even moderately elevated suspended sediment levels may contribute to the slow decline of imperiled fish populations, exacerbating the continual homogenization of regionally distinct fish assemblages in the southern Appalachians and elsewhere.

<u>Potential effects of ambient SSC on spotfin chub stress response</u>

By examining the flow and sediment regime typical of lotic systems harboring spotfin chubs and other sensitive fishes, we can put these results into context and estimate the impact of increased suspended sediment concentration. The upper Little Tennessee River (LTR; Swain

Co. and Macon Co., NC) is one of only 5 upland river systems with extant populations of the spotfin chub, and the reach upstream of Fontana Reservoir (~70 km) has been designated as critical habitat for this species (USFWS 1983). We examined the flow regime for upper LTR for June – Sept. 2003, to estimate the potential exposure duration of YOY spotfin chubs to elevated SSC. This time period was chosen because this is when YOY fishes produced in early summer (as is the case for spotfin chubs) are presumably most vulnerable to elevated suspended sediment levels. These first months of life are crucial for the long-term viability of most fish species, with the success of newly emerged young being one of the most important determinants of inter-annual population dynamics (Wooten 1990).

A sediment rating curve was created using discharge (cms; m^3/s) and SSC (mg/L) measured in the upper LTR during June and July 2001 (USGS 2001). Based on this rating curve, storms > 22 m^3/s are sufficient to elevate turbidity above 100 mg/L (SSC = 26.24 *discharge - 241.3, $R^2 = 0.59$, $P = 0.02$; USGS 2001). From June – Sept. 2003 there were > 17 storm events in the upper LTR > 22 m^3/s (Figure 4). During this period mean monthly river discharge ranged between 24 – 43 m^3/s (range = 14 – 166 m^3/s; USGS 2003). These storms resulted in elevated sediment concentration during approximately 75% of the summer. During most years this would be an overestimate of elevated SSC exposure duration, because mean discharge for this period was ~ 50% higher than the 57 year recorded median stream flow (USGS Needmore gauge 03503000; USGS 2003). However, the mean daily flow for water years 1944 – 2003 exceeded 23 m^3/s approximately half the time (USGS 2003). This means that in an average year in the upper LTR, spotfin chub early life stages may experience sub lethal stress due to elevated SSC for ~ 50% of the time.

This study showed that moderate SSC (100 mg/L) can cause a stress response in young-of-year spotfin chubs 3-fold higher than resting levels and within the range reported for rainbow trout larvae exposed to acute handling and thermal stress (Barry et al. 1995). In a recent study of four upper Little Tennessee River (LTR) tributaries, stormflow suspended sediment was found to regularly exceed this value (Sutherland et al. 2002). Disturbed streams (78 – 87% forested land cover) exceeded 100 NTU (nephelometric turbidity units) 67 – 100% of the time (note: 100 NTU = 116 mg/L SSC; based on upper LTR sediment rating curve: Sutherland 2002). Perhaps more surprising, stormflow turbidity samples in the 2 reference streams (97 – 99% forested) exceeded 100 NTU 33 – 40% of the time. One of these two reference streams, Tellico Creek, is known to harbor spotfin chubs (McLarney 2000, Sutherland 2002). Tellico Cr. is considered a relatively unimpacted stream, with fairly good water quality (Braatz 2000, Sutherland 2002). However, during the period Nov. 1990 to Mar 1994, 20% of stormflow SSC samples exceeded 1960 mg/L, and 50% exceeded 159 mg/L (Braatz 2000). Therefore, even within this relatively unimpacted refuge, SSC during storms regularly exceeds levels shown to increase stress response in spotfin chubs 3-fold above control levels.

Excessive sedimentation not only causes loss and fragmentation of habitat, but may also have sub lethal but severe direct impacts for native fishes. This study suggests that only moderate levels of suspended sediment are necessary to markedly increase the stress response of native fishes, including the imperiled spotfin chub. This research adds to our understanding of a potential mechanism (i.e., stress) linking SSC, a commonly measured environmental stressor, and observed trends in non-game fish imperilment and assemblage change. When combined with flow data typical of many upland rivers and streams, suspended sediment appears to represent a significant chronic environmental stressor. Land use practices that reduce sediment inputs, and thereby reduce the amount of time SSC exceeds moderate levels, should benefit native fish populations by reducing direct and indirect effects due to stress.

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Table 3.1: One-way analysis of variance (ANOVA) measuring the effects of suspended sediment concentration (SSC; mg/L) on whole-body cortisol concentration (ng/g) of spotfin chubs (4-months old) and whitetail shiners (2-months old and 8-months old).

ANOVA					
Source		d.f.	Sum of squares	*F*-statistic	*P*-value
Trial	Spotfin chubs (4 mo.)	3	133.9	0.44	0.728
	Whitetail shiners (8 mo.)	1	35.8	1.23	0.275
	Whitetail shiners (2 mo.)	1	576.0	0.69	0.411
SSC (mg/L)	Spotfin chubs (4 mo.)	4	6494.2	91.56	< 0.0001
	Whitetail shiners (8 mo.)	4	817.2	23.27	< 0.0001
	Whitetail shiners (2 mo.)	4	11999.3	8.81	< 0.0001

Table 3.2: Two-factor analysis of variance (ANOVA) measuring the effect of life stage (2-months old versus 8-months old *Cyprinella galactura*) and species (*C. galactura* versus *Erimonax monachus*) on the magnitude of stress response (i.e. whole-body cortisol concentration; ng/g) to suspended sediment concentration (SSC; mg/L).

Source	d.f.	Sum of squares	*F*-statistic	*P*-value
Life stage (*Cyprinella galactura*)	4	3487.4	4.99	0.0014
species (*C. galactura* versus *E. monachus*)	4	1001.9	8.33	< 0.0001

Table 3.3: Student's t-tests for species comparison (2-months old *Cyprinella galactura* versus 4-months old *Erimonax monachus*) and life stage comparison (*C. galactura* 2-months old versus 8-months old) of whole-body cortisol concentration (ng/g) at each suspended sediment concentration (SSC; mg/L).

Source	SSC (mg/L)	*T*-statistic	Critical value	*P*-value
life stage	0	2.33	1.78	0.0581
	25	2.92	1.76	0.0112
	50	4.87	1.76	0.0002
	100	6.95	1.76	< 0.0001
	500	12.36	1.76	< 0.0001
species	0	2.44	1.72	0.1465
	25	2.74	1.72	0.0885
	50	7.13	1.72	0.0014
	100	5.16	1.72	0.0051
	500	13.51	1.72	< 0.0001

Table 3.4: Analysis of covariance (ANCOVA) measuring the difference in relationship of suspended sediment concentration (SSC; mg/L) and whole-body cortisol concentration (ng/g) between *Cyprinella galactura* life stages (2-months old and 8-months old) and species (*Erimonax monachus* versus *C. galactura*).

Source	d.f.	Sum of squares	*F*-statistic	*P*-value
life stage (*Cyprinella galactura*)	1	23927.06	102.66	< 0.0001
species (*C. galactura* versus *E. monachus*)	1	19330.37	98.37	< 0.0001

Figure 3.1: Displacement curves for ELISA kit cortisol standards and serial dilutions of whitetail shiner whole-body homogenates. Each point represents four measurements. B = absorbance reading of sample or standard. B_0 = absorbance reading of zero standard.

Figure 3.2: Results of Tukey-Kramer multiple comparison tests, of whole-body cortisol concentration (ng cortisol/gram fish) at different suspended sediment concentration (SSC; mg/L). Means comparisons are presented for 2-months old and 8-months old *Cyprinella galactura* and 4-months old *Erimonax monachus*. Note the difference in scales. Bars with different letters above them are significantly different ($\alpha = 0.05$). Sediment treatments presented as initial SSC added to each tank.

Figure 3.3: Regressions of individual replicates of whole-body cortisol concentration (ng cortisol/gram fish mass) versus suspended sediment concentration (log SSC; mg/L), for 2-months old and 8-months old *Cyprinella galactura* and 4-months old *Erimonax monachus*. The regression equations are as follows: 2-months old *Cyprinella galactura*: whole-body cortisol = 22.48 (log SSC) + 5.6; ($R^2 = 0.46$; $P < 0.0001$); 8-months old *Cyprinella galactura*: whole-body cortisol = 5.42 (log SSC) -1.09; ($R^2 = 0.56$; $P < 0.0001$); 4-months old *Erimonax monachus*: whole-body cortisol = 8.384 (log SSC) + 1.861; ($R^2 = 0.40$; $P < 0.0001$). Sediment concentrations used in regression analyses are SSC estimated from sediment settling curves.

Figure 3.4: Upper Little Tennessee River hydrograph for water year 2003 (Oct. 2002 – Sept. 2003). Discharge (cms; m^3/s) was measured at the USGS Needmore gauge (station 03503000) and is presented on a log scale. Dotted line represents 22 m^3/s, the discharge corresponding to 100 mg/L SSC in the upper LTR.

Figure 3.1

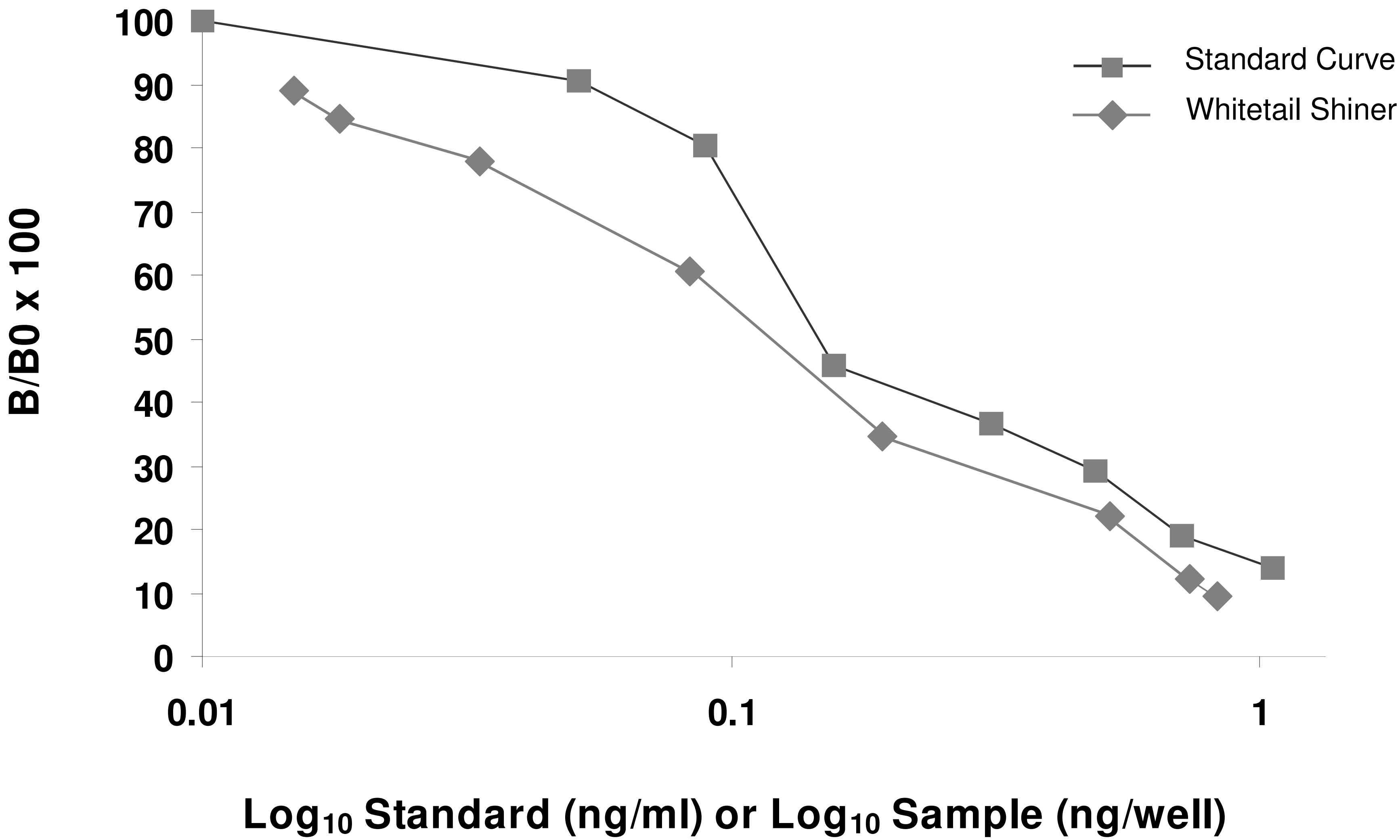

100
90
80
70
60
50
40
30
20
10
0
B/B0 x 100
Standard Curve
Whitetail Shiner
0.01
0.1
1
Log10 Standard (ng/ml) or Log10 Sample (ng/well)

Figure 3.2

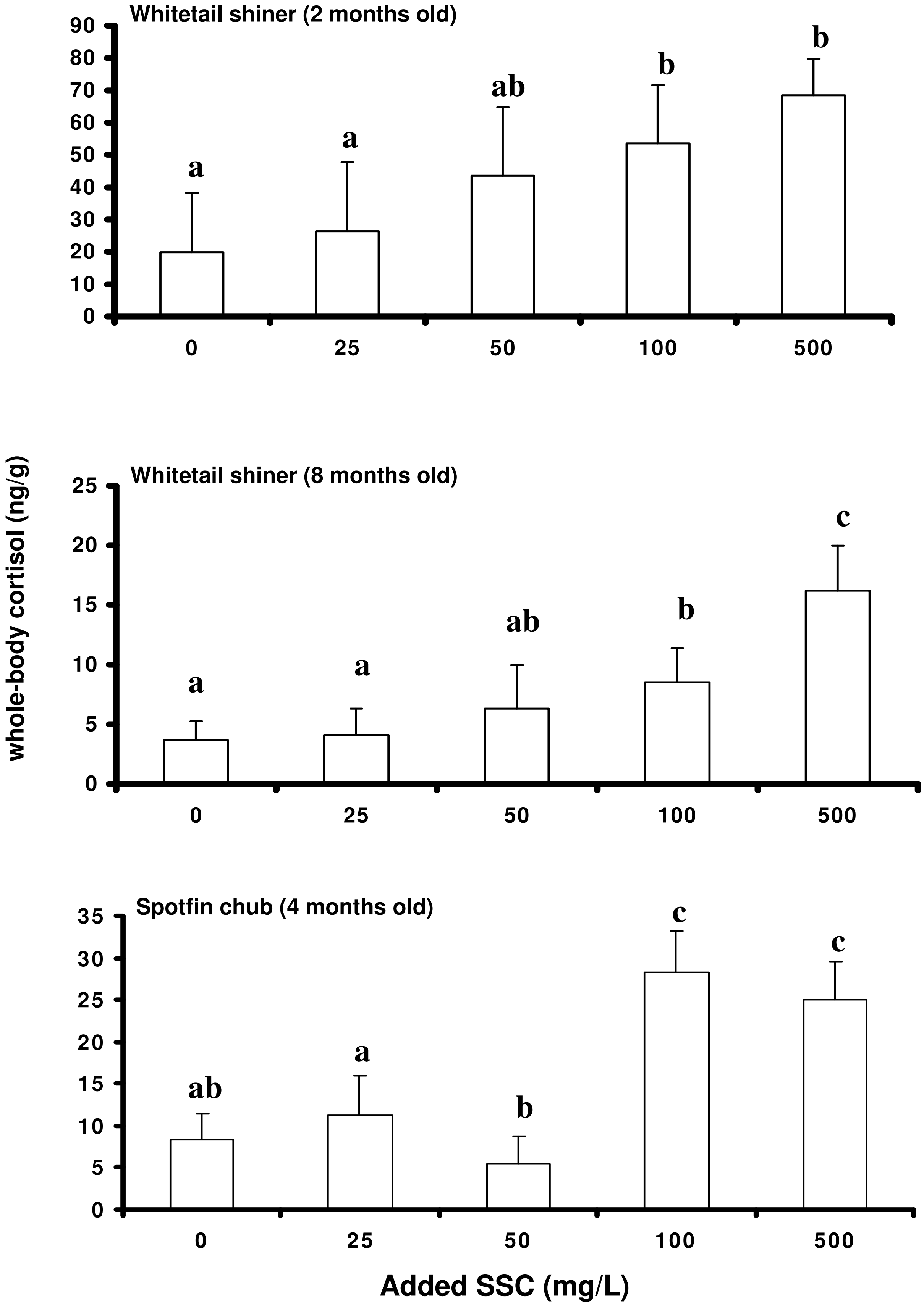
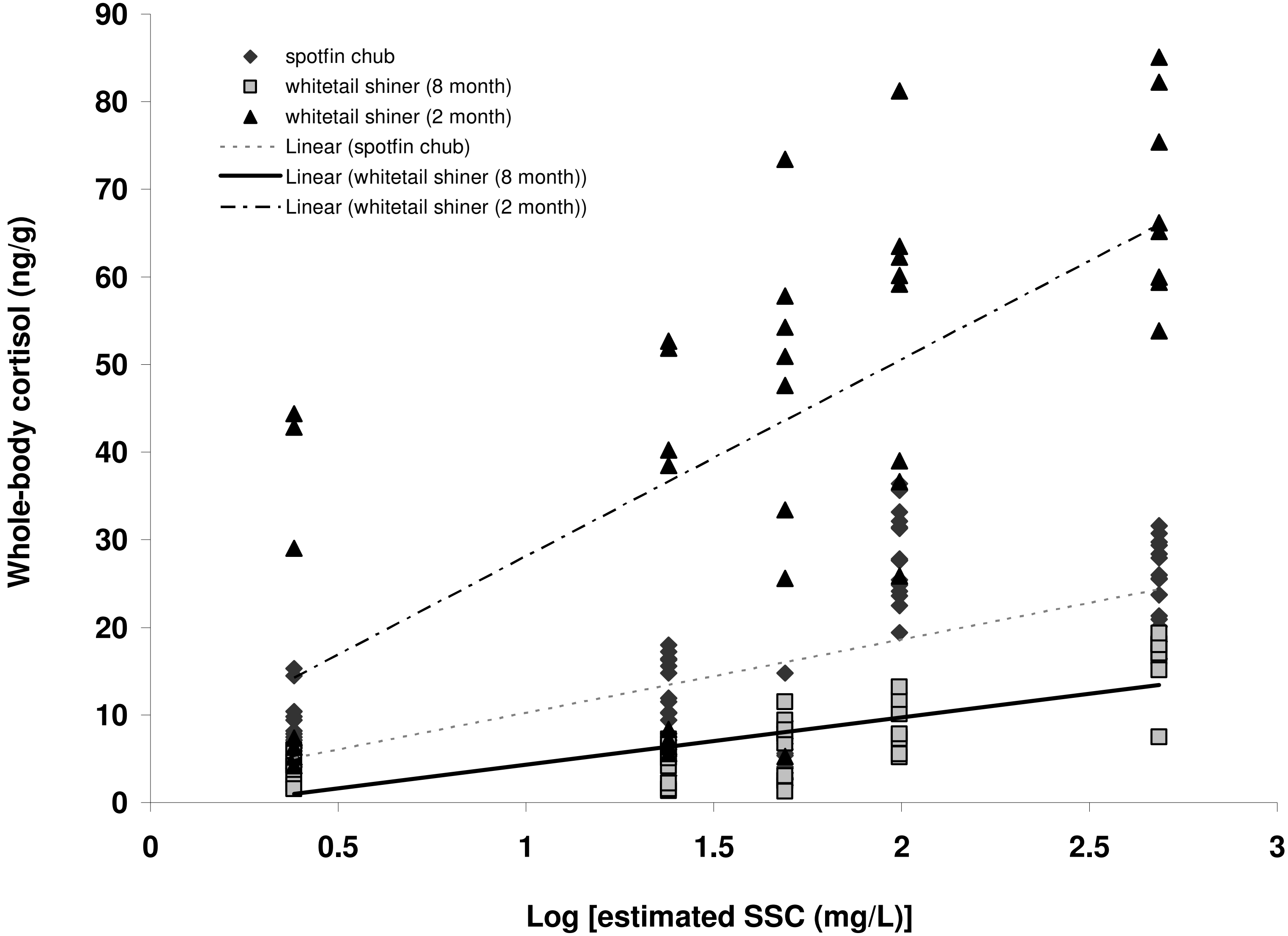
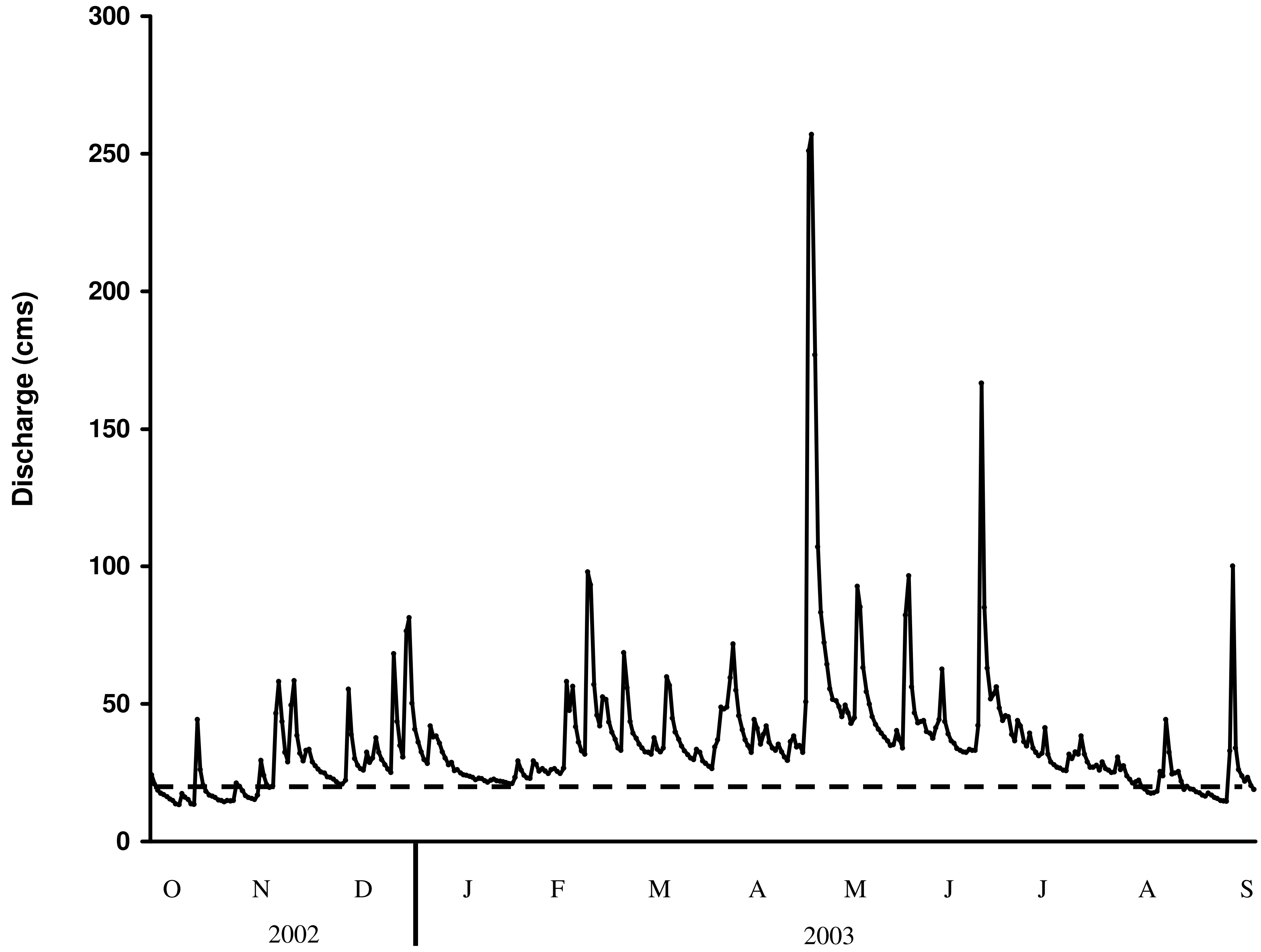

Whitetail shiner (2 months old)
90
80
70
60
50
40
30
20
10
0
a
a
ab
b
b
0
25
50
100
500
whole-body cortisol (ng/g)
Whitetail shiner (8 months old)
25
20
15
10
5
0
a
a
ab
b
c
0
25
50
100
500
Spotfin chub (4 months old)
35
30
25
20
15
10
5
0
ab
a
b
c
c
0
25
50
100
500
Added SSC (mg/L)

Figure 3.3



90
80
70
60
50
40
30
20
10
0
Whole-body cortisol (ng/g)
spotfin chub
whitetail shiner (8 month)
whitetail shiner (2 month)
Linear (spotfin chub)
Linear (whitetail shiner (8 month))
Linear (whitetail shiner (2 month))
0
0.5
1
1.5
2
2.5
3
Log [estimated SSC (mg/L)]

Figure 3.4


300
250
200
150
100
50
0
Discharge (cms)
O
N
D
J
F
M
A
M
J
J
A
S
2002
2003

CHAPTER 4

EFFECTS OF INCREASED SUSPENDED SEDIMENT CONCENTRATION ON GROWTH RATE AND GILL CONDITION OF TWO SOUTHERN APPALACHIAN MINNOWS, *Erimonax monachus* AND *Cyprinella galactura*[3]

[3]A.B. Sutherland and J. Meyer. To be submitted *Environmental Biology of Fishes*

Synopsis

Despite the recognition that increased suspended sediment concentration (SSC) is a primary pollutant of streams, research is limited on the effects of SSC on non-game fishes. This study adds to our limited knowledge of sediment impacts on fish growth and gill condition. Specific growth rate (i.e. percent change in mass per day) and gill condition (i.e. lamellar thickness and interlamellar area) were measured in young-of-year (YOY) whitetail shiners (*Cyprinella galactura*) and federally threatened spotfin chubs (*Erimonax monachus*) exposed for 21 days to increased SSC (0, 25, 50, 100, and 500 mg L^{-1}). Exposure to elevated SSC caused a significant decrease in specific growth rate in both species and at all life stages tested. In general, specific growth rates were greatest in younger fish. (i.e. 2-3-months old whitetail shiners). The effect of increased SSC was greatest in spotfin chubs, which exhibited a 15-fold decrease in specific growth rate at the highest treatment (500 mg L^{-1}). Effects of increased SSC were least for 8-months old whitetail shiners, which had growth rates similar to controls for 25, 50 and 100 mg L^{-1} treatments. The rate of response to increasing SSC differed from what has been observed in salmonids. These minnows exhibited a greater response at low to moderate SSC, and a lower response at higher sediment levels. Gill damage was minimal at the three lowest treatment levels, moderate at 100 mg L^{-1} and severe at the highest treatment. Gill interlamellar area was inversely related to gill lamellar thickness. Gill analyses suggest that respiratory surfaces of upland minnows may be much more sensitive than other species previously tested. Specific growth rate decreased significantly with increasing gill lamellar thickness, suggesting respiratory impairment and the resulting stress response as a possible mechanism for reduced growth rate.

Introduction

Increased sedimentation of rivers and streams has been linked to the decline of imperiled fishes throughout the US (Walsh et al. 1995, Burkhead et al. 1997, Warren et al. 2000). Sediment-induced habitat loss and habitat fragmentation are associated with fish assemblage homogenization and loss of sensitive endemic species in the southeastern US (Burkhead et al. 1997, Scott & Helfman 2001, Sutherland et al. 2002, Walters et al. 2003). Direct impacts of excessive sediment loading may be contributing to the decline of native fishes. Among these are sub-lethal effects such as growth rate reduction and abrasion of gill tissue and subsequent respiratory and osmoregulatory impairment. An abundant literature focuses on the lethal impacts of high suspended sediment concentrations on game-fishes (primarily salmonids; see Newcombe & MacDonald 1991). In contrast, relatively few studies have explored the effects of lethal and sub-lethal concentrations of sediment on non-game species.

Within the southeastern US, cyprinids are the second most diverse fish family (~30% of species) and among the most imperiled (Walsh et al. 1995, Warren et al. 2000). Southeastern cyprinid diversity is greatest in the southern Appalachians (Walsh et al. 1995) and within this region one of the primary threats to minnows is excessive sedimentation (Burkhead et al. 1997, Warren et al. 2000). Despite these facts, very few studies have investigated sediment effects on cyprinids (e.g. Gradall & Swenson 1982, Burkhead & Jelks 2001).

The research on non-game fishes that exists has focused on adults, with even less known about direct effects of sediment on young-of-year (YOY) non-game and imperiled fishes. This is an important area of research because the events that occur in the first few months of life are crucial for the survival of most fish species (Wooten 1990, Helfman et al. 1997). Along with reproductive success, survival of sensitive early life-stages is one of the most important determinants of interannual population dynamics (Helfman et al. 1997). Age and size play critical roles in affecting survivorship. YOY mortality rates are inversely related to size (Wooten 1990). Fish with lower growth rates will spend more time at a smaller size and are hence more susceptible to predation and removal by floods.

Excessive sedimentation has other deleterious impacts as it can affect growth rates by reducing visual acuity (Sigler et al. 1984, Newcombe & MacDonald 1991), prey capture success, and feeding efficiency (Barrett et al. 1992). Increased levels of suspended sediment may also reduce growth rates of YOY salmonids by increasing scour, physiological stress and metabolic rates, and by reducing feeding rates (Sigler et al. 1984, Redding et al. 1987, Newcombe & MacDonald 1991). One objective of this study was to determine if increased suspended sediment impacts the growth of non-salmonid YOY fishes.

Another direct effect of increased suspended sediment concentration (SSC) is respiratory impairment. However, the effects of SSC on gill condition have not been quantified for non-salmonid species, and the literature on salmonid gill condition presents an unclear picture. Sediment-induced gill abnormalities suggest that increased SSC may cause gill abrasion, hyperplasia and hypertrophy, which in turn may cause decreased fitness and growth rate (Herbert & Merkens 1961, Bruton 1985, Berg & Northcote 1985, McLeay et al. 1987, Goldes et al. 1988, Servizi & Martens 1992). Abrasion by sediment particles may increase the chance of infection of gill epithelium, thereby increasing susceptibility of fish to disease (Herbert & Merkens 1961). Conversely, other studies suggest minimal impact, even at very high SSC. A second objective of this study was to explore these effects in species other than salmonids by determining sediment effects on gills of cyprinid species.

As part of the US Fish and Wildlife Service Spotfin Chub Recovery Plan (per objective 1.3.1; USFWS 1983), we investigated the effects of excessive sedimentation on the federally threatened spotfin chub (*Erimonax monachus*). Specifically, we examine the effect of increased SSC (0, 25, 50, 100 and 500 mg L^{-1}) on the growth rate and gill condition of two southern Appalachians minnows, the spotfin chub, and the whitetail shiner (*Cyprinella galactura*), a closely related surrogate for the spotfin chub.

Materials and methods

<u>Growth Trials</u>

Study Organisms

Four growth trials were conducted using the whitetail shiner, which is phylogenetically similar to the federally threatened spotfin chub as detailed in Chapter 3. One whitetail shiner growth trial was conducted using ~ 8-months old juveniles and three trials were conducted using ~ 2-months old post-larvae. All were propagated from adults collected in the upper Little Tennessee River (Swain Co. & Macon Co., NC), and reared in the laboratory (Chapter 3). YOY were fed brine shrimp nauplii (*Artemia* spp.) and a high-protein micro-encapsulated commercial starter diet (< 100 µm; Zeigler® larval diet).

Four growth trials were conducted using YOY spotfin chubs. Larvae were obtained from Conservation Fisheries Inc. (CFI; Knoxville, TN), who reared them from eggs spawned by adults collected in the Buffalo River (Lewis Co., TN). We reared larvae in 30-liter flow-through tanks and fed them the diet described above. Spotfin chubs used in growth trials ranged in age from 4 – 6 months, and were reared for this time period to ensure their transition from a benthic to pelagic life stage. It was assumed that the experimental apparatus paddles would be very stressful to the benthic stage of this fish.

Experimental Procedure

The apparatus used for these experiments consisted of slow moving (~ 3 – 5 mm sec^{-1}) motor-driven paddles within each of 20 experimental tanks (30-liter tanks; Chapter 2). Paddles were each fitted with two baffles that slowly sweep the floor of a given tank, while also delivering a column of compressed air that resuspended any settled particles.

Sediment used in growth experiments was collected from the Little Tennessee River basin (Macon Co., NC) and wet-sieved to obtain the < 45 µm fraction. Sediments were free of metal or organic contamination (Chapter 3; Appendix 1). SSC treatments used in this study (0, 25, 50, 100 and 500 mg L^{-1}) are within the range of conditions observed in the Little Tennessee River (turbidity range: 10 – 1500 mg L^{-1}, W. O. McLarney unpublished data).

The ages of whitetail shiners varied slightly because they were randomly chosen from different cohorts based on size similarity to minimize variability of initial fish mass. The initial growth trial lasted 30 days, and the following three trials each lasted 21 days. In each growth trial three whitetail shiners were reared in each of 20 experimental tanks. Five suspended sediment treatments (0, 25, 50, 100 and 500 mg L^{-1}) were randomly assigned to the 20 experimental tanks (four replicates per treatment).

At the start of each growth trial, all tanks and paddles were cleaned thoroughly. Tanks were filled with 30 liters of well water, warmed in a head-tank to 25°C. A sulfa-based antibiotic (Sulfa-4; Fishy Farmacy, Tucson, AZ) was then added to each tank to prevent bacterial blooms. Individual unanesthetized fish were briefly and carefully placed on a dry towel to reduce excess water weight, placed in a pre-weighed beaker of water, and weighed to the nearest 0.1 mg. Each fish was introduced randomly into a tank and allowed to acclimate for 48 hours before sediment was added. Starting on the second day of acclimation, fish were fed twice a day at a rate of 10%

initial body mass per day. Fish in the first trial were fed a diet of dry pelleted Purina® AquaMax (D04; 1.5mm), and fish in the three subsequent trials were fed a high-protein micro-encapsulated commercial starter diet (400 μm; Zeigler® larval diet).

Maintaining suspended sediment concentrations precluded the use of biological filtration in the experimental tanks. This requirement made periodic water changes necessary to minimize water quality problems. During the first growth trial water and sediment were replaced on days 9, 17 and 25. For the remaining three trials sediment and water changes were performed on days 7 and 14. During water changes, fish were removed to one of four temporary holding tanks for approximately 10 minutes while fresh well water warmed in head tanks and new sediment were added after cleaning each tank. Fish were then returned to the tanks.

At the end of each growth trial, fish were anesthetized by adding 10 ml of eugenol (a 1:5 mixture of eugenol in ethanol) to each tank, which anesthetized larval and juvenile whitetail shiners within 2 minutes. Each fish was then removed and weighed. Because individuals could not be identified, initial and final weights used to calculate growth rates are the sum of weights from the three fish in each tank. Specific growth rates were calculated as the percent change in mass per initial mass per day (100 x [(final wt – initial wt)/initial wt]/days).

For the spotfin chub growth trials, a single fish was placed in each of 20 tanks as described above. Fish were treated and tested as described for whitetail shiners. After the final weighing, spotfin chubs were placed in scintillation vials containing a 10% solution of neutral buffered formalin, to preserve for later determination of gill condition. Each treatment replicate represents the daily specific growth rate of one spotfin chub.

Gill Condition

The effects of increased suspended sediment on gill condition was determined for spotfin chubs reared in the first growth trial. To avoid confusion with terminology, primary gill lamellae are henceforth referred to as “filaments”, secondary lamellae are referred to as “lamellae”, and “interlamellar” refers to the space between secondary lamellae. Two measures of gill condition were determined: mean thickness of lamellae and mean space between adjacent lamellae. These two metrics of gill impairment were chosen because lamellar thickening and reduction in interlamellar space may reduce capacity for respiration and reduce osmoregulatory performance. Although gill thickening is often associated with decreasing interlamellar space, both parameters were measured because the latter may also occur due to excess mucous production.

The right operculum was removed from preserved spotfin chubs and the first gill arch was excised. Gill arches were stained for 30 min using fluorescein dye (excitation = 488nm; emission = 530nm). After rinsing off excess dye, 2 – 5 filaments were removed from the excised gill arch and placed on a hydrophobic-coated glass slide (Cel-Line® HTC™). Micrographs of gill lamellae were created using a spectral confocal microscope (Leica TCS SP2 with Coherent Ti:sapphire multiphoton laser; Mira Optima 900-F), with a 40X water immersion objective. Optical sectioning of fluorescent gill lamellae was standardized by always capturing the optical section at 50% of lamellar height (i.e. vertical thickness, +/- 2 μm).

Micrographs of lamellae generated by confocal microscopy were analyzed using Image-Pro Plus© software (Version 4.5.1, Media Cybernetics Inc., Silver Springs, MD). Gill thickness (μm) was measured perpendicular to the long axis of each lamella (Figure 1). Fifty gill thickness measurements were taken for each fish using 10 – 20 lamellae and 1 – 6 measurements on each lamella. The number of lamellae and measurements per lamella were a function of micrograph quality. Interlamellar area (μm^2) was determined as the space between adjacent lamellae for 25 interlamellar regions per fish (Figure 1).

Data Analyses

Specific growth rates were compared using analysis of variance (ANOVA; JMP, SAS Institute, Cary, NC), followed by pairwise comparisons using the Tukey-Kramer test ($\alpha = 0.05$). Differences among experimental trials were determined using ANOVA blocked by trial, after using Levene's test to assure that group variances were equal. Differences in mean specific growth rates were determined between species (i.e. whitetail shiners versus spotfin chubs) and between life stages (i.e. whitetail shiner 2 months olds versus 8 months olds) using two-factor ANOVA. If two-factor ANOVA showed a significant effect, each treatment was compared (i.e. between species and between life stages) using the Student's t-test. Slopes of the regression of mean specific growth rate as a function of SSC were compared between life stages and species using analysis of covariance (ANCOVA).

As expected, a small fraction of sediment settled during the course of each growth trial, so SSCs were not constant. Therefore, sediment settling was estimated during the first whitetail shiner growth trial, and the first spotfin chub growth trial as described in Chapter 2. These sediment settling curve data were used to estimate the average SSC during the course of a growth trial. These estimated average SSC were used for the previously described regression analyses.

Mean gill lamellar thickness and mean interlamellar area were compared among sediment treatments using analysis of variance (ANOVA) followed by pairwise comparisons using the Tukey-Kramer test ($\alpha = 0.05$). Linear regression was used to determine if there was a significant relationship between estimated SSC and gill lamellar thickness or interlamellar area, between gill condition and natural log (mean specific growth rate), and between gill lamellar thickness and the more time consuming measure, interlamellar area ($\alpha = 0.05$).

Results

Growth Rates

Specific growth rates (% d^{-1}) of young whitetail shiners (2-3 months) did not differ significantly among experimental trials (ANOVA; $P = 0.435$; Table 1), so all trials were combined in further analyses. Specific growth rates were significantly different among suspended sediment treatments (ANOVA; $P < 0.0001$; Table 1). Growth rates at the highest SSC were significantly lower than at all other SSC (Figure 2, Tukey-Kramer multiple comparison; $\alpha = 0.05$). Furthermore, specific growth rate was significantly and inversely related to increasing SSC (Figure 3, $R^2 = 0.47$, $P < 0.0001$).

Specific growth rates for older whitetail shiners (8-9 months) were significantly different among SSC treatments (ANOVA; $P = 0.001$; Table 1). Growth rates at the highest SSC were significantly lower than at all other SSC (Figure 2, Tukey-Kramer multiple comparison; $\alpha = 0.05$). Specific growth rate was significantly and inversely related to increasing SSC (Figure 3, $R^2 = 0.41$, $P < 0.0001$).

Specific growth rates for spotfin chubs (4-6 months) did not differ significantly among experimental trials (ANOVA; $P = 0.729$; Table 1), so all trials were combined in further analyses. Growth rates for spotfin chubs were significantly different among suspended sediment treatments (ANOVA; $P < 0.0001$; Table 1). Spotfin chub growth rates decreased steadily with increasing SSC; all treatments (except 25 and 50 mg L^{-1}) were significantly different from each other (Figure 2, Tukey-Kramer multiple comparison; $\alpha = 0.05$). Specific growth rate was significantly and inversely related to increasing SSC (Figure 3, $R^2 = 0.79$, $P < 0.0001$).

Species and Life Stage Differences in Growth Rates

Specific growth rate (% d^{-1}) differed significantly between species (i.e. 2-3-months old whitetail shiners vs. 4-6-months old spotfin chubs; two-factor ANOVA; $P < 0.0001$; Table 2). Growth rates were significantly different between species at the four lowest treatments, but not at 500 mg L^{-1} (Table 3). Whitetail shiner (2-3-months old) growth rate declined more with increasing SSC than did spotfin chub (4-6-months old) growth rate (ANCOVA; $P = 0.008$; Table 2); growth rate decrease in young whitetail shiners was approximately 2 times greater than that of spotfin chubs (Figure 3).

Specific growth rate also differed significantly between whitetail shiner life stage (i.e. 2-3-months old vs. 8-9-months old whitetail shiners; two-factor ANOVA; $P = 0.0247$; Table 2). Growth rates were significantly different between whitetail shiner life stage at the four lowest treatments, but not at 500 mg L^{-1} (Table 3). The decrease in growth rate of young whitetail shiners was significantly different than for older whitetail shiners (ANCOVA; $P = 0.012$; Table 2); growth rate decreased in young fish at approximately 2 times the rate of older fish (Figure 3).

Gill Condition

Confocal microscopy provided not only gross observations, but also allowed quantification of gill lamellae changes of fish exposed to elevated SSC. In general, the gill tissue of spotfin chubs reared in the three lowest sediment concentrations appeared similarly undamaged when viewed with the naked eye or under a dissecting scope (Figure 4). Gill cavities appeared free of sediment and mucous, and individual gill filaments were readily discernable. Gills of fish grown in 100 mg L^{-1} were similar to those of lower treatments, but slightly more opaque, and individual filaments were less discernable. Gills of spotfin chubs reared at 500 mg L^{-1} appeared very different from all other treatments. Gill cavities were filled with mucous and sediment. Some gill arches and filaments were fused, making it difficult to discern individual filaments. While gross appearance (i.e. dissecting microscopy) of gills of fish exposed to 100 mg L^{-1} appeared similar to lower treatments, gill micrographs (i.e. confocal microscopy) revealed moderate epithelial hyperplasia (increased cell growth), gill fusion and other abnormalities (Figure 5). Gill micrograph analysis also suggested severe gill epithelial hypertrophy (i.e. thickening) for fish exposed to 500 mg L^{-1} (Figure 5).

Gill lamellar thickness differed significantly among treatments (ANOVA; $P < 0.001$) and was significantly greater for spotfin chubs reared at the highest sediment concentration (Figure 6). Interlamellar area also differed significantly among treatments (ANOVA; $P < 0.01$). Space between gill lamellae was significantly smaller for spotfin chubs reared at 100 mg L^{-1} than for the three lowest treatments (Figure 6). Space between lamellae was smallest at the 500 mg L^{-1} treatment; interlamellar area for this treatment was significantly lower than for all other treatments (Figure 6). Gill lamellar thickness increased significantly with increasing SSC (Figure 7, $R^2 = 0.99$, $P = 0.0004$). Gill interlamellar area was significantly and inversely related to increasing gill thickness (Figure 7, $R^2 = 0.97$, $P = 0.003$). The natural log of specific growth rate was significantly and inversely related to increasing gill thickness (Figure 8, $R^2 = 0.95$, $P = 0.005$).

Discussion

Sediment-related declines in fish populations have been documented for over a century (Waters 1995). Streams with elevated SSC (20-300 mg L^{-1}) had seven times smaller populations of cutthroat (*Oncorhynchus clarki*), rainbow (*O. mykiss*) and brown trout (*Salmo trutta*), when compared to nearby clear streams (Peters 1967). Others have also reported similar sediment-related population declines in salmonids (Herbert et al. 1961, Herbert & Merkins 1961, Ritchie

1972). Increasingly, excessive sedimentation has also been related to fish assemblage homogenization and the loss of sensitive and endemic fish species (Berkman & Rabeni 1987, Burkhead et al. 1997, Scott & Helfman 2001, Sutherland et al. 2002, Walters et al. 2003). One way that increased sedimentation and turbidity has been shown to alter fish assemblages and decrease local populations is by inducing an alarm reaction and causing avoidance and emigration (Sigler et al. 1984, Newcombe & MacDonald 1991; Henley et al. 2000). However, for those species that remain in impacted areas, increased suspended sediment can threaten their growth and survival (Waters 1995). Despite increasing correlational evidence of these harmful effects of sediment on fish, we lack a clear understanding of the mechanisms behind these observations, especially for non-salmonid species. Therefore, it is important to identify and quantify under laboratory conditions the mechanisms responsible for observed effects of elevated sediment on declining native fish populations.

Growth Rates

One of the primary ways in which SSC may affect fish populations is by reducing individual growth rates (Waters 1995). Reduced growth rates of YOY fish negatively impact both the fitness and survivability of individual fish, and affect year-class strength through reduced recruitment . The positive relationship between size of YOY fish and recruitment is well established (Miller et al. 1988, Wooten 1990). Visual performance (e.g. visual acuity and reactive distance) is directly related to size of fish larvae. Risk of predation is inversely related to size, as smaller fish have slower swimming speed and a reduced ability to escape predators (Miller et al. 1988). Reduced growth rates also prolong vulnerability of larvae and juveniles to gape-limited predators.

Most experimental studies relating sediment concentration to fish growth rates have explored the acute lethal effects of high SSC (i.e., 10,000s – 100,000s mg L^{-1}) (Newcombe & MacDonald 1991). Studies investigating the effects of lower SSC (10s – 100s mg L^{-1}) are less common. However, relatively low turbidity levels have been shown to reduce growth rates of YOY salmonids (Sykora et al. 1972, Crouse et al. 1981, Sigler et al. 1984, MacKinley 1987), golden redhorse (*Moxostoma erythrurum*), and spotted bass (*Micropterus punctulatus*) (Gammon 1970). Conversely, the growth of larval lake whitefish (*Coregonus artedii*) was not affected by relatively low SSC (1-28 mg L^{-1}; Swenson & Matson 1976).

The present study contributes to further understanding of the impacts of low SSC on fish growth. In general, exposure to elevated but still relatively low SSC caused a significant decrease in growth rate for both life stages of whitetail shiners and for spotfin chubs. Growth rates are within the same order of magnitude (0.01 – 0.25 g d^{-1}) of those previously documented for YOY steelhead (*Oncorhynchus mykiss*) and coho salmon (*O. kisutch*) reared in similar SSC (84 mg L^{-1}; Sigler et al. 1984). However, when growth rate change relative to controls is compared, differences between the two studies become apparent. At 100 mg L^{-1} spotfin chubs and 2-months old whitetail shiners exhibited 4 – 5 fold greater decreases in growth rate, than did YOY steelhead (Sigler et al. 1984). In contrast, steelhead YOY reared in 265 NTU, and whitetail shiners and spotfin chubs reared in 500 mg L^{-1} (= 411 NTU; test sediment NTU = 0.81 * SSC + 5.83), exhibited similar reductions in growth rate relative to controls. These findings suggest that the rate of response to increasing SSC differs between upland minnows and salmonids, with minnows exhibiting a greater response at lower treatment levels.

Several potential mechanisms link increased SSC to decreases in fish growth rates. Many studies relate increasing SSC with decreasing feeding efficiency of fish. Two interrelated mechanisms are sediment-induced decreases in reactive distance and feeding efficiency. As reactive-distance decreases, more time is needed to search a given volume of water. This reduced feeding efficiency results in higher energy expenditure per prey captured, thus potentially reducing growth. Turbidity as low as 30-60 NTU has been shown to reduce the reactive distance of juvenile coho salmon (Berg & Northcote 1985). Others have also documented an inverse relationship between turbidity and reactive distance of bluegill (*Lepomis macrochirus*), and rainbow trout (Gardener 1981; Barrett et al. 1992). Cutthroat trout stopped feeding when exposed to SSC as low as 35 mg L^{-1} (Wilber 1983). Increased turbidity also negatively affected feeding rates for two large cyprinids (*Barbus* spp. & *Labeo* spp.; Bruton 1985) and for bluegill (Gardener 1981). Several studies documented reduced feeding ability of salmonids exposed to high SSC (McLeay et al. 1987, Redding et al. 1987, Reynolds et al. 1989). In turbid prairie streams, feeding efficiency was lower for minnows not usually associated with high turbidity, and higher for those species historically found in turbid streams (Bonner & Wilde 2002).

Increased SSC may also inhibit normal feeding by increasing physiological stress (Redding et al. 1987). Suspended sediment concentrations used in the present study were sufficient to severely stress both YOY whitetail shiners and spotfin chubs (Chapter 3). Stress-induced inhibition of normal feeding may thus reduce performance capacity and growth rate (Redding et al. 1987, Waters 1995). Highly stressful environments have been associated with growth rate suppression in fishes (Schreck et al. 1997). Other research suggests that suspended sediment-induced physiological stress may negatively affect fish growth more than indirect effects such as decreased prey abundance (Shaw & Richardson 2001). Hence, stress may play a role in the observed growth reduction of whitetail shiners and spotfin chubs at elevated SSC.

Gill Condition

Sediment-related increase in stress response and reduction of growth rates may both be partially due to increased gill damage which could operate via respiratory impairment (Schreck et al. 1981, Waters 1995). Some research suggests that increased suspended sediment causes gill thickening and fusion, presumably due to continual abrasion and irritation of gill lamellae (Herbert & Merkens 1961). Thickening of lamellae and reduction in interlamellar space may result in reduced respiratory surface area and reduced capacity for ion regulation. The present study showed a strong inverse relationship between gill thickness and specific growth rate (Figure 8), suggesting that tissue damage and subsequent impairment of respiratory function may be a possible mechanism for reduced growth rate.

The results reported here do not support the assessment by some researchers that acute gill damage occurs only after exposure to very high levels of suspended sediment (i.e. many g L^{-1}; see Henley et al. 2000). In fact, results from other studies vary considerably and present no clear pattern. Some studies report an effect on gill thickening at low SSC, some report effects only at high SSC, and some report no effect even at very high SSC (Table 4). Some studies present qualitative results, reporting that increased SSC results in clogging of gill filaments and gill rakers (Bruton 1985). Others mention behavioral changes that suggest gill irritation, such as increased gill flaring and coughing (Berg & Northcote 1985, Servizi & Martens 1992). Both gill flaring and coughing are thought to remove excess sediment particles and concomitant excess mucous lodged in fish gills.

Although some studies report sediment-induced thickening of gill lamellae, the severity of these effects is generally much less than documented here. Brown trout (*Salmo trutta*) exhibited gill epithelial thickening similar to what we observed but only when exposed to SSC > 1000 mg L^{-1} (Herbert et al. 1961). In general, gill abnormalities in the present study are more similar to results reported for YOY brown trout exposed to acidic (pH = 4.9 – 5.4) stream water containing aluminum (Ledy et al. 2003), and juvenile channel catfish (*Ictalurus punctatus*) exposed to high levels of ammonia (Mitchell & Cech 1983). Belontiids (*Colisa fasciatus*) with similarly severe gill hyperplasia and lamellar fusion were exposed to sub lethal chromium concentrations (48 ppm; Nath et al. 1997).

Other factors that may cause differential effects of SSC on gill condition are the characteristics of sediments used in experiments, particularly their abrasiveness. Angular sediment particles are known to increase stress response (e.g. increased hematocrit) relative to more rounded particles (Lake & Hinch 1999). Sharp, angular sediment is more abrasive and can become lodged more easily in gill lamellae. This can cause excessive mucous discharge, causing further respiratory problems such as reduction or loss of ion regulation capacity. One of the few studies that found sediment-induced gill damage similar to the present study, involved brown trout exposed to china-clay waste water that contained a large amount of angular mica particles (Herbert et al. 1961). The sediment used in the present study consisted of clay and silt. The silt-sized particles (2 – 45 μm) were composed of mica-based and quartz, and as such were very sharp and angular. These particles also have a high electrostatic charge, making them potentially harder for fish to expel from their gills.

<u>Dose Response</u>

Growth rates and gill condition measured in the present study may also vary from previous research due to differences in sediment dose. Suspended sediment dosage (i.e. concentration times exposure duration), may better explain sediment-induced impacts to fish than concentration alone (Newcombe & MacDonald 1991, Newcombe & Jensen 1996, Shaw & Richardson 2001). One of the few studies testing the effects of exposure duration determined that the mass and length of rainbow trout were negatively correlated with increased duration when SSC was held constant (Shaw & Richardson 2001). Recognizing the importance of sediment dose, Newcombe & Jensen (1996) developed a series of models that predict impairment due to both sediment concentration and exposure duration. Some researchers have found that these models consistently predict their observed results (Shaw & Richardson 2001). Other researchers found that these models underestimated the severity of sediment-induced impairment (Burkhead & Jelks 2001). When our SSCs and experiment duration were used as input data, Newcombe and Jensen's model predicted 20 – 60% mortality; we observed none. This difference may arise because that the model was developed for younger more sensitive life stages (i.e. eggs and larvae), whereas the fish used in the present study were juveniles.

Few studies have reported the extent of gill damage we observed (although see Herbert et al. 1961). The severity of impairment (i.e. severe gill damage but no mortality) we found seems to fall midway between the model prediction (i.e., substantial mortality) and previous observations of minimal impact to gills of game fish species. Model inaccuracy may result from differences in sediment tolerances among fish families coupled with the fact that data from only a few families were used for model creation (Salmonidae, Centrarchidae and Clupeidae; Newcombe and Jensen 1996). Supporting this idea of family- or species-specific sediment tolerance, YOY arctic grayling (*Thymallus arcticus*) exhibited growth rates twice as high as in our study despite being exposed to similar SSC for twice the duration (McLeay et al. 1987). Also, lethal levels of suspended sediment are known to vary greatly among fish species and life stage (Newcombe & Jensen 1996).

Potential growth of spotfin chubs under ambient sediment conditions

Our results indicate that spotfin chubs exposed for 21 days to 100 and 500 mg L^{-1} exhibit 3- and 15-fold reductions in growth rate, respectively. In the upper Little Tennessee River (LTR), which harbors one of the few remaining populations of spotfin chubs, stormflow > 22 m^3/s is sufficient to elevate turbidity above 100 mg L^{-1}, and 48 m^3/s is sufficient to elevate turbidity above 500 mg L^{-1} (SSC = 26.24 discharge - 241.3, $R^2 = 0.59$, $P = 0.02$; USGS 2001). During 1964 - 2003, daily discharge in the upper LTR exceeded 23 m^3/s for ~ 50% of the time, and exceeded 48 m^3/s > 10% of the time (water years 1964 – 2003; USGS 2003). Our experiments were conducted for 21 days, so it is also useful to consider the number of times the upper LTR exceeded 23 and 48 m^3/s for a continuous three week period. Between 1964 and 2003, discharge exceeded 23 m^3/s for a three week period 258 times and 48 m^3/s 23 times (USGS 2003). Based on these discharge data, our results suggest that YOY spotfin chubs in the upper LTR have been exposed to sediment doses sufficient to reduce growth rates 3-fold for ~ 38% of the time (i.e., ~ 6 times per year), and sufficient to reduce growth rates 15-fold for ~ 3% of the time (i.e., ~ once every 2 years).

Summary/Conclusions

An objective of the US Fish and Wildlife Service recovery plan for the federally threatened spotfin chub is to determine the effects of excessive sediment on this species (USFWS 1983). This study has demonstrated under laboratory conditions how increased suspended sediment concentrations may directly affect declining spotfin chub populations. Chronic exposure to high SSC (i.e. 500 mg L^{-1}) may overwhelm the ability of spotfin chubs to remove excess sediment from their gills, resulting in severe gill damage. This negative impact on gill condition can cause elevated physiological stress (see Chapter 3) thereby reducing spotfin chub growth rates. Despite phylogenetic similarities between spotfin chubs and whitetail shiners, spotfin chubs are more sensitive to moderate and high levels of suspended sediment, which may help explain why spotfin chubs and not sympatric whitetail shiners have undergone population declines throughout their native habitat.

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Table 4.1: One-way analysis of variance (ANOVA) measuring the effects of suspended sediment concentration (SSC; mg/L) on specific growth rate (% initial mass per day) of *Erimonax monachus* (4-6 months old) and *Cyprinella galactura* (2-3 months old and 8-9 months old). ANOVA results measuring differences in growth rate due to experimental trial are presented, as well as results of Levene's test for homogeneity of variance.

ANOVA						*Levene's Test*		
	Source	d.f.	Sum of squares	*F*-statistic	*P*-value	d.f.	*F*-ratio	*P*-value
SSC (mg/L)	*C.galactura* (2-3 mo.)	4	0.4188	20.86	<0.0001	4	3.387	0.02
	C. galactura (8-9 mo.)	4	0.0187	8.21	0.001	4	2.782	0.065
	E. monachus (4-6 mo.)	4	0.2067	77.34	<0.0001	4	2.376	0.059
Trial	*C.galactura* (2-3 mo.)	2	0.02	0.8449	0.4349	2	2.422	0.0978
	E. monachus (4-6 mo.)	3	0.0043	0.435	0.7289	3	2.159	0.0998

Table 4.2: Two-factor analysis of variance (ANOVA) measuring the effect of life stage (2-months old versus 8-months old whitetail shiners) and species (whitetail shiner versus spotfin chub) on the magnitude of response of specific growth rate (% initial mass per day) to suspended sediment concentration (SSC; mg/L). Also presented are results of analysis of covariance (ANCOVA) measuring the difference in relationship of suspended sediment concentration (SSC; mg/L) and specific growth rate between life stage and between species.

Two-factor ANOVA

Source	d.f.	Sum of squares	*F*-statistic	*P*-value
life stage	4	0.0485	2.982	0.0247
species	4	0.0752	7.495	<0.0001

ANCOVA

Source	d.f.	Sum of squares	*F*-statistic	*P*-value
life stage	1	0.0309	6.636	0.012
species	1	0.0284	7.134	0.008

Table 4.3: Student's t-tests for species comparison (whitetail shiner versus spotfin chub) and life stage comparison (2 months versus 8 months whitetail shiner) of specific growth rate (% initial mass per day) at each suspended sediment concentration (SSC; mg/L).

Source	SSC (mg/L)	*T*-statistic	Critical value	*P*-value
life stage	0	7.42	1.86	<0.0001
	25	7.11	1.76	<0.0001
	50	4.1	1.78	<0.001
	100	1.98	1.78	0.035
	500	0.001	1.76	0.499
species	0	7.32	1.75	<0.0001
	25	9.02	1.75	<0.0001
	50	7.91	1.76	<0.0001
	100	4.84	1.78	0.0002
	500	1	1.77	0.168

Table 4.4: Studies documenting effects of elevated suspended sediment concentration (mg/L) on fish gills. Fish life stages are as follows: A = adult; J = juvenile; YY = young-of-year. Experiment durations are in days (d).

Species	Life Stage	SSC (mg/L)	Duration (d)	Effect	Reference
Rainbow trout	A	270	13	gill thickening	Herbert and Merkens 1961
White perch	A	650	5	gill thickening; increase in goblet cells	Sherk et al. 1975
Rainbow trout	A	810	21	gill thickening	Herbert and Merkens 1961
Brown trout	A	1,040	730	gill thickening	Herbert et al. 1961
Arctic grayling	YY	1,250	2	moderate gill damage	Simmons 1982
Arctic grayling	YY	1,388	4	gill hyperplasia and hypertrophy	Simmons 1982
Coho salmon	J	1,547	4	gill damage	Noggle 1978
Rainbow trout	J	4,887	64	slight gill thickening	Goldes et al. 1988
Sockeye salmon	YY	9,850	4	gill hyperplasia, hypertrophy, separation and necrosis	Servizi and Martens 1987
Coho salmon	J	40,000	4	distal deterioration of gill filaments	Lake and Hinch 1999
Arctic grayling	YY	250,000	4	no gill damage	McLeay et al. 1987

shows gill cavity filled with mucous and sediment; note severe gill fusion, and the presence of mucous and sediment in *E – H*.

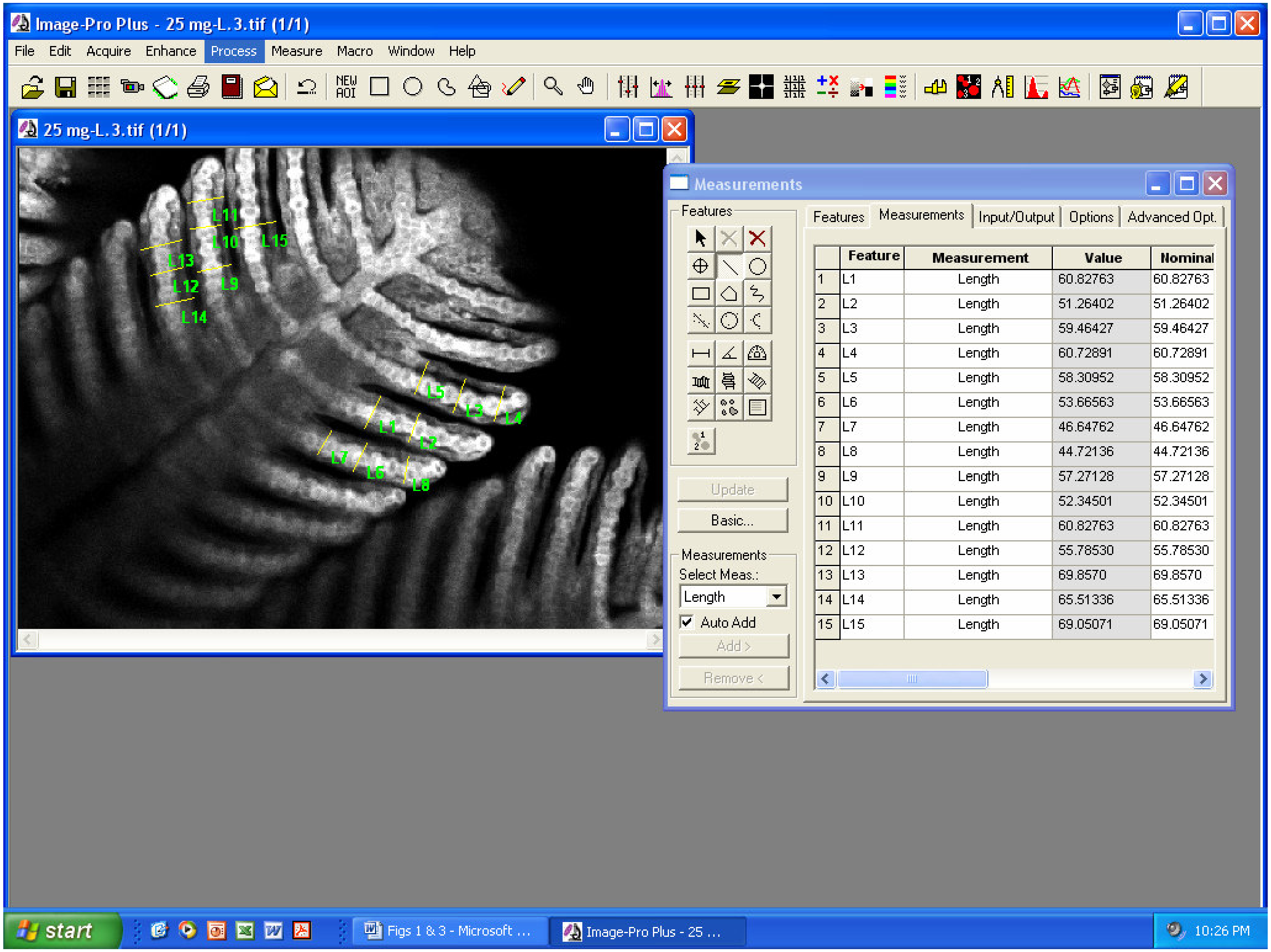
Figure 4.5: Spectral confocal micrographs of gill filaments of spotfin chubs reared for 21 days at each of the five sediment treatments (0, 25, 50, 100, and 500 mg/L). Micrograph *A* shows gill lamellae typical of fish reared in lowest three treatments (0, 25, and 50 mg/L). Micrographs *B* and *C* show lamellae typical of fish reared in 100 and 500 mg/L, respectively. Micrographs *D – G* show typical abnormalities of fish reared in highest two treatments. Note arrows indicating hyperplasia in micrograph *E*, and lamellar fusion in micrographs *F* and *G*.

Figure 4.6: Results of Tukey-Kramer multiple comparison tests of spotfin chub gill lamellae thickness (μm) versus suspended sediment concentration (SSC; mg/L), and interlamellar area (μm^2) versus SSC. Bars with different letters above them are significantly different ($\alpha = 0.05$). Sediment treatments presented as initial SSC added to each tank.

Figure 4.7: Regressions of gill lamellar thickness (μm) versus suspended sediment concentration (SSC; mg/L) and gill lamellar thickness (μm) versus interlamellar area (μm^2), for spotfin chubs (4-6 months old) reared for 21 days in growth trial 1. The regression equations are as follows: lamellar thickness = 0.05 (SSC) + 23.2 ($R^2 = 0.99$, $P = 0.0004$); lamellar thickness = -0.0365 (interlamellar area) + 45.389 ($R^2 = 0.97$, $P = 0.003$). Sediment concentrations used in first regression analysis are SSC estimated from sediment settling curves.

Figure 4.8: Regression of natural log of specific growth rate (% initial mass (g) per day) versus gill lamellae thickness (µm), for spotfin chubs (4-6 months old) reared for 21 days in growth trial 1. The regression equation is as follows: ln specific growth rate = -0.1159 (lamellae thickness) + 0.428 ($R^2 = 0.95$, $P = 0.005$).

Figure 4.1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L11
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L12
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L14
L5
L3
L4
L1
L2
L7
L6
L8
Measurements
Features
Features Measurements Input/Output Options Advanced Opt.
Feature Measurement Value Nominal
1 L1 Length 60.82763 60.82763
2 L2 Length 51.26402 51.26402
3 L3 Length 59.46427 59.46427
4 L4 Length 60.72891 60.72891
5 L5 Length 58.30952 58.30952
6 L6 Length 53.66563 53.66563
7 L7 Length 46.64762 46.64762
8 L8 Length 44.72136 44.72136
9 L9 Length 57.27128 57.27128
10 L10 Length 52.34501 52.34501
11 L11 Length 60.82763 60.82763
12 L12 Length 55.78530 55.78530
13 L13 Length 69.8570 69.8570
14 L14 Length 65.51336 65.51336
15 L15 Length 69.05071 69.05071
Update
Basic...
Measurements
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Length
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Figs 1 & 3 - Microsoft ...
Image-Pro Plus - 25 ...
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Figure 4.2

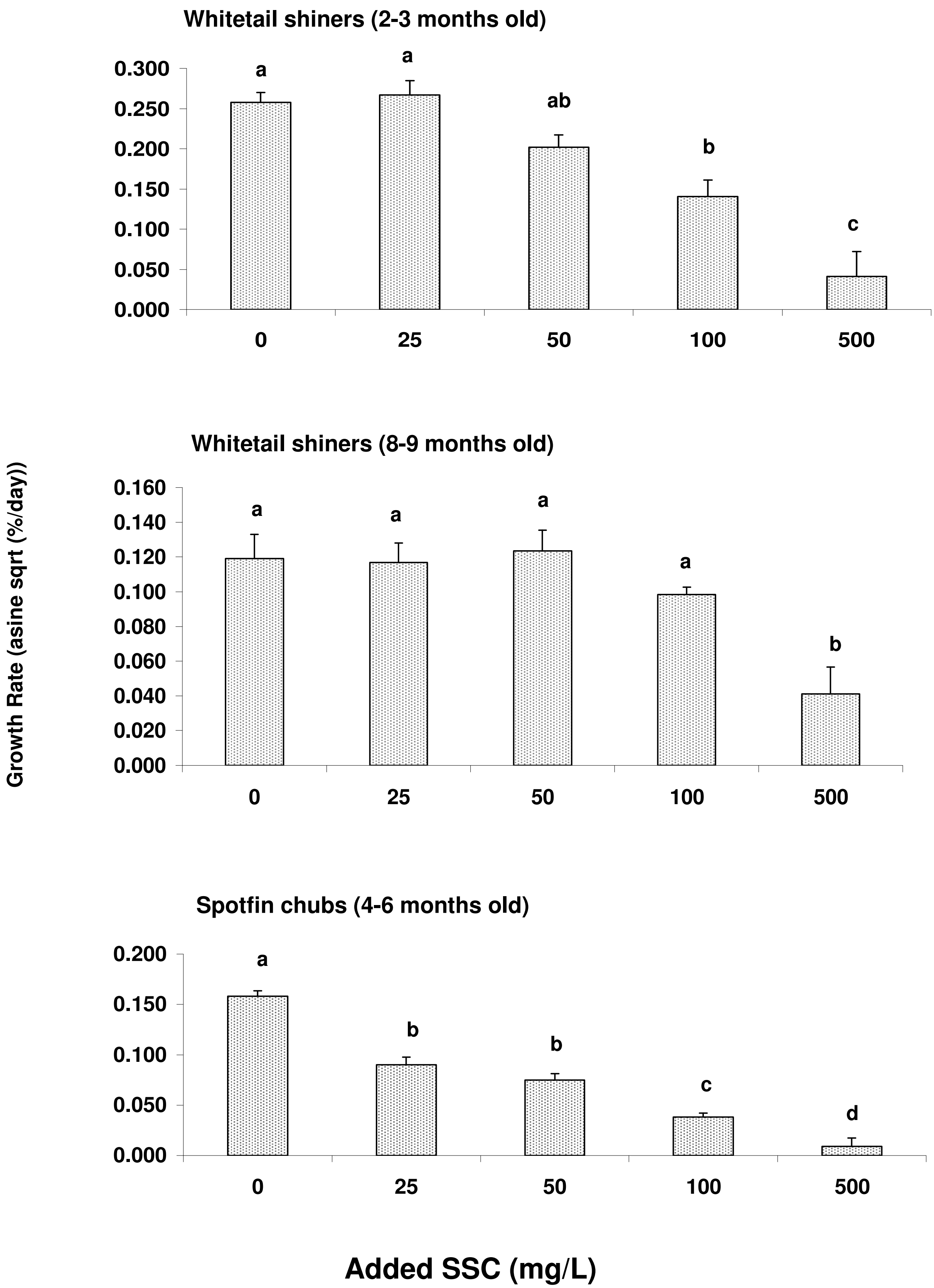
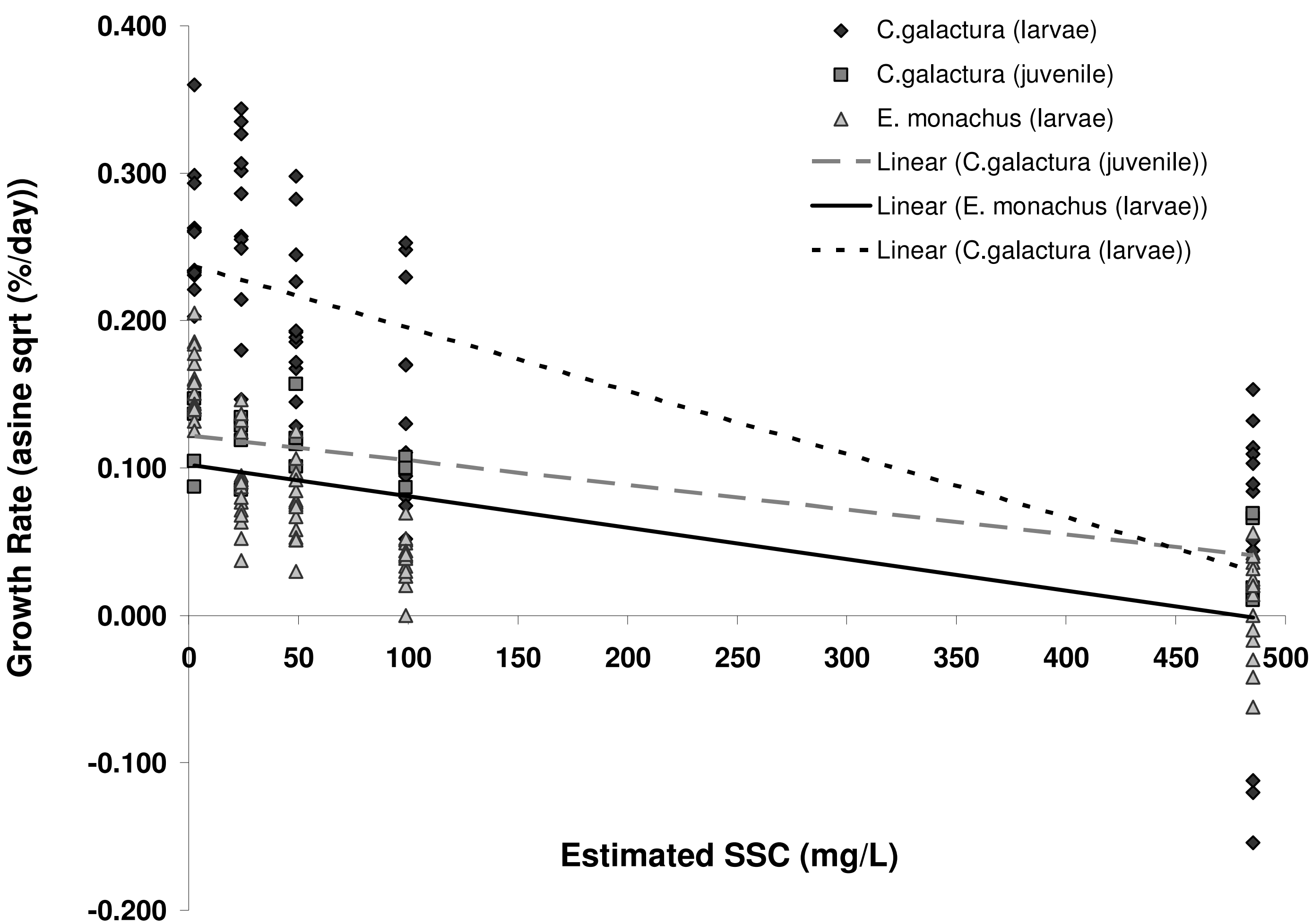

Whitetail shiners (2-3 months old)
0.300
0.250
0.200
0.150
0.100
0.050
0.000
a
a
ab
b
c
0
25
50
100
500
Whitetail shiners (8-9 months old)
0.160
0.140
0.120
0.100
0.080
0.060
0.040
0.020
0.000
a
a
a
a
b
0
25
50
100
500
Spotfin chubs (4-6 months old)
0.200
0.150
0.100
0.050
0.000
a
b
b
c
d
0
25
50
100
500
Growth Rate (asine sqrt (%/day))
Added SSC (mg/L)

Figure 4.3



Growth Rate (asine sqrt (%/day))
0.400
0.300
0.200
0.100
0.000
-0.100
-0.200
0
50
100
150
200
250
300
350
400
450
500
Estimated SSC (mg/L)
C.galactura (larvae)
C.galactura (juvenile)
E. monachus (larvae)
Linear (C.galactura (juvenile))
Linear (E. monachus (larvae))
Linear (C.galactura (larvae))

Figure 4.4

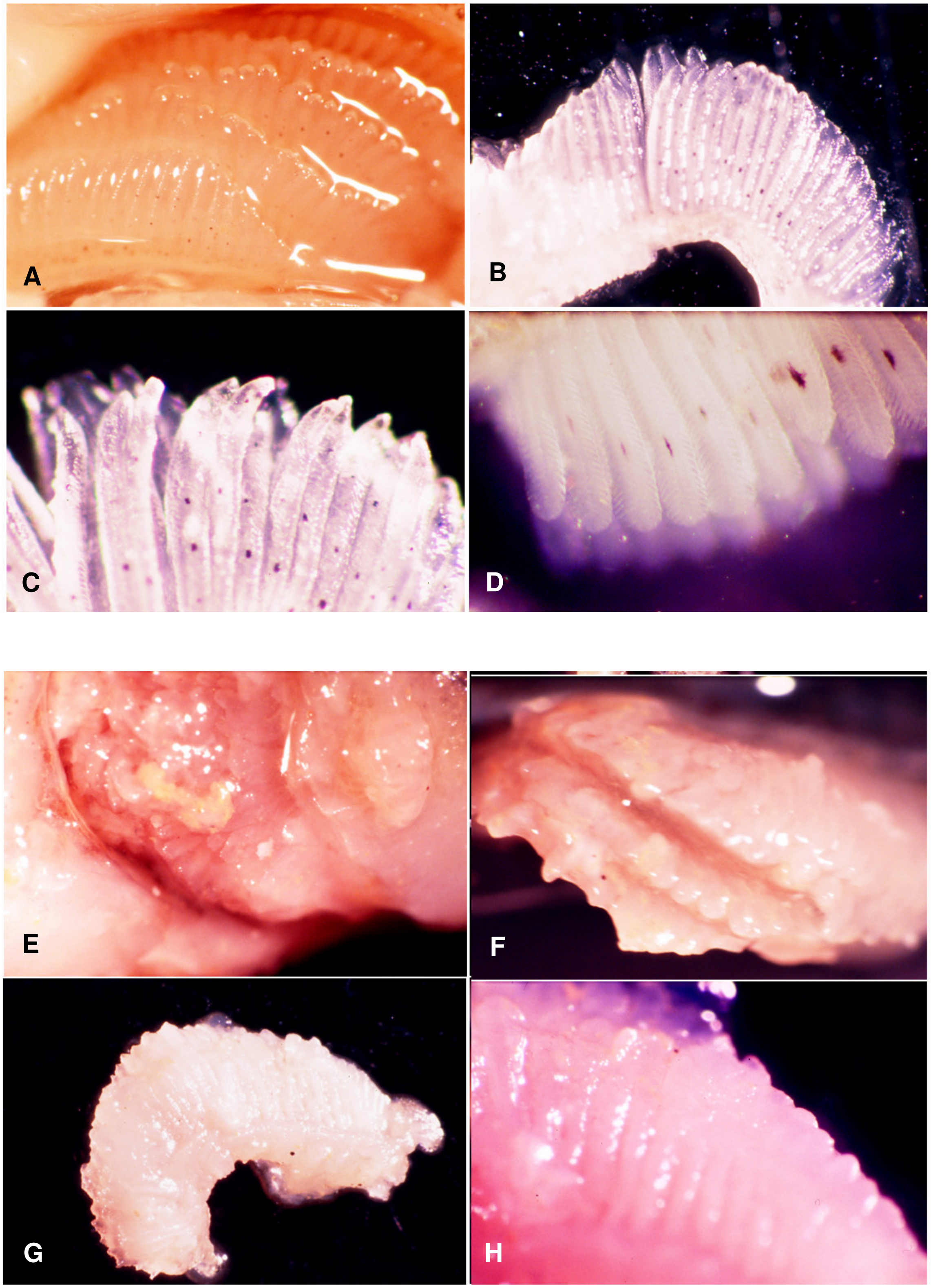


Figure 4.5

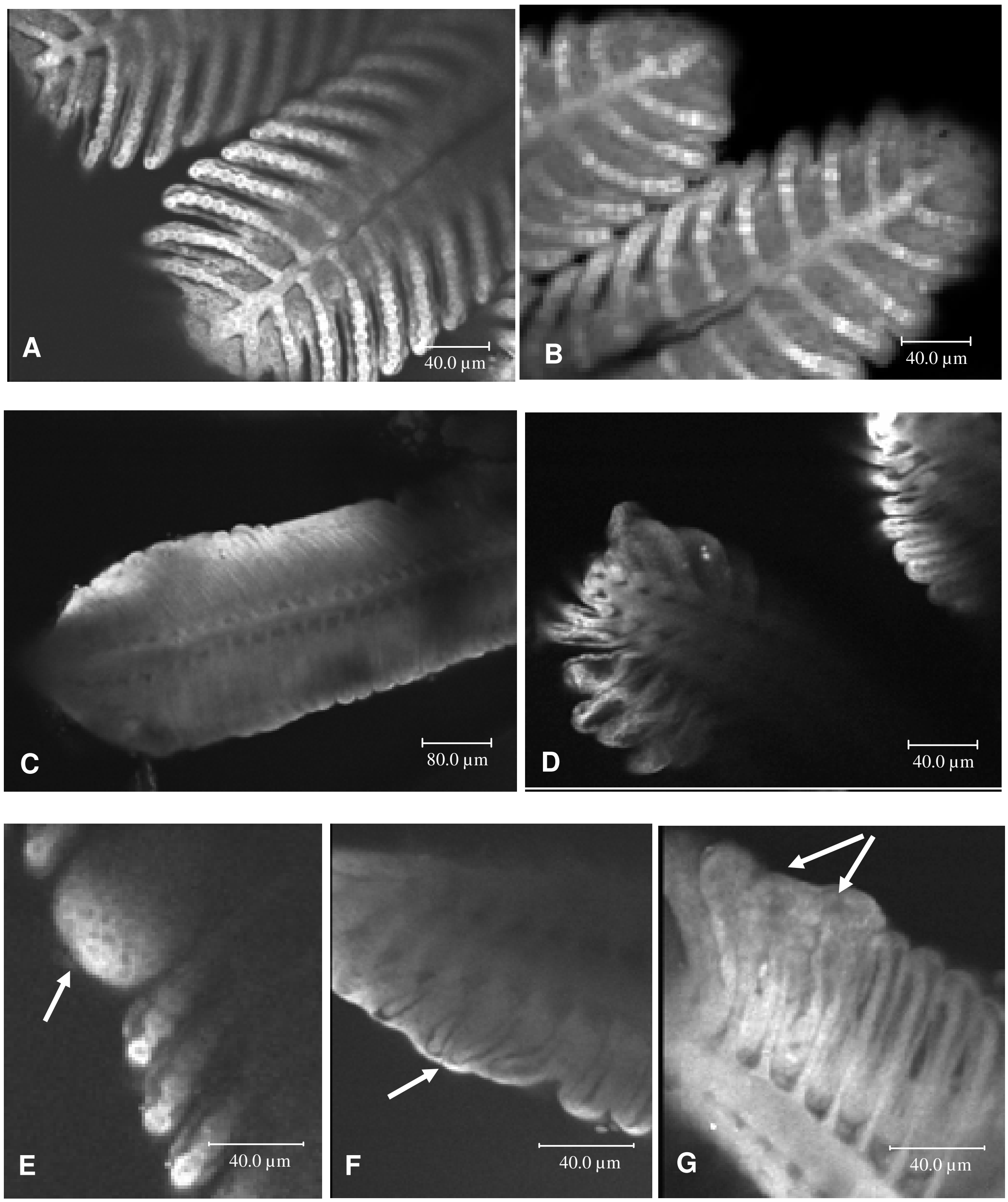
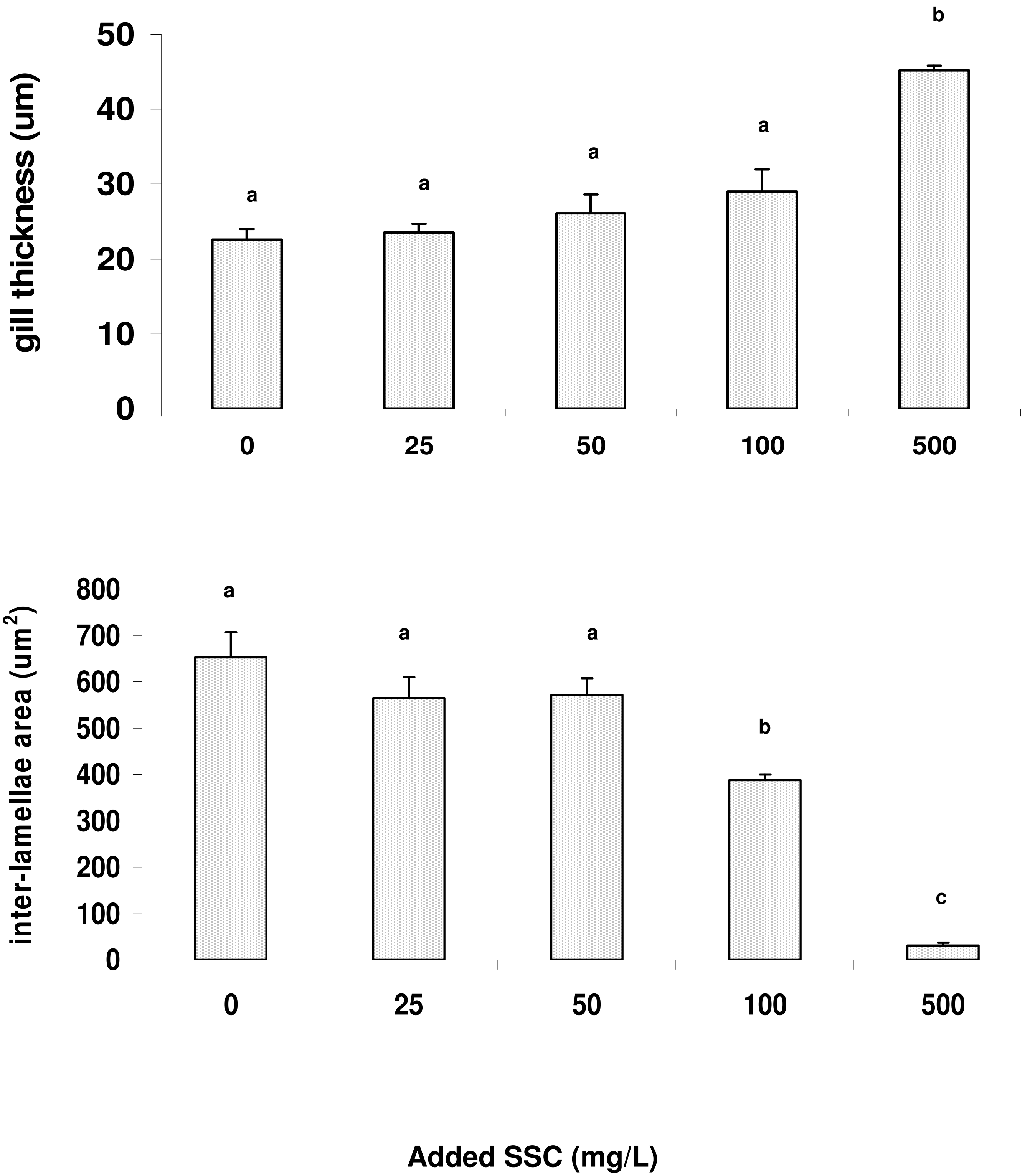
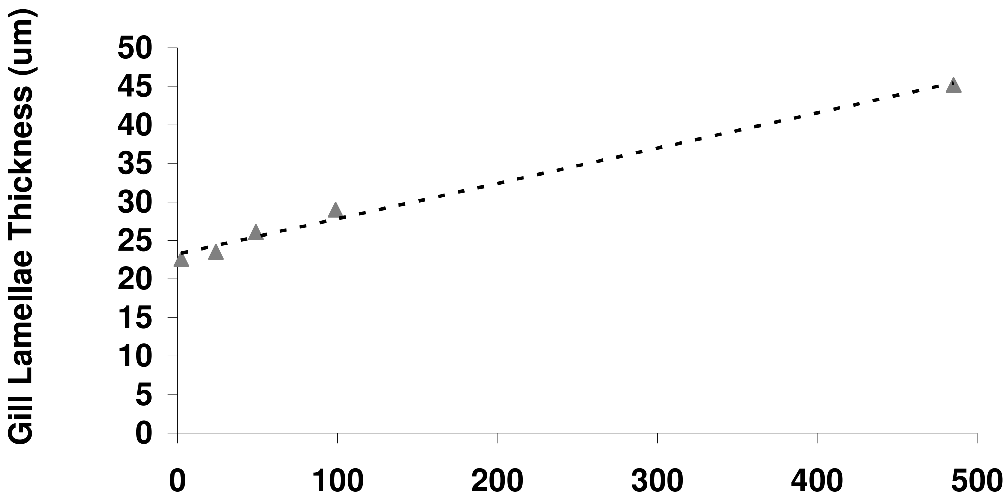


Figure 4.6

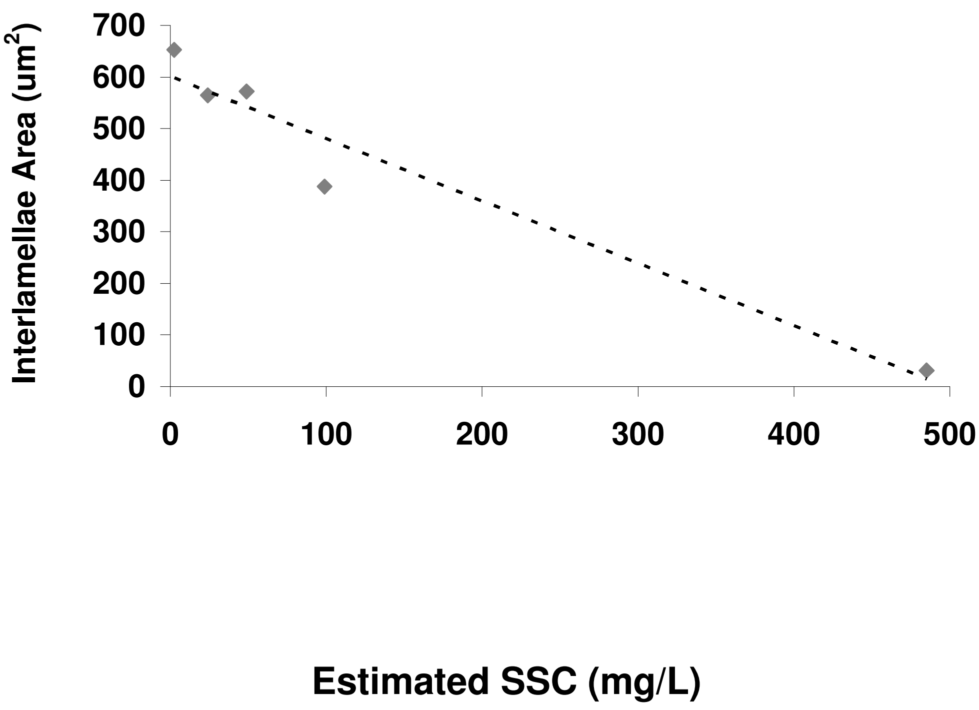


gill thickness (um)
50
40
30
20
10
0
a
a
a
a
b
0
25
50
100
500
inter-lamellae area (um²)
800
700
600
500
400
300
200
100
0
a
a
a
b
c
0
25
50
100
500
Added SSC (mg/L)

Figure 4.7

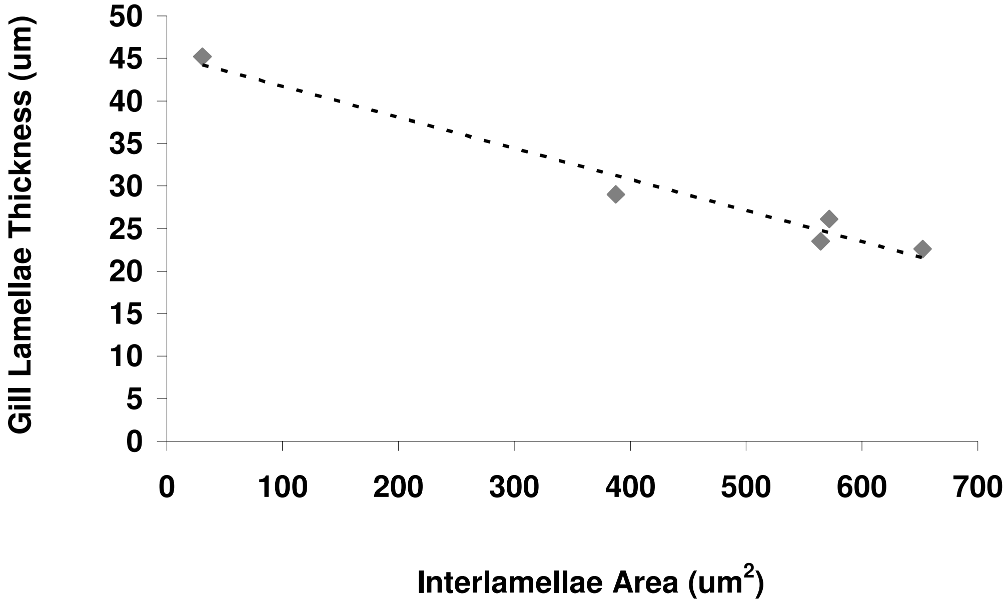


Gill Lamellae Thickness (um)
50
45
40
35
30
25
20
15
10
5
0
0
100
200
300
400
500

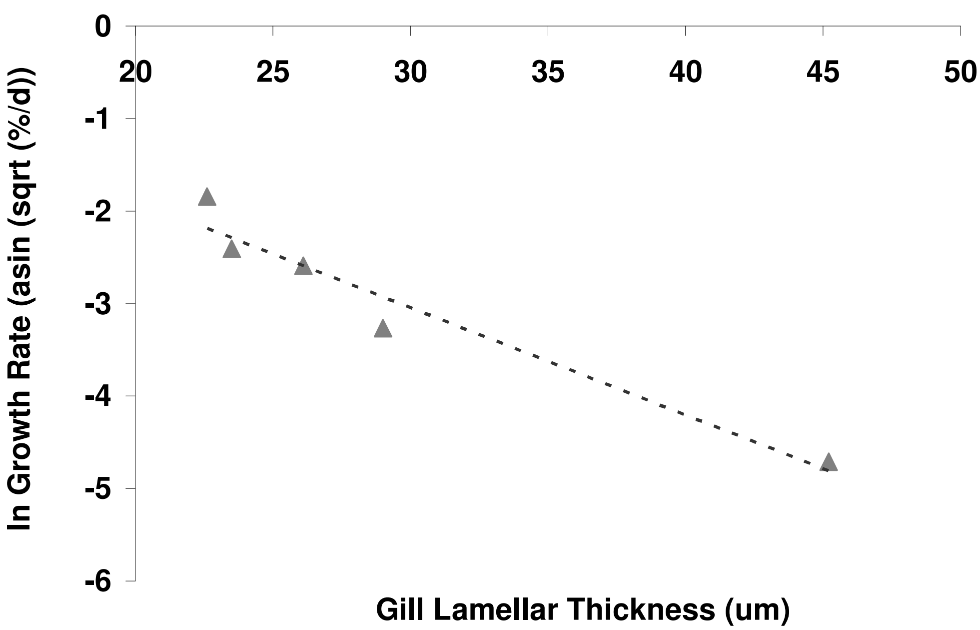


Interlamellae Area (um2)
700
600
500
400
300
200
100
0
0
100
200
300
400
500
Estimated SSC (mg/L)

Figure 4.8



Gill Lamellae Thickness (um)
50
45
40
35
30
25
20
15
10
5
0
0
100
200
300
400
500
600
700
Interlamellae Area (um2)



ln Growth Rate (asin (sqrt (%/d))
0
-1
-2
-3
-4
-5
-6
20
25
30
35
40
45
50
Gill Lamellar Thickness (um)

CHAPTER 5

EFFECTS OF INCREASED SUSPENDED SEDIMENT CONCENTRATION ON SPAWNING SUCCESS OF THE WHITETAIL SHINER (*Cyprinella galactura*)[4]

[4]A.B. Sutherland. To be submitted *Transactions of the American Fisheries Society*

Abstract

Little is known about the effects of elevated suspended sediment on the reproductive behavior of fishes, especially non-game fishes of the southern US. I investigated the effects of increased suspended sediment concentration (SSC; 0, 25, 50, 100 and 500 mg/L) on the spawning success of the crevice-spawning whitetail shiner (*Cyprinella galactura*), a phylogenetically similar species to the federally threatened spotfin chub (*Erimonax monachus*). During two week-long experiments, spawning success was measured as spawning effort (the number of replicates where spawning occurred) and spawning output (number of propagules [clear eggs, eyed eggs and larvae] spawned). Above a threshold of 25 mg/L, spawning effort decreased significantly with increasing SSC. Spawning effort decreased from 7 of 8 control tanks to 4 of 8 tanks at 500 mg/L. Total mean number of propagules at 500 mg/L was 10 – 14% of output in the other treatments. The number of eyed eggs and clear eggs spawned were significantly higher than the number of larvae spawned, indicating a delay in reproduction until SSCs had declined in all treatments. A comparison of propagule developmental stage with sediment settling curves allowed an estimation of mean SSC when propagules were spawned. The number of propagules spawned was inversely and significantly related to mean SSC during spawning. Whitetail shiner spawning success was moderately affected by the SSCs used in this study. Comparison of these results with a similar study on the tricolor shiner (*Cyprinella trichroistia*) suggests that whitetail shiners may be slightly more tolerant of excessive sedimentation, but nonetheless show reduced spawning success at SSCs commonly observed in the upper Little Tennessee River, where whitetail shiners and spotfin chubs naturally occur.

Introduction

North America has the highest diversity of temperate freshwater fishes in the world, and the southeastern US is the center of this rich fish fauna, harboring over 600 species (Warren et al. 2000). Within the southeast, the greatest diversity (70%) is in upland rivers and streams of the Appalachian Mountains (Walsh et al. 1995). In addition to high diversity, the southeastern US has very high rates of fish imperilment (28%; Warren et al. 1997, Master 1998, Warren et al. 2000). In the southern Appalachians 21% of darters (Percidae) and minnows (Cyprinidae) are imperiled (Walsh et al. 1995). Southeastern rivers and streams are negatively affected by numerous anthropogenic stressors. Excessive sedimentation, as the primary pollutant, is responsible for ~ 40% of fish imperilment (Etnier 1997). Elevated sediment deposition from poor land-use practices results in the fragmentation, degradation and elimination of suitable habitat for many benthic fish species (Neves and Angermeier 1990, Walsh et al. 1995, Burkhead et al. 1997, Richter et al. 1997, Johnston 1999, Burkhead and Jelks 2001). Although habitat destruction from deposited sediment is the primary sediment-induced impact in southeastern riverine systems (Burkhead et al. 1997, Richter et al. 1997, Warren et al. 2000), some evidence suggests that turbidity-related effects on reproductive behavior may severely impact the population stability and longevity of southeastern fishes (Burkhead and Jelks 2001).

Twenty years ago Bruton (1985) noted that our understanding of the effects of sediment on fish reproduction was poor; today we know only slightly more, especially regarding effects on non-game fishes. A high percentage of southeastern non-game fishes with declining abundance and range are benthic-specialized species that require unembedded heterogeneous substrate for reproduction (Jenkins and Burkhead 1994, Burkhead et al. 1997, Warren et al. 2000). However, despite this, very little research has been conducted on the relationship between sediment and spawning success of these species. The majority of research on the effects of sediment on fish reproduction has involved game fishes (primarily Salmonidae and Centrarchidae). Other studies have focused on egg and fry survival in spawning redds (Chapman 1988, Montgomery et al. 1996), on overwinter success and production of various life stages (Hartman and Scrivener 1990), and on habitat of various life stages (see review by Waters 1995).

Most of what is known about the relationship between increased sediment and game-fish reproduction deals with habitat modification due to sediment deposition. Less is known about the effects of increased suspended sediment on fish reproductive behavior. However, some researchers have found that excessive siltation caused cutthroat trout (*Oncorhynchus clarkii*) to abandon spawning grounds (Wilber 1983) and delayed timing of spawning in several families of warmwater fishes (Muncy et al. 1979). Some researchers suggest that sediment-induced habitat degradation and physiological stress affect fishes more severely than behavioral effects (Newcombe and Jensen 1996). However, one study suggests that by disrupting spawning behavior, increased suspended sediment concentration may impact the population stability of some benthic-specialized fishes (Burkhead and Jelks 2001). Reproductive success of the tricolor shiner (*Cyprinella trichroistia*) was shown to be negatively affected by high levels of suspended sediment (100 – 600 mg/L), presumably because visual cues necessary to induce spawning behavior, were disrupted by increased turbidity (Burkhead and Jelks 2001).

The purpose of this study was to increase understanding of the effects of suspended sediment on non-game fish reproduction. The spotfin chub, *Erimonax monachus* (Cope), is typical of imperiled, benthic-specialized non-game fishes in the southern Appalachians. Objectives of the USFWS recovery plan for this species include assessment of potential threats and determination of reproductive biology (USFWS 1983). The whitetail shiner, *Cyprinella galactura* (Cope), was chosen as a surrogate for the spotfin chub because both share habitat, reproductive ecology and phylogeny (Burkhead and Bauer 1983, Mayden 1989, Jenkins and Burkhead 1994). Similar to spotfin chubs, whitetail shiners typically inhabit clear upland montane streams. Whitetail shiners are common throughout the Tennessee and Cumberland river drainages and are also found in the southern Ozarks (Jenkins and Burkhead 1994). They are known to hybridize with spotfin chubs (Burkhead and Bauer 1983), and both species spawn fractionally (i.e. multiple clutches over protracted spawning period) in bedrock and boulder crevices (Jenkins and Burkhead 1994). The objective of this study was to determine the effects of elevated suspended sediment on the spawning success of the whitetail shiner. Specifically, the objectives were to determine the relationship between suspended sediment concentration (SSC; 0, 25, 50, 100 and 500 mg/L) and whitetail shiner spawning effort (the number of tanks where spawning occurred) and spawning output (the number of propagules spawned).

Methods

<u>Experimental Procedure</u>

Two spawning trials were conducted, each for one week (168 h) at 25° C. At that temperature, a week is sufficient time for eggs hatched in controls to develop into larvae (personal observation and Noel Burkhead, USGS, pers. comm.). Each trial consisted of four replicates of five suspended sediment treatments (0, 25, 50, 100 and 500 mg/L). SSCs were within the lower range of conditions observed in the upper Little Tennessee River (LTR; turbidity range: 10 – 1500 mg/L, W. O. McLarney unpublished data). The apparatus used for experiments consisted of slow moving (~ 3 – 5 mm/sec) motor-driven paddles within each of twenty 30 L experimental tanks (Chapter 2). Each paddle was fitted with two baffles that slowly swept the floor of a given tank, while also delivering a column of compressed air that resuspended settled sediment particles. Sediment used in growth experiments was collected from the Little Tennessee River basin (Macon Co., NC) and wet-sieved to obtain the < 45 µm fraction. Sediments were free of metal and organic contamination (Chapter 3; Appendix 1).

Spawning substrate within each tank consisted of a stack of 5 unglazed tiles separated by metal washers, held together by two stainless steel bolts (Figure 1). Similar tile 'towers' have been used with success to spawn crevice-spawning *Cyprinella* species (Gale and Gale 1977, Rakes et al. 1999, Burkhead and Jelks 2001). Instead of resting on the bottom of the tank, towers were suspended from a wooden board spanning the top of each tank. The bottom tile of each tower was 18.5cm x 30cm, the next tile was 11.5cm x 30cm and the final three tiles were 10cm x 30cm. Suspending the tower enabled the paddle to move freely below the bottom tile. The bottom tile was made larger than the rest so that it would serve as a 'false bottom', thereby inducing the fish to spawn in the crevices above it. Each tank was equipped with a small powerhead pump to generate current over the spawning towers, similar to Burkhead and Jelks (2001).

Whitetail shiner adults used in spawning experiments were dipnetted while snorkeling in the upper LTR (Swain County, North Carolina). All fish used in this study were collected on two separate days. An attempt was made to collect only nuptial males (i.e., tuberculate with pale blue iridescence) and gravid females. After transporting fish to the laboratory, males and females were kept in separate 220 gallon holding tanks at 20 - 22° C, and fed frozen chironomid larvae, frozen *Artemia* adults and a dry pelleted prepared food (Purina® AquaMax D04; 1.5mm). Each fish was used only one time, insuring that all fish used in experiments were behaviorally naïve.

Normal operation of the experimental apparatus caused ~ 5 – 10% of suspended sediment to settle over the course of one week (Chapter 2). The presence of the tile towers within each tank caused a marked increase in sediment settling during each 7-day spawning trial. Suspended sediment dissipation was estimated by measuring turbidity each day for one week in two replicates of each sediment treatment. Sediment settling experiments were conducted in tanks containing no fish. Turbidity data were converted to suspended sediment concentrations using the following rating curve developed for test sediment:

$$SSC = 1.2316(t) - 6.8426$$

where SSC = suspended sediment concentration (mg/L), t = turbidity (nephelometric turbidity units [NTU]); $R^2 = 0.99$, $P < 0.001$. During sediment settling experiments 58 – 71% of suspended sediment settled. SSC at spawning was estimated by comparing sediment settling curves (i.e., SSC over time) and time (h) necessary to attain three developmental stages (clear eggs, eyed eggs, and larvae). Similar to Burkhead and Jelks (2001), development intervals used were 45 h for eye development, and 120 h for hatching; intervals were based on published rates for other *Cyprinella* spp. at similar temperatures. (Gale and Gale 1977, Snyder 1993).

At the start of each trial, two females and one male were taken from their respective holding tanks and randomly assigned to each experimental tank. Fish with more advanced secondary sexual characteristics (i.e. coloration and tubercles for males; abdomen swelling for females) were preferentially selected. Males ranged in size from 120 – 140 mm, and females were 90 – 110 mm. After fish were placed in each tank, sediment treatments were added. Fish were not fed during spawning trials. Experimental trials were conducted with a constant photoperiod of 14 h light and 10 h dark and water temperatures were increased from holding tank temperatures to ~ 25° C. At the end of each trial, fish were removed from tanks and tile towers were removed and examined for propagules. Then the water from each tank was siphoned through a net, and the net examined for propagules. All propagules were preserved in a 10% solution of buffered formalin.

Data Analyses

Regression analysis was used to determine if there was a significant relationship between suspended sediment concentration and spawning effort, defined as number of tanks per treatment in which spawning occurred. Time intervals (h) necessary for development of each of the three developmental stages were plotted on sediment settling curves to estimate sediment concentration at initiation of spawning. Regression analysis was used to determine if there was a significant relationship between mean SSC at initiation of spawning and the number of propagules spawned. The numbers of each developmental stage spawned (larvae, eyed eggs and clear eggs) were compared using analysis of variance (ANOVA; JMP, SAS Institute, Cary, NC).

Results

Spawning effort decreased with increasing SSC (Figure 2), although no change in spawning effort occurred until SSC was above 25 mg/L. Above this threshold, spawning effort decreased significantly ($R^2 = 0.95$, $P = 0.03$; Figure 2). Fish spawned in only half of the tanks at 500 mg/L and only 2 of 8 tanks at that treatment had more than 3 eggs.

Comparison of propagule developmental stage with sediment settling curves allows an estimation of the conditions under which the propagules were spawned (Figure 3). For example, in the 500 mg/L tanks, only clear eggs were found. Because eye development occurs after ~ 45 hours in *Cyprinella* spp. (Gale and Gale 1977), this indicates that spawning in these tanks took place after 123 hours (i.e. 168 – 45 h). Therefore, this indicates that eggs in the 500 mg/L tanks were spawned when the mean SSC was ~ 209 mg/L. Only eyed and clear eggs were found in 100 mg/L tanks. Clear eggs were spawned after 123 hours, and eyed eggs were spawned between 48 and 123 hours after trial initiation. Therefore, in the 100 mg/L tanks, clear eggs were spawned when mean SSC was ~ 44 mg/L, and eyed eggs were spawned when mean SSC was ~ 54 mg/L. The number of propagules spawned was inversely and significantly related to log (mean SSC) (R^2 = 0.36, P = 0.02; Figure 4).

In addition to spawning effort decreasing with increasing SSC, mean spawning output (i.e. the mean number of propagules spawned per treatment) declined consistently with increasing SSC (Tables 1 & 2). For each developmental stage, and for total propagules spawned, mean spawning output at 500 mg/L was 10 – 14% of the output at the four lower SSCs. The number of eyed eggs and clear eggs spawned were significantly higher than the number of larvae produced (ANOVA; F = 5.29, P = 0.006), indicating a delay in timing of reproduction.

Discussion

Whitetail shiner spawning success was moderately affected by increasing SSC. Spawning effort decreased significantly above a threshold of 25 mg/L SSC. The total number of propagules decreased significantly with increasing SSC. The fact that significantly more eggs than larvae were observed suggests that spawning was delayed by increasing SSC.

Whitetail shiners appear to be slightly less sensitive to increasing SSC than the only other crevice-spawning minnow whose spawning response to elevated SSC has been studied, the tricolor shiner (*Cyprinella trichroistia*; Burkhead and Jelks 2001). Tricolor shiner spawning effort, output and timing were all significantly affected by increased SSC (Burkhead and Jelks 2001). At the highest treatment (600 mg/L) tricolor shiner spawning effort dropped to 25%, as opposed to 50% in 500 mg/L for the whitetail shiner. The mean number of tricolor shiner eggs in 100 mg/L tanks decreased to 50% of controls; the number of whitetail shiner propagules at the same SSC decreased to 70% of controls.

The moderate impact of elevated SSC on whitetail shiner reproduction supports previous anecdotal evidence of sediment tolerance in this species (Jenkins and Burkhead 1994). Previous research has documented whitetail shiner persistence in heavily sedimented tributaries (Sutherland et al. 2002), and nowhere are they known to be in jeopardy (Warren et al. 2000). Sediment tolerance may partly explain the success of whitetail shiners relative to other upland crevice-spawning minnows, which are experiencing dramatic declines in population and range (e.g. the spotfin chub and the blue shiner *Cyprinella caerulea*). However, some other aspect of life history may also give whitetail shiners an edge in disturbed systems. Life history differences have been suggested as an explanation for the differential success of four crevice-spawning *Cyprinella* species of the upper Coosa River system (Burkhead and Jelks 2001). The two species that are widespread spawn in swift, deep riffles and are thus able to survive in river reaches affected by chronic sedimentation. The two marginal species spawn in slow riffles, a habitat more vulnerable to sedimentation. Just as 'sediment tolerance' appears to be related to life history of the two widespread *Cyprinella* species in the Coosa River, this may also be the case for the whitetail shiner. Whitetail shiners spawn in a variety of habitats including under trash (e.g. tires, sheet metal, plywood), and in logs located above the substrate (Jenkins and Burkhead 1994; personal observations). Burkhead and Jelks (2001) note that spawning in swift current may be advantageous to some *Cyprinella* species in sedimented systems. The ability to spawn in crevices above the riverbed may give whitetail shiners a similar advantage. Just as spawning in swift current does not correspond to *Cyprinella* phylogeny (Mayden 1989), perhaps neither does sediment intolerance.

Whitetail shiners may also be less sensitive to elevated SSC relative to other *Cyprinella* spp. because of a lower reliance on visual cues during spawning. Tricolor shiners are thought to delay spawning until SSCs decline because they rely on visual cues during spawning (Burkhead and Jelks 2001). Visual cues are thought to be important to the reproductive success of sexually dimorphic, brightly colored, displaying fish (Muncy et al. 1979, Kodric-Brown 1998, Burkhead and Jelks 2001). Whitetail shiners are less brilliantly colored than other fishes for which visual cues are presumed to be important. Even in turbid water, the white pigment on their caudal peduncle and base of caudal fin is evident (personal observations). Although whitetail shiners did delay spawning at high SSCs, this delay was less pronounced than was observed with tricolor shiners.

The moderate response of whitetail shiners to elevated SSC is within the range of impairment predicted by a sediment dose-response model (Newcombe and Jensen 1996). This model, which uses SSC (mg/L) and exposure duration (h) to predict severity of impairment to various fish taxa and life stages, predicted moderate physiological stress when mean SSC, and duration from the present study were applied. In general, the results from this study agree with the model's prediction of sub lethal impairment. Although impairment to larvae and eggs is not lethal, sediment effects on reproductive behavior may still severely harm the long-term stability and longevity of native fish populations (Burkhead and Jelks 2001). Because sediment-induced alterations of spawning reduces or precludes the production of eggs and larvae, reproductive behavior effects are more severe than is indicated by sediment-related egg and larvae mortality alone.

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Table 5.1: Total number of propagules spawned by whitetail shiners in two one-week spawning trials at 5 suspended sediment concentrations (SSC; mg/L). Mean spawning output and standard errors across eight replicate tanks are presented at each SSC.

			SSC (mg/L)				
total propagules	trial	replicate	0	25	50	100	500
	1	1	110	0	120	167	19
		2	103	166	145	0	0
		3	48	16	0	0	0
		4	78	111	0	68	0
	2	1	115	2	23	26	37
		2	0	130	67	0	3
		3	81	100	131	134	0
		4	94	22	65	19	2
		mean	79	68	69	52	8
		standard error	14	23	21	23	5

Table 5.2: Total number of each developmental stage spawned by whitetail shiners in 2 one-week spawning trials, for five suspended sediment concentrations (SSC; mg/L). Mean spawning output across eight replicate tanks is presented for each developmental stage at each SSC. Standard errors are also presented.

			SSC (mg/L)				
larvae	trial	replicate	0	25	50	100	500
	1	1	0	0	107	0	0
		2	0	0	0	0	0
		3	48	0	0	0	0
		4	0	0	0	0	0
	2	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	43	32	0	0
		4	15	0	0	0	0
		mean	8	5	17	0	0
		standard error	6	5	13	0	0

			SSC (mg/L)				
eyed eggs	trial	replicate	0	25	50	100	500
	1	1	57	0	13	104	0
		2	65	112	41	0	0
		3	0	0	0	0	0
		4	0	34	0	51	0
	2	1	0	2	23	0	0
		2	0	25	0	0	0
		3	81	57	51	27	0
		4	59	0	65	0	0
		mean	33	29	24	23	0
		standard error	13	14	9	13	0

			SSC (mg/L)				
clear eggs	trial	replicate	0	25	50	100	500
	1	1	53	0	0	63	19
		2	38	54	104	0	0
		3	0	16	0	0	0
		4	78	77	0	17	0
	2	1	115	0	0	26	37
		2	0	105	67	0	3
		3	0	0	48	107	0
		4	20	22	0	19	2
		mean	38	34	27	29	8
		standard error	15	14	14	13	5

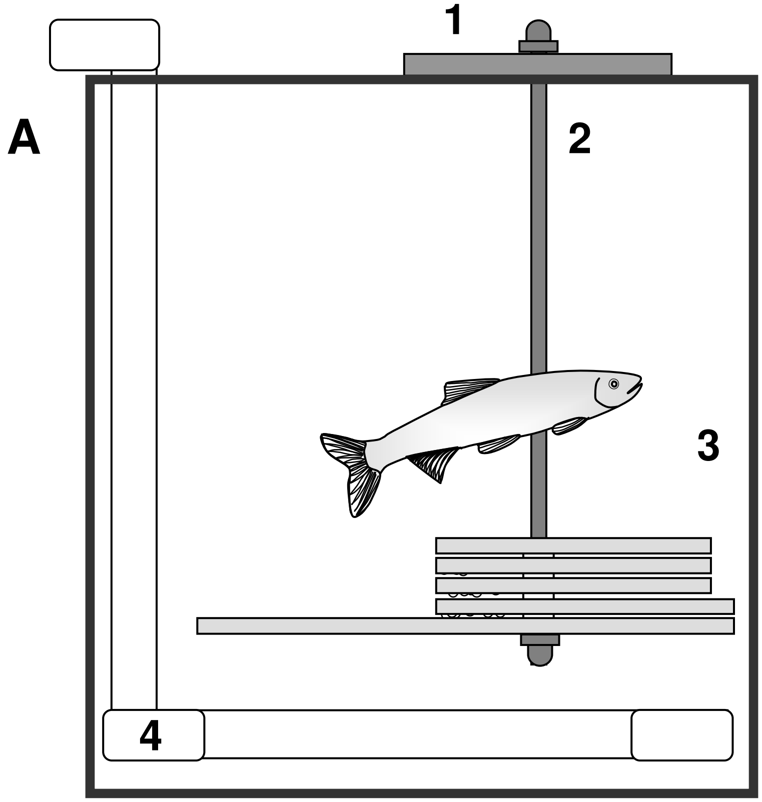
Figure 5.1: Schematics showing 'tower' of spawning tiles used in whitetail shiner spawning experiments. *Figure A:* Side view of experimental tank showing orientation of paddle and tile tower. No. 1: wooden board which spans the tanks, and from which the tile tower is suspended; No. 2: one of two metal bolts used to suspend tower; No. 3: tile tower made of five non-glazed tiles (x); No. 4: PVC apparatus paddle. *Figure B:* Top view of experimental tank showing orientation of tile tower and powerhead pump. No. 1: wooden board from which tower is suspended; No. 2: powerhead pump attached to side of tank (velocity = ~ 20 cm/s).

Figure 5.2: Regression of whitetail shiner spawning effort (number of tanks where spawning occurred) versus suspended sediment concentration (SSC; mg/L). The following regression equation was fit to the four highest SSCs (25, 50, 100 and 500 mg/L): spawning effort = -2.281(log SSC + 1) + 9.96 ($R^2 = 0.95$, $P = 0.03$). Suspended sediment concentrations used in regression analyses are initial SSC treatment values.

Figure 5.3: Sediment settling curves for five suspended sediment treatments over a 7 day trial duration. The hatched region represents the approximate period from spawning required to produce larvae at the end of a 7-day experimental trial. The shaded region is the period from spawning required to produce eyed eggs, and the clear region is the period required to produce clear eggs. Eyed egg and larvae development times are based on the literature (Gale and Gale 1977, Snyder 1993, Noel and Burkhead 2001).

Figure 5.4: Regression of number of propagules spawned versus suspended sediment concentration (SSC; mg/L). Each data point used for regression analysis represents the total number of larvae, eyed eggs or clear eggs spawned in 8 replicate tanks, at each of five initial sediment concentrations. SSCs used in regression analyses are mean values measured during sediment dissipation experiments. The regression equation is as follows: number of propagules =-89.564 (log mean SSC) + 273.9 (R^2 = 0.36, $P = 0.02$).

Figure 5.1



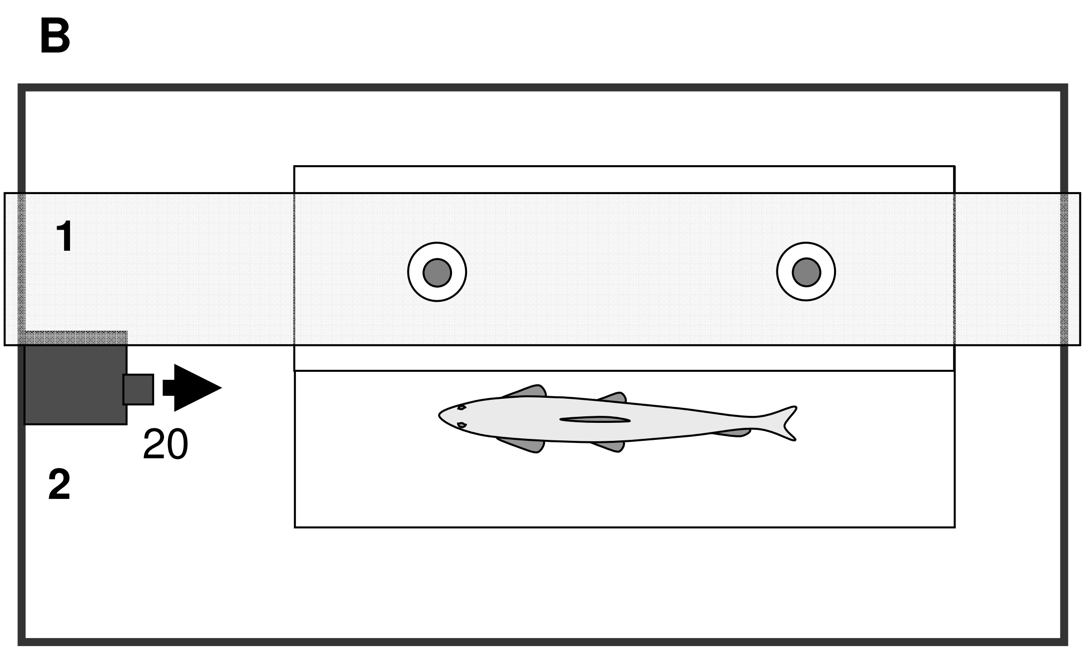
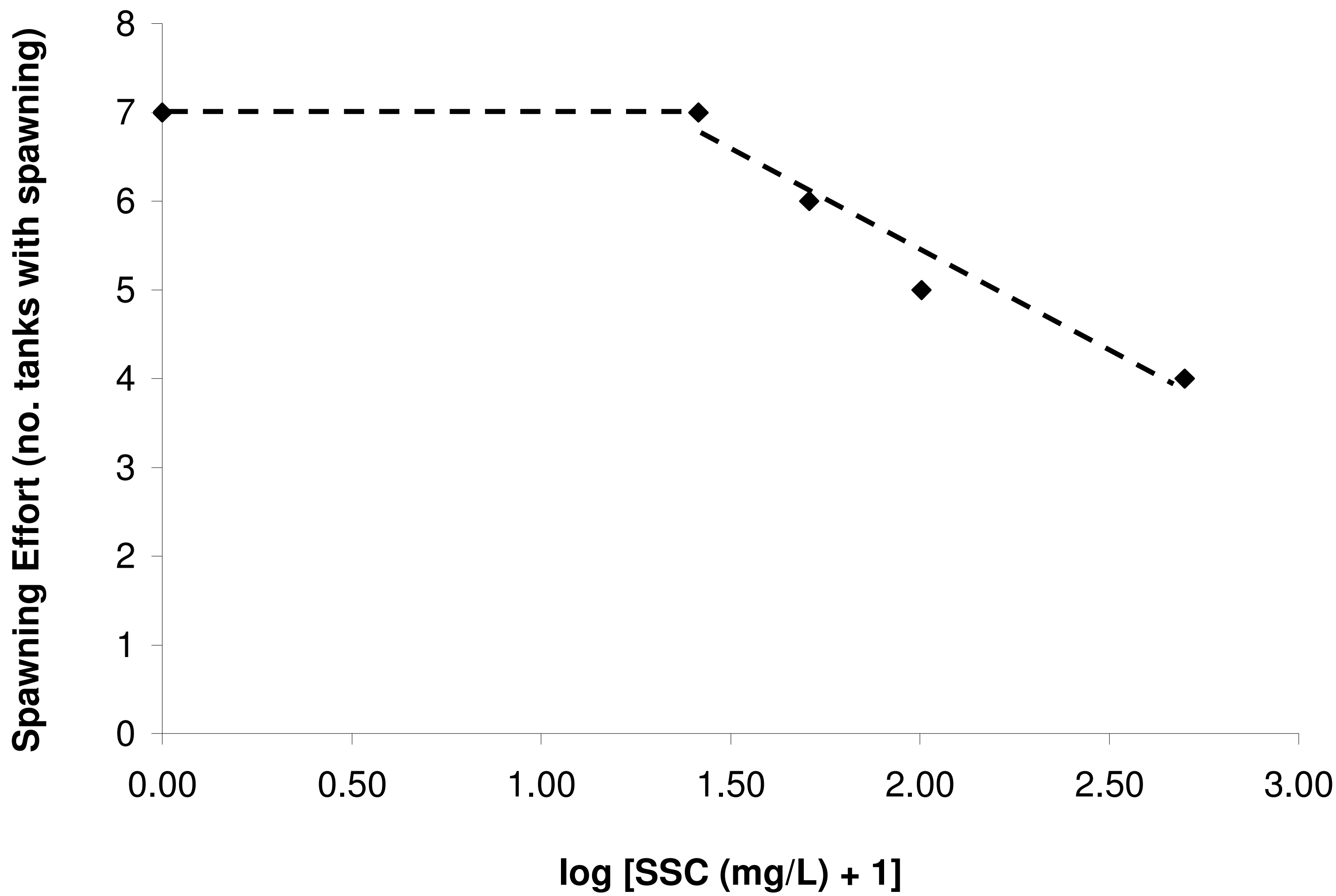


Figure 5.2



Spawning Effort (no. tanks with spawning)
8
7
6
5
4
3
2
1
0
0.00
0.50
1.00
1.50
2.00
2.50
3.00
log [SSC (mg/L) + 1]

Figure 5.3

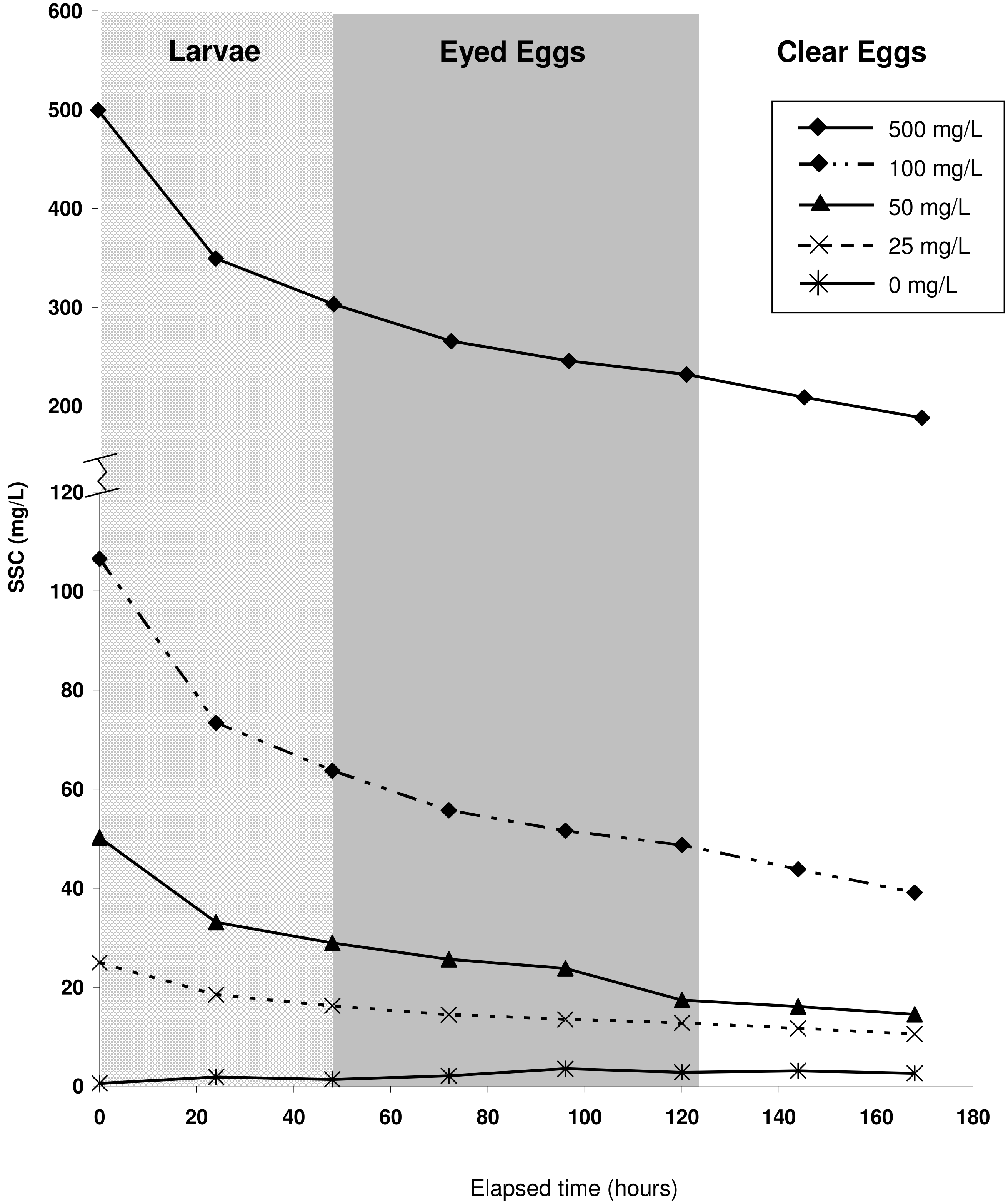
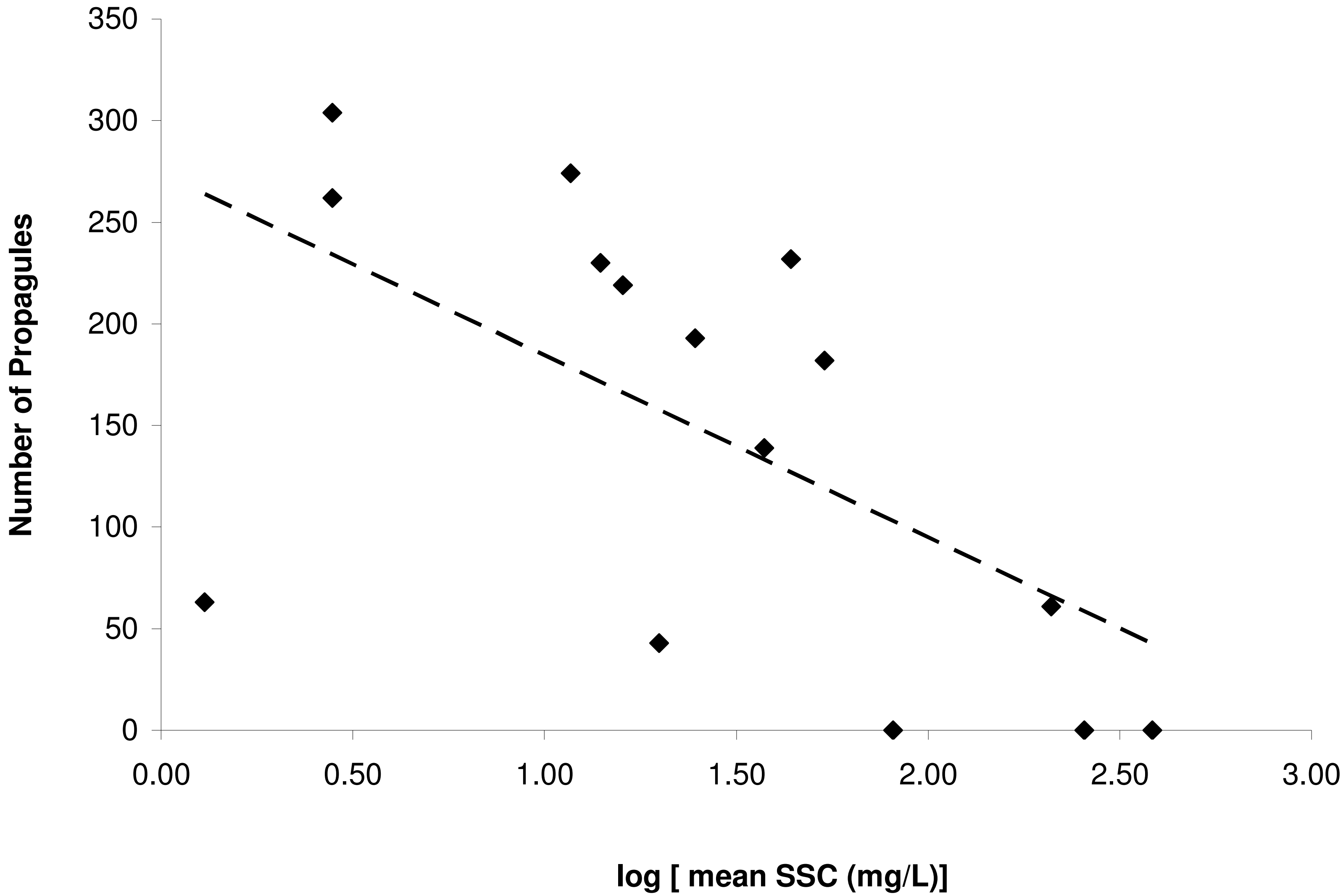

Larvae
Eyed Eggs
Clear Eggs
500 mg/L
100 mg/L
50 mg/L
25 mg/L
0 mg/L
SSC (mg/L)
Elapsed time (hours)
0
20
40
60
80
100
120
140
160
180
200
300
400
500
600

Figure 5.4


Number of Propagules
350
300
250
200
150
100
50
0
0.00
0.50
1.00
1.50
2.00
2.50
3.00
log [mean SSC (mg/L)]

CHAPTER 6

SPOTFIN CHUB (*Erimonax monachus*) SPAWNING HABITAT AND BEHAVIOR IN THE UPPER LITTLE TENNESSEE RIVER, NORTH CAROLINA[5]

[5]A.B. Sutherland. To be submitted *Environmental Biology of Fishes*

Synopsis

Spawning habitat characterization is necessary for the long-term recovery and maintenance of the spotfin chub (*Erimonax monachus* (Cope)), an imperiled crevice-spawning minnow inhabiting upland rivers of the southern Appalachians. Spawning habitat character and extent were determined for the spotfin chub within the Needmore Tract of the upper Little Tennessee River (LTR). The feasibility of supplementing spawning habitat through the creation of artificial spawning sites was also tested. In general, spotfin chub spawning was located in swift (0.8 m s^{-1}), moderately deep (0.5 – 0.6 m) bedrock riffles, with very little to no fine sediment. In addition to spawning under smooth loose cobbles lying on bedrock, spotfin chubs were regularly found spawning in mid-channel bedrock crevices covered with riverweed (*Podostemum ceratophyllum*). Other newly observed spotfin chub spawning behaviors include: a nest located in a boulder high off the riverbed, males performing milting displays in several crevices simultaneously, the use of spawning rocks twice the size of those previously reported, and spawning in areas where flow is low (0.35 m s^{-1}) and bedrock is 25 – 50% covered with fine sediment. Quantification of areal extent of spawning habitat in the upper LTR revealed that although only ~ 4.4% of the riverbed was suitable for spotfin chub reproduction, this area is twice as high as previous estimates. Spawning habitat patches were ~ 10 – 100 m apart. The distance between distinct groups of spawning habitat patches ranged from 194 – 1840 m. Vast areas of siltation between patches may hamper spotfin chub reproduction and population connectivity within the upper LTR. Spawning habitat enhancement was moderately successful; of 50 supplemented spawning rocks, 1 was used for a nest and 2 more were guarded by nuptial males. Spawning habitat supplementation may prove to be a simple and inexpensive means of mitigating the effects of excessive sedimentation on spawning habitat of small riverine fishes.

Introduction

North America's rich freshwater fish fauna continues to decline at an alarming rate, due primarily to increasing degradation and loss of habitat (Williams et al. 1989, Warren and Burr 1994, Richter et al. 1997). This trend is particularly acute in the southeastern United States, which harbors the most diverse temperate freshwater fish fauna in the world (Warren et al. 2000). Rapid regional population growth, combined with a lack of planning for the protection of vulnerable species, has contributed to a growing percentage of this diverse fauna becoming vulnerable to extinction (Neves and Angermeier 1990, Walsh et al. 1995, Warren et al. 2000). In the past two decades alone, the rate of imperilment of southeastern fishes has increased by 125% (Warren et al. 2000). The declining abundance and range of these fish populations is inextricably linked to rapid urbanization and concomitant widespread lotic habitat degradation, fragmentation and loss (Walsh et al. 1995, Warren et al. 2000, Burkhead and Jelks 2001). The leading causes of habitat destruction include excessive erosion and sedimentation, widespread reservoir construction, channelization, urbanization and other forms of pollution (Neves and Angermeier 1990, Warren and Burr 1994, Burkhead et al. 1997, Richter et al. 1997, Allan 2004).

Within the southeastern US, one of the most pervasive and harmful impacts to aquatic fauna is the homogenization of stream substrate through the deposition of fine sediment (Walsh et al. 1995, Burkhead et al. 1997). Nonpoint-source pollution, primarily siltation, causes ~ 40% of fish imperilment in the southeast (Etnier 1997). As a result, imperilment of fish within this region is closely linked to benthic specialization (Neves and Angermeier 1990, USFWS 1996, Burkhead et al. 1997; Johnston 1999). Of the 188 vulnerable, threatened or endangered fish species in the southern US, the majority are small-bodied benthic invertivores such as darters (Percidae: Etheostomatinae) and minnows (Cyprinidae) (Warren 2000). A disproportionate number of southeastern fishes declining in range and abundance are benthic spawners (Warren et al. 2000, Burkhead and Jelks 2001). On this growing list of vulnerable, benthic-specialized species is the federally threatened spotfin chub (*Erimonax monachus* Cope), which requires abundant, heterogeneous, silt-free substrate for reproduction (Jenkins and Burkhead 1984, Jenkins and Burkhead 1994). Previous observations suggest that spotfin chubs spend a limited amount of time over sand-covered habitats, and may completely avoid areas covered by sediment finer than sand (i.e. silt and clay; Jenkins and Burkhead 1994). Evidence suggests that this is especially true when spotfin chubs spawn; they seem to prefer silt-free crevices for breeding (McLarney 1989, McLarney 1990). Due to historical land disturbance (e.g. agriculture, mining and silviculture) and current suburban development, much of the spotfin chub's native habitat has been covered with fine sediment (Jenkins and Burkhead 1984, USFWS 1996).

The upper Little Tennessee River (LTR; Macon and Swain Counties, North Carolina) is one of only five river systems still harboring the spotfin chub. Previous research suggests that maintenance and distribution of spotfin chubs in the upper LTR may be limited by the availability of suitable spawning habitat (USFWS 1983, McLarney 1989, McLarney 1990, Rakes et al. 1999). The U.S. Fish and Wildlife Service (USFWS) spotfin chub recovery plan states as its ultimate goal the restoration of viable populations of spotfin chubs to a significant portion of their historical range (USFWS 1983, Winston 1998). Objectives central to this goal include characterization of required habitat, with emphasis on spawning habitat and determination of extent of required habitat (USFWS 1983). In particular, as part of its spotfin maintenance and recovery efforts in the upper LTR, the USFWS has identified as important quantification of suitable spawning habitat within the designated critical habitat (i.e. ~70 km reach upstream of Fontana Reservoir; pers. comm. M. Cantrell, USFWS, Asheville, NC). Also considered necessary for the recovery and maintenance of spotfin chubs is the development of techniques and sites for spawning habitat enhancement (USFWS 1983). Habitat enhancement is a common management tool for the rehabilitation of game fisheries (Cowx 2000, Rubin et al. 2004). Recent research has shown that spawning habitat of non-game benthic invertivores can also be successfully and inexpensively supplemented (*Cottus* and *Etheostoma spp*.; Piller and Burr 1999, Knaepkens et al. 2002, Knaepkens et al. 2004).

Recovery and conservation of threatened fishes necessitates the determination of habitat requirements for all life-history stages (Rosenberger and Angermeier 2003, Gibson et al. 2004). Reproductive success is one of the most important determinants of inter-annual population dynamics (Wooten 1990). Therefore, spawning habitat preservation is an important step in the long-term maintenance of spotfin chub populations. To aid this effort, the first objective of this study was to characterize suitable spawning habitat and describe spawning behavior for the spotfin chub in the mainstem of the upper LTR. The reach studied flows through the Needmore Tract (Swain Co., NC), a protected portion of the LTR designated critical habitat, where spotfin chubs are most abundant (Alderman 1987, McLarney 1989, 1990). The second objective was to use observed spawning habitat characteristics to locate and estimate the areal extent of suitable spawning habitat in the mainstem upper Little Tennessee River (in the Needmore Tract). The final objective was to test the feasibility of supplementing spawning habitat through the creation of artificial spawning sites within a river reach that lacked suitable spawning substrate.

Materials and Methods

<u>Study species natural history</u>

The spotfin chub (*Erimonax monachus*) is a small cyprinid (adults 55 – 90 mm SL) endemic to warm medium-sized rivers within the upper and middle Tennessee River system (Jenkins and Burkhead 1994, Winston 1998). Once widespread throughout warm, clear upland rivers in this system, both their abundance and distribution have decreased significantly, primarily as a result of human-induced habitat loss and fragmentation (USFWS 1983, Jenkins and Burkhead 1984, Jenkins and Burkhead 1994). Formerly distributed in five states, four physiographic provinces and 13 tributary systems of the Tennessee River, the spotfin chub now exists only in localized populations within five systems: the Emory, Buffalo, North and Middle Forks of the Holston and the Little Tennessee (Jenkins and Burkhead 1984, Jenkins and Burkhead 1994, USFWS 1996, Winston 1998). Remaining populations exist in isolated, fragmented habitat, and the continued existence of two or more of these populations is tenuous (Jenkins and Burkhead 1994). Because of continued decline and disjunctive distribution, *E. monachus* was designated as federally threatened in 1977 (Federal Register 1977).

Spotfin chubs were thought to be extirpated from the Little Tennessee River until an individual specimen was collected by the Tennessee Valley Authority in 1975 (McLarney 2000). Some suggest that the state-status in North Carolina should be elevated from threatened to endangered status (Rohde et al. 1998). However, other evidence suggests that the upper LTR population is robust in a 70 km section between Fontana Reservoir and Lake Emory, North Carolina (P. Rakes, Conservation Fisheries Inc., Knoxville, TN, and W. McLarney, Little Tennessee Watershed Assoc., pers. comm.). Because of the relative strength of this population, it serves as the source of brood stock for USFWS reintroduction of spotfin chubs into LTR tributaries in the Great Smoky Mountains National Park. However, despite its apparent resurgence, the upper LTR population of spotfin chubs is still beset by many problems. Primary among these impacts is excessive sedimentation resulting from current and historical land-disturbing activities (USFWS 1996).

Until recently, little was known about the spawning behavior of the spotfin chub. Serious research on this species did not begin until after 1970 (USFWS 1983). Due to its rarity and fragmented, localized distribution, only a few researchers have observed the reproductive behavior of the spotfin chub (McLarney 1990, Jenkins and Burkhead 1994, Rakes et al. 1999). The spotfin chub is a crevice spawner, depositing eggs into boulder and bedrock fissures (Jenkins and Burkhead 1984, Winston 1998). Fish also spawn in the crevices formed at the interface of unanchored stones (primarily large cobbles) and the underlying bedrock (McLarney 1990, Jenkins and Burkhead 1994). Nest sites are chosen in areas of moderate depth and moderate to swift flow, and are typically free of fine sediment.

It is, therefore, likely that increased sedimentation negatively affects this species by embedding substrate with fine sediment, thereby reducing the number of potential nest sites. However, the closely related and sympatric whitetail shiner (*Cyprinella galactura*) also spawns in crevices and appears to be less vulnerable to excessive sedimentation (Jenkins and Burkhead 1994; personal observations). Differences in sediment tolerance may be a function of other crevice-spawning species spawning in crevices higher off the streambed or in woody debris or other substrates. In contrast, spotfin chubs are thought to prefer the lowermost crevices and therefore may be more sensitive than other fishes to substrate embeddedness (Rakes et al. 1999).

The spawning period for spotfin chubs is protracted, extending from early to late summer. This long reproductive period may be a result of fractional spawning. The exact timing of reproduction varies slightly among river systems, starting as early as mid-May in some systems and extending through early September in others (Jenkins and Burkhead 1984, McLarney 1990). Some research suggests that in the upper LTR, spotfin chub spawning may extend from early June to early September, although nuptial males begin to develop coloration and tuberculation and may begin to choose nesting sites as early as mid-May (McLarney 1990, personal observation).

Study area

This study was conducted in the upper LTR, within a section that flows through the Needmore Tract, a protected corridor in Macon and Swain Counties, North Carolina (Figure 1). The study reach extends ~12 km from river kilometer (RKM) 148 (upstream of the head of Fontana Reservoir) to RKM 160 (confluence of Burningtown Creek), and flows through the lowermost 1620 ha contiguous parcel of the 1860 ha Needmore Tract. The drainage area for the LTR at Needmore is ~ 1118 km^2, and the mean daily discharge is 34 m^3/s (Simmons 1988).

The upper LTR in the Needmore Tract is a hotspot of aquatic diversity within the southern Blue Ridge physiographic province. This reach is inhabited by half of North Carolina's freshwater fish species, and is the only major Blue Ridge river to harbor all of its original native fishes. As well as the imperiled spotfin chub, the upper LTR is inhabited by the highest diversity of redhorse suckers (6 spp. of *Moxostoma*) of any river in North America. The upper LTR also harbors the greatest diversity of freshwater mussels in North Carolina, and two other sensitive species, the Little Tennessee River crayfish (endemic to the upper LTR; *Cambarus georgiae*), and the hellbender (*Cryptobranchus alleghenienisis*).

Designated critical habitat in the upper LTR consists of two differing sections, divided by the city of Franklin, NC. Within Franklin, the river is impounded by Porter's Bend Dam, which creates Lake Emory (0.8 ha). The reach of river extending upstream of Lake Emory is characterized by severe erosion of incised banks, high stormflow suspended sediment load, and high substrate embeddedness and homogenization (personal observations). Spotfin chubs have not been recorded upstream of Porter's Bend Dam (McLarney 1989). Downstream of the dam the river widens, is less embedded and has greater habitat heterogeneity. Lake Emory serves as a sediment trap, reducing the amount of fine sediment transported through the Needmore Tract. Despite this reduction however, sediment transport is still very high through the lower reach of the LTR, averaging approximately 110,000 tons per year at Needmore (Simmons 1988). Nearly 40,000 tons of this annual load may be contributed during baseflow (pers. comm. D. Braatz, Duke Power Co.) Extent of suitable spawning habitat was quantified exclusively in the Needmore Tract, which makes up over half of the designated critical habitat between Lake Emory and the head of Fontana Reservoir (Figure 1).

<u>Spawning Habitat Characterization and Behavioral Observations</u>

Field observations of spotfin chub reproductive behavior were made between May and September 2001, 2002 and 2004. High flow and high turbidity precluded observations during the entire 2003 spawning period. Flow was monitored using a USGS gaging station located near the downstream end of the study reach (Figure 1; Needmore Gage, Station No. 03503000). In general, water column visibility was poor when river discharge was above ~ 20 m^3/s, fair when between ~ 11.5 – 20 m^3/s, and good when below ~ 11.5 m^3/s. Frequent periods of high flow, and thus high turbidity, limited observations to a relatively small subset of days within each year's spawning period (USGS 2001, 2002, 2003, 2004; Figure 2). Due to the high inter-annual variability of summer precipitation, the number of days available for observation varied each year.

Field observations were made by slowly walking and/or snorkeling upstream, typically between 10am and 4pm. During 2001 and part of 2002 spotfin chub nuptial males and spawning sites were located using previously published nest site descriptions (Alderman 1987, McLarney 1989, McLarney 1990, Jenkins and Burkhead 1994). By the end of summer 2001, nest sites and/or nuptial male displaying behavior could be located relatively easily and reliably. Based on observations made during summer 2001, knowledge of nest habitat enabled me to bypass large expanses of river where spotfin chubs are never observed (e.g. deep, slow reaches covered with fine sediment), and instead target specific meso-habitat types in which females, males displaying spawning behavior, and/or nests were located. Based on previous research (McLarney 1989, McLarney 1990), nest surveys conducted during 2001 and part of 2002 were restricted to deep fast riffles near shore with smooth bedrock and with mean depth of 0.3 – 1.0 m and mean velocities of 0.5 – 1.0 m sec^{-1}. Surveys were widened to include mid-channel bedrock covered with riverweed (*Podostemum ceratophyllum*), after the 19 June 2002 discovery of a spotfin chub nest within this habitat.

After locating an area of potentially suitable habitat, more detailed surveying was conducted by snorkeling. Many times, however, nuptial males were located within a specific meso-habitat by simply standing on a high bank or boulder and observing while wearing polarized sunglasses. Nuptial spotfin chub males were easy to locate, even within turbulent water, due to their brilliant iridescent turquoise coloration. Upon locating a nest, spawning observations were made when possible. After spawning behavior was observed and/or videotaped, physical habitat was characterized by measuring the following microhabitat characteristics: temperature; water column velocity and near-bed velocity (~ 5 cm from bed) using a Marsh-McBirney® flowmeter; water depth; spawning rock size, spawning crevice size (i.e. vertical height of crevice in cm), and spawning crevice orientation relative to flow. In addition, I measured % coverage with fine sediment (< 2 mm) of substrate within 1 m^2 of spawning rock on a scale of 1 to 4 (1 = 0-25%, 2 = 26-50%, 3 = 51-75%, 4 = 76-100%), and % substrate embeddedness within 1 m^2 of spawning rock on the same four point scale. Mean discharge (cms; m^3/s) was estimated from the USGS Needmore gage (Figure 1).

While characterizing spawning habitat, spotfin chub spawning behavior was documented when visible. In some cases, nests were located, but due to high turbidity, very little specific spawning behavior was seen or recorded. However, on 27 occasions over 3 summers detailed spawning behavior was observed and recorded. On three occasions the complete spawning sequence was observed. On other occasions only part of spawning was observed. This included interactions between nuptial males, interactions between males and females, and interspecific interactions between spotfin chubs and whitetail shiners (*Cyprinella galactura*). In summer 2004 spawning activity was recording using an underwater video camera (Sony® DCR-TRV 900 inside an Amphibico® housing). Length of observation time per nest varied from 10 minutes to over an hour. Approximately 10 hours of spawning behavior was recorded.

<u>Spawning Habitat Quantification</u>

Spotfin chub nest characteristics measured during habitat surveys were used to estimate areal extent of suitable spawning habitat within most of the Needmore Tract reach. Spawning habitat was mapped during September and October 2004, when river discharge ranged from 10.8

– 27.8 m^3/s. Upon location of suitable spawning habitat, the approximate boundary of each habitat patch was mapped by recording points along the perimeter using a Rockwell® PLGR-96 global positioning system (GPS) receiver. GPS data (~ 1 m resolution) were overlaid on USGS digital orthophotoquads (1 m resolution) in ArcView (Environmental Systems Research Institute, Inc.), to create a map of potential suitable spawning habitat for spotfin chubs within the Needmore Tract of the upper LTR.

Spawning Site Enhancement

Artificial spawning sites were created during May 2004. Spawning nest measurements taken during summer 2001 and 2002 were used to determine the specific characteristics of artificial spawning sites. Spawning habitat surveys and previous literature indicated that nests are composed of a single large cobble resting on smooth bedrock within a bedrock chute (Figure 3A). Artificial spawning sites were created by placing 50 appropriately sized (0.5 – 1.0 m) 'spawning rocks' 2 – 3 m apart (in each direction) on a large stretch of bedrock within the Needmore Tract (RKM 156.5). This large area (~200 – 300 m^2) of smooth bedrock is of appropriate depth and velocity, but lacks large cobbles of the type typically used for spawning by spotfin chubs. The lack of cobble-sized substrate within this reach is presumably due to its location on the outside of a constricted meander bend; shear stress during bankfull floods likely removes all but the largest boulders. This particular bedrock outcrop was chosen for two reasons: first, it is devoid of spawning rocks, and second, it is located upstream and downstream of areas where spotfin chubs were regularly observed during summer 2002. Spawning rocks were placed on the bedrock outcrop within the appropriate depth and velocity ranges, and monitored for spawning activity 11 times during the 2004 spawning period (May – September).

Results

Spawning Behavior

Spotfin chubs were observed spawning in crevices formed by the intersection of unanchored stones resting on bedrock, in bedrock and boulder cracks and fissures, and in crevices formed between layers of overlying bedrock. The complete spawning behavior was witnessed on three occasions. In all other cases only part of the spawning ritual was observed, including characteristic nest defense, inter- and intraspecific male aggression and male displaying swims. Most nest sites were associated with one nuptial male and 1 – 5 females. However, at three nest sites, two males were associated with the same nest, along with numerous females. The following description is of one of the three complete spotfin chub spawning acts observed. The other two complete observations are very similar to this one. Observations from the many partial spawnings are included in cases where there are behavioral differences or when specific details add unique information.

The spawning act described below occurred at a 'spawning rock' nest (see below for details of this type of nest; Figure 4A), located by noticing a brightly colored nuptial male swimming in moderate-swift flow over a large outcrop. This male was typical of most nuptial males observed: tuberculate with very bright iridescent turquoise coloration and two characteristic white triangular-shaped bars on each side. He was swimming approximately 1m downstream of the spawning rock (i.e. nest). Every 10 – 20 seconds the male would swim to the spawning rock and either circle it or insert himself underneath the left leading (i.e. relative to flow) edge. After approaching the spawning rock, the male would swim in a circle (~1 m radius) around the rock and then resume his position 1 m downstream of the rock. After approximately 3 minutes of this behavior, 2 females swam into the area of the spawning rock and then left. For several minutes the male continued to approach the spawning crevice while the females swam in a larger circle (2-3 m radius) around the nest area, periodically approaching the nest rock from downstream. Many times both male and females left the immediate area and then returned within < 1 minute. Although they would temporarily disappear from view, I assumed that the fish returning were the same as those that left.

After several minutes of the solo male and two females entering the area and approaching the spawning rock, the male entered the crevice from downstream. Once in the crevice he rotated his body so that his ventral side was against the ceiling of the crevice (at ~ 45° angle). Inverted, he wriggled violently, shifting from side to side, eventually exiting the upstream end of the crevice. This mock or displaying swim was performed twice, after which the male entered the crevice with one female. With the female entering first from downstream, both pressed their ventral side to the crevice ceiling, wriggled violently, and then departed. The female left the area and was not seen for several minutes. The male continued to either approach the spawning rock, or to enter the crevice and perform a presumably mock milting. The female then entered the crevice alone and pressed her ventral side to the crevice ceiling. She left the area and was out of view, but then returned with the male and both entered the crevice as before. This pattern continued for ~ 10 minutes, with the male continuing to swim in large circles around the nest and entering the crevice periodically. Many times both the male and one or two females entered and then exited the crevice without inverting. At some nests females would remain away from the nest for up to 10 – 15 min before returning and resuming spawning behavior.

During the occasion described above, it was too turbid to see gamete release. At several other nests, male milting was observed. Ovipositing by females was never witnessed, usually because it was impossible to view the inside of the spawning crevice, or because of elevated turbidity. At two separate spawning sites a single female was observed entering a nest crevice alone, pressing the ventral side of her body against the crevice ceiling and wriggling violently, as if depositing eggs. Usually, however, females entered either at the same time as a male or shortly before. On several occasions eggs were seen leaving the downstream end of the crevice. These were quickly eaten by spotfin chubs, whitetail shiners (*Cyprinella galactura*), warpaint shiners (*Luxilus coccogenis*), or other fish. Many species commonly congregated downstream during spawning, consuming eggs that escaped from the nest.

In addition to eating eggs that escaped the nest, large male whitetail shiners often entered the nest area and were aggressive towards spotfin chub males (Figure 4B). Whitetail shiner males frequently entered the spawning crevice and continually patrolled the nest until chased away by the spotfin chub male. On several occasions whitetail shiner males and females entered nests, chased away the spotfin chub males and females, and proceeded to spawn (or at least mock display). At one nest a very large whitetail shiner male chased the resident spotfin chub male away, and he remained away. After ~ 2 minutes, the whitetail shiner left and the spotfin chub male returned and resumed patrolling and displaying activity. In many cases male whitetail shiners entered the spawning area (~ 2 m radius around nest) while spotfin chubs were engaged in spawning behavior. The male spotfin chubs would take 30 seconds to several minutes to chase them away, and then resume spawning. However, on one occasion, the female spotfin chubs swam away immediately, the spotfin chub male chased away the whitetail shiner, and no fish returned.

When not spawning during the summer, male spotfin chubs were seen alone or with groups of females. They were often observed schooling and feeding in shallow riverweed-covered bedrock chutes with many other minnow and darter species. Prior to the first spawning observation in summer 2002, a nuptial male was observed swimming and feeding with a group of approximately 8 – 10 spotfin females; several of the females seemed to be gravid. Nuptial males were rarely seen with other males. However, on three occasions two males were observed at the same nest (Figure 4C). In one instance a relatively small nuptial male was observed swimming in place ~ 2 m from an active nest. When the primary, much larger, male would leave the nest, the smaller male would quickly and briefly enter the crevice, sometimes displaying mock spawning behavior. The large male would then return and chase the smaller male up to 5 – 6 m from the nest. This occurred repeatedly for ~10 minutes. On another occasion two nuptial males were observed attempting to occupy the downstream area of a large cobble where two areas of flow intersected. They violently attacked each other, writhing and biting each other's dorsal and pectoral fins repeatedly. One or both males made a high-pitched clicking noise during each attack (observations made while snorkeling). The two males spun in rapid circles continually biting at each other. One male also repeatedly lunged its nose at the side of the other male, seemingly trying to hit the opercular area.

In most cases a male spotfin chub guarded, displayed and spawned in a single spawning crevice. However, one observation was made of a male guarding a large area (~ 2 m radius) displaying in and along three bedrock ledge crevices. He would swim in each crevice in turn, sometimes invert and display mock spawning, and then return to patrolling or foraging nearby. When females entered the area he would increase swimming speed and display in all three crevices. When females exited, he resumed feeding or patrolling.

<u>Spawning Habitat Characterization</u>

Spotfin chub spawning habitat surveys were conducted during the summer (mid-May to mid-September) for 10, 22, and 25 days during 2001, 2002, and 2004, respectively.

Approximately 5 hours were spent per day searching for spotfin chub nests (i.e. walking and/or snorkeling). Total time spent varied from ~ 50 hours during summer 2001, to ~ 110 hours during 2002 and ~ 125 h during 2004. The majority of spawning sites were located in June, July and August. One nest was found in May (2004), and no nests were found in September.

In 2001, 10 nuptial males (i.e. tuberculate, with spawning coloration) were seen, and 6 nests were found between RKM 153 and RKM 154.5. All nest sites were found in July 2001; the first nuptial male was seen on July 9, and the last was seen on August 15. During 2002, 28 nuptial males were located, the first in early June. In 2002, 13 nests were found between RKM 153 and RM 156. The first nest was located in mid-June and the last in mid-August. Nuptial males were seen until the end of August, but no more nests were located. No observations were made between June 19 and July 20, 2002 due to elevated turbidity resulting from the removal of a sunken sand barge in Lake Emory. During 2004, 19 nuptial males were located, the first in mid-May. From late May to late July, 14 nests were located between RKM 145 and RKM 160. A total of 32 spotfin chub nests were located and characterized during the three summers (Tables 1 & 2). Nests were found at temperatures ranging from 20.5 – 29.5° C; temperatures were significantly different among years (Table 3).

Spawning Rock Nests

The predominant nest type consisted of a single large stone sitting on bedrock; 21 of 32 nests were of this type. This type of spawning site is hereafter called a spawning rock nest. Water column velocity (0.6 depth) at spawning rock nests was significantly higher in 2001 than the following two years (Table 3). During 2001 nests were found in swift riffles with mean water column velocity 0.89 m s^{-1}. Water column velocities were not significantly different between 2002 and 2004 (Table 3). Near-bed velocity was moderate, and did not differ significantly among years (Table 3). During 2001 nest site depth was significantly greater than the following two years.

The size of rocks chosen by spotfin chubs for spawning rock nests ranged from 0.3 – 1.0 m long (mean = 0.5 m). Spawning rock shapes ranged from smooth egg-like cobbles to rough, angular rocks. One spawning rock was very different from all others; it was long (0.9 m), rectangular, in relatively slow flow (0.35 m s^{-1}), covered with *Podostemum* and surrounded by sand and gravel. One other spawning rock nest was covered with riverweed, however most were smooth and located on large expanses of smooth rounded bedrock. They were most often within constricted regions of the bedrock, where flow was non-turbulent and very swift.

Crevices were formed at the intersection of spawning rocks and the basal bedrock they were lying on (Figure 3A). Crevice heights ranged from 1 – 2 cm and crevices were approximately 1 – 4 times as long as male fish. In most cases, crevice depth was slightly larger than that of one male fish. Some were large enough for two fish to completely insert their body into the crevice. All except one spawning rock crevice were oriented parallel to the main flow. One spawning rock nest crevice was nearly perpendicular to the main flow direction; this spawning rock was covered with *Podostemum* and surrounded by sand (Figure 3B). Percent substrate embeddedness and percent coverage of the river bed with fine sediment (< 2 mm) were similarly low for all sites (Tables 1 & 2). Percent fines was 0 – 25% at all spawning rock nests, except for two which were 26 – 50%. In most cases fines were present only in the lee of the spawning rock or trapped in adjacent riverweed.

Bedrock Crevice Nests

A second type of spotfin chub nest consisted of a crevice within a continuous outcrop of bedrock (Figure 3C). This nest type is hereafter called a bedrock crevice nest; 7 of 32 nests were of this type. Bedrock crevices were found in smooth and angular bedrock outcrops, the majority of which were mid-channel and covered with *Podostemum*; 5 of 7 were covered with riverweed. Crevices within this nest type consisted of fractures in the bedrock, and were usually formed within the side walls of bedrock chutes (Figure 3C). Although the surrounding bedrock was completely covered with *Podostemum*, the crevices themselves were clean.

Mean water column velocity at bedrock crevice nests was moderate to swift and was significantly higher in 2002 than in 2004 (Table 3). Substrate velocity was moderate and was also significantly higher in 2002 than in 2004 (Table 3). Depth was not significantly different between years at bedrock crevice nest sites (Table 3).

Bedrock crevice nest sites consisted of cracks and fissures within the parent bedrock (Figure 3C). Similar to spawning rock nests, bedrock crevice heights ranged from 1 – 2 cm and crevices were approximately 1 – 3 times as long as male fish. Bedrock crevices were, in general, more shallow than those formed by spawning rocks lying on bedrock. In most cases, fish did not insert their entire bodies into the spawning crevice. All bedrock crevices were approximately parallel to the main flow. Percent substrate embeddedness was very low at all bedrock crevice nests (Table 2). At all except one site, fine sediment coverage was very low and fines were located primarily among the base of riverweed. In the one nest with appreciable fine sediment, none was present in the spawning crevice.

Bedrock Ledges and Boulder Crevice

Four nest sites did not fit within the first two categories. Three of these nest sites were bedrock crevices consisting of one layer of bedrock forming a ledge over the basal bedrock; these are referred to as bedrock ledge nests (Figure 5). The final nest type consisted of a crevice within a large boulder; one nest was of this type. This site was unique because the crevice was located at approximately 50% water depth (as opposed to near the river bed), on a nearly vertical rock face.

Mean water column velocity, substrate velocity and depth varied markedly among the three bedrock ledge nests (Table 2). Mean water column and substrate velocities and depth for the boulder crevice nest were similar to velocities measured for bedrock crevice nests. Percent substrate embeddedness was very low for all four nests, and percent fines was very low for all except one bedrock ledge nest (Table 2). Crevice heights ranged from 1 – 3 cm and crevice lengths were 1 – 4 times the length of male fish. Bedrock ledge crevices were approximately perpendicular to the main flow, although one was at a slight angle. The boulder crevice was parallel to the main flow direction. *Podostemum* was present on two bedrock ledge nest sites (i.e. on the top layer of bedrock; the crevice was free of riverweed).

Spawning Habitat Quantification

Spotfin chub spawning habitat occurred exclusively within moderate to swift boulder and bedrock riffles. Of the 847,980 m^2 surveyed, ~4.4% (37,670 m^2) was determined to be suitable spawning habitat for spotfin chubs. A total of 135 distinct spawning habitat patches were located, ranging in size from 14 to 2540 m^2. Mean habitat patch size was 279 m^2 (SD = 298), and mean distance between patches was 90 m (SD = 196; range: 10 – 1840 m). Some habitat patches were grouped tightly together while others were more evenly spaced over a long distance (Figure 6). Over 75 patches were within ~ 10 – 30 m of adjacent spawning habitat, and 16 patches were ~ 80 – 100 m from adjacent habitat. Spawning habitat was located within ~ 11 groups that were 150 – 1840 m from adjacent habitat groups.

Many of the larger habitat patches were bedrock ledges extending across the entire width of the river, or extending longitudinally for over 10 – 100 m. Each of these large ‘patches’ could presumably support many nest sites, if crevices or spawning rocks were present.

Spawning Site Enhancement

Fifty spawning rocks were placed on a large bedrock outcrop (RKM 156.5) in mid-May 2004, and monitored 11 times throughout the summer. The majority of spawning rocks were large smooth, clean cobbles averaging 30 – 45 cm in length. Although spotfin chubs (males and females) were regularly seen upstream and downstream of this area, nuptial males were not seen among the artificial spawning rocks until mid-June. After mid-June males and females were seen occasionally within the 200-300 m^2 artificial spawning rock area.

During the entire summer, three males were seen engaged in what appeared to be spawning behavior in the supplemented habitat area. On all three occasions a single nuptial male was seen hovering near a spawning rock. The first two males exhibited typical spawning behavior, consisting of stationary swimming ~ 1 m behind the spawning rock with periodic approaches and crevice entry. The final male was located on 22 July and behaved differently from the first two. He spent relatively more time near the spawning rock and in the crevice, especially when approached. It became apparent that this male was exhibiting nest guarding behavior. Upon removing and inspecting the spawning rock, a nest of ~ 400 eggs was found.

Females were never associated with a specific spawning rock, but were seen in the general area. Spotfin chubs were last seen within the artificial habitat area in early August. In late July it became evident that storms had moved several of the spawning rocks from their original placement. On 8 September, a large storm (> 295 m^3/s) washed away many of the spawning rocks; no monitoring was conducted after this date.

Discussion

Spotfin chubs are typical of benthic-specialized fish which rely on unembedded, high-quality substrate for successful reproduction (Jenkins and Burkhead 1984, Jenkins and Burkhead 1994). This type of habitat has become increasingly rare and fine sediment has become more abundent in the upper LTR as a result of second home development, urban sprawl and small scale agriculture.

Prior research suggests that spotfin chubs in the upper LTR spawn almost exclusively in crevices formed by individual loose cobbles resting on smooth bedrock (McLarney 1989, McLarney 1990). Previously, nest sites were thought to always be near shore, devoid of fine sediment and vegetation, located in constricted areas with high flow (mean water column velocity = 0.5 – 1 m s^{-1}, mean depth = 0.3 m; McLarney 1990), and always very close to the streambed (Rakes et al. 1999). Finally, spotfin chubs were thought to always perform milting or displaying swims (i.e. solo runs) within a single crevice (Jenkins and Burkhead 1994).

While the present study corroborates some findings from previous research (66% of nests were under 'spawning rocks' in deep swift bedrock riffles), it also suggests that spotfin chub reproduction may be more complicated than once believed. Instead of spawning only under spawning rocks, spotfin chubs were found to make nests in 4 distinct types of substrate: under cobbles resting on bedrock, in bedrock fissures, under bedrock ledges and in boulder crevices. The utilization of bedrock and boulder crevices and bedrock ledges greatly increases the amount of usable spawning habitat for spotfin chubs in the upper LTR. Mean length of spawning rocks was twice that previously reported (McLarney 1989). While spawning rocks were usually located on smooth bedrock within swift constricted flow, some nests were found in relatively slow flow (0.35 m s^{-1}), surrounded with gravel and some fine sediment.

Other new findings include the fact that, contrary to previous assumptions, nuptial males will perform displaying swims within multiple crevices. In addition, spotfin chubs were found spawning in crevices high up in the water column; this nest consisted of a crevice within a large boulder and was located at ~1/2 water column depth. Therefore, although spotfin chubs usually chose crevices near the river bed, they are also able to take advantage of crevices far above the substrate. This finding suggests that, on occasion, spotfin chubs may be able to take advantage of crevices with low likelihood of being embedded with fine sediment.

This study found that the Needmore Tract contains a larger amount of spawning substrate than previously thought, and that spotfin chubs are able to reproduce within a wider range of depths and velocities. Mean depth at nest sites was two times higher than previously reported (0.6 vs. 0.3 m), and some nests were located in water as deep as 0.9 m. While mean velocity (0.8 m s^{-1}) for nest sites was similar to previous studies (0.48 – 0.96 m s^{-1}; McLarney 1990), spotfin chubs located in this study were found to utilize a greater range of velocities (0.35 – 1.05 m s^{-1}). Near-bed velocities were fairly high, but these values overestimate the conditions experienced by spawning fish within the crevices, or by the eggs because it was impossible to take velocity readings underneath spawning rocks or in bedrock crevices; velocities were measured at ~ 5cm from substrate.

Timing of spotfin chub reproduction was similar to previous accounts, with the majority of spawning occurring between early June and mid-August. In 1988 the first nuptial male in the upper LTR was seen on June 3 and the last on July 9 (McLarney 1989). During 1990 the first and last nuptial males were seen on June 4 and August 15 (McLarney 1990). During the present study, nuptial males were seen over a period of 76 days in 2002 (June 12 to August 26) and 59 days in 2004 (May 27 to July 24). The difference in length of spawning period between these two years may be a function of temperature. Summer 2002 was warm; the average temperature when nests were located was 28°C. However, it is worth noting that the phylogenetically similar whitetail shiner has also been recorded spawning at 28°C (Jenkins and Burkhead 1994). Summer 2004 was cooler, with an average temperature at nests of 25°C.

Spawning habitat character differed significantly among years (Table 3). Spawning rock nests located in 2001 were on average in faster, deeper water than those found in 2002 and 2004. Bedrock crevice nests in 2002 were located in faster riffles than those found in 2004. These differences were likely influenced by the fact that different sections of river were surveyed each year. During 2004, the majority of bedrock crevice nests were found within one section of river which was wide and slow relative to the section surveyed in 2002. *A priori* decisions regarding which meso-habitats and river sections to search likely contributed to among-year differences in observed spawning habitat characteristics.

Previously, spotfin chubs were presumed to prefer only near-shore spawning substrate that remained free of *Podostemum* due to riparian shading (McLarney 1989, McLarney 1990). Contrary to this, over 20% of nests located in this study were found in or near the middle of the river, and many of these were at least partially covered with riverweed. One bedrock crevice nest was in an area so thick with riverweed that the spawning crevice was almost obscured from view. In this case the fish seemed to be spawning in riverweed, but upon closer inspection a clean crevice was located. It is probable that only a small fraction of the areal extent of these *Podostemum*-covered bedrock outcrops in the upper LTR will be suitable for spawning, because they must also have the requisite spawning crevice within the appropriate range of depth and velocity. However, much of the mid-channel bedrock outcrops are covered with riverweed; this suggests that much more of the river may be available for spawning than previously believed.

A lack of suitable spawning habitat was presumed to limit the success of spotfin chub populations in the upper LTR (McLarney 1989). Because spotfin chubs were found utilizing a greater variety and thus a greater amount of available substrate, suitable spawning habitat may be less of a limiting factor than previously thought. The areal extent of usable spawning habitat has previously been estimated at ~ 1 - 2% of the substrate in the Needmore Tract reach of the upper LTR (McLarney 1990). This estimate was determined by measuring the extent of smooth bedrock, of appropriate depth and velocity, located ≤ 8 m from shore. In the present study suitable spawning habitat was estimated at ~ 4.4% of the riverbed. This estimate is higher because it included mid-channel bedrock outcrops and bedrock ledges partially or entirely covered with *Podostemum*.

The amount of spotfin chub spawning habitat is a function of river discharge, which influences both riffle depth and velocity. Extent of suitable spawning habitat was estimated using GPS in Fall 2004, when mean river discharge was ~ 18 m^3/s (USGS 2005). Mean summer discharge in the upper LTR can vary interannually by an order of magnitude, from 6 to 60 v (USGS gaging station No. 03503000; water years 1944 - 2004). However, during most years summer discharge in the upper LTR ranges between 16 – 25 m^3/s, suggesting that 4.4% may be a reliable estimate. Most of the Needmore Tract of the upper LTR is unsuitable for spotfin chub reproduction. Much of the river is too deep and too slow, and vast reaches are completely covered with fine sediment. While even in an undisturbed state only a fraction of the upper LTR would provide suitable spawning habitat, the amount of usable riverbed is now much lower than historically, due to excessive sediment inputs.

Sedimentation not only reduces the number of potential nest sites; it also fragments habitat patches. In the Needmore Tract, spawning habitat patches are separated by large expanses of sediment-covered riverbed. These low gradient, heavily embedded areas are devoid of all but the most tolerant invertebrates and fish (e.g. aquatic worms, midge larvae and bass; personal observations). Studies have also shown that sediment-induced modification and destruction (i.e. embedding) of riffle habitats may reduce the range of other imperiled minnows (Propst and Bestgen 1991). Spotfin chubs are known to avoid these areas of fine sediment (McLarney 1990, Jenkins and Burkhead 1994; personal observations), which may mean that dispersal across these sediment 'deserts' is limited for this species.

Researchers have shown that blockage of preferred spawning habitat by impoundments is detrimental to populations of large mobile riverine fishes (Cooke and Leach 2004). However, less is known about how sediment-induced fragmentation of habitat may affect these river species. In the case of the spotfin chub, sediment may isolate individuals from each other, making reproduction more difficult and the stability of the upper LTR population more tenuous. A recent study on the movement patterns of the crevice-spawning blue shiner (*Cyprinella caerulea*) found that a small proportion of individuals moved primarily between adjacent habitat patches, and that the distance between patches ranged from 3.1 to 93.5 m (Johnston 2000). Other studies also suggest that small riverine fishes have restricted movement, except for colonizing individuals which may move long distances (Hill and Grossman 1987, Freeman 1995). Blue shiners seem to exist as isolated subpopulations; while dispersal between patches is infrequent, when it occurs intermediate habitat patches are used as “stepping stones” (Johnston 2000). In the upper LTR, the distance within groups of spawning habitat patches (i.e. patches clustered close together) was similar to the distances moved by blue shiners among habitat patches (~ 10 – 100 m). However, the distance between 11 groups of spawning habitat patches in the upper LTR ranged from 194 – 1840 m (Figure 6). Some adjacent groups of spawning patches have few intervening “stepping stones” to assist dispersal; therefore, heavily silted areas may act as barriers, isolating subpopulations from each other. If spotfin chub mobility and movement patterns are similar to blue shiners, these vast patches of sedimented habitat may hamper the maintenance of spotfin chub population connectivity within the upper LTR. Although spotfin chubs are thought to move long distances up tributary streams (McLarney 2000), the source of these migrating fish may be individual mainstem subpopulations adjacent to the tributary confluence.

In addition to limiting reproduction through elimination of spawning habitat, sedimentation also smothers nests, abrades eggs and disrupts spawning cues through elevated turbidity (Waters 1995, Henley et al. 2000, Burkhead and Jelks 2001). The fact that spotfin chubs are fractional spawners may mitigate this latter problem (Gale and Gale 1977, Jenkins and Burkhead 1984). The production and spawning of multiple clutches of eggs throughout a protracted spawning period enables spotfin chubs to suspend reproduction during high turbidity. Spotfin chub eggs may also be more sensitive to sediment deposition, relative to those of other egg-attaching species (e.g. those that attach in a monolayer such as *Pimephales* spp.) because they are attached in clumps. This increases the likelihood that the entire egg mass may become detached. On two occasions an entire spotfin chub egg mass (hundreds of eggs) was observed lying detached on the bedrock below a spawning rock. Eggs that become detached are more likely to become covered with sediment, or washed out of the crevice.

Once in a stream or river, excessive sedimentation may affect the recovery of fish populations for many decades (Harding et al. 1998). However, imperiled fish populations may receive short term benefit from immediate measures such as spawning habitat enhancement. Artificial substrate has been used successfully to supplement spawning habitat for small riverine fishes (Piller and Burr 1999, Knaepkins et al. 2002, Knaepkins et al. 2004). While not an overwhelming success, the spawning habitat enhancement in the present study shows that spotfin chub reproduction can be augmented by relatively simple, inexpensive means. Spawning habitat supplementation in the upper LTR is likely only limited by the areal extent of swift bedrock riffles. However, this too could decrease over time with continued influx of sediment into the river. If current sediment sources to impacted rivers are reduced, habitat enhancement may aid in the preservation of imperiled fishes during the time necessary for excess sediment to be removed.

Summary/Conclusions

Long term maintenance of the upper LTR population of spotfin chubs will rely on the preservation of suitable spawning habitat. While this research increases understanding of spawning requirements, much is left to be learned about other life-history requirements of this imperiled species. Successful restoration of declining species necessitates the determination of habitat requirements throughout the life history of a species, as well as the ecosystem functions that maintain these habitats (Rosenberger and Angermeier 2003). The preservation of a heterogeneous mix of habitat types is necessary for the recovery and maintenance of small upland fishes (Burkhead et al. 1997, Rosenberger and Angermeier 2003). The importance of diverse habitats to spotfin chubs is evidenced by their migration to and use of small headwater tributaries within the upper LTR basin (McLarney 2000, SFC 2002). Spotfin chub foraging habitat and young-of-year habitat is markedly different from spawning habitat (personal observations; Jenkins and Burkhead 1984, McLarney 1989). The preservation of these and other habitats, as well as access to these habitats, is likely essential to this species. Many imperiled riverine fishes require heterogeneous habitat free of excessive fine sediment (Burkhead et al. 1997, Burkhead and Jelks 2001, Warren et al. 2000). Many fish species also require a mosaic of high quality habitat patches connected over large spatial scales (McLarney 2000, Johnston 2000, Rosenberger and Angermeier 2003, Scheurer et al. 2003). It has been argued that the preservation of habitat that supports imperiled riverine fishes would presumably benefit many other species (Stephens and Mayden 1999). This is certainly true for the spotfin chub, which co-occur with a wide variety of southeastern stream fishes. A watershed-scale management approach that attempts to minimize sediment sources into lotic ecosystems and preserves habitat heterogeneity is needed for the recovery and long-term maintenance of spotfin chubs and other imperiled fishes in the southern Appalachians.

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Table 6.1: Habitat characteristics for spotfin chub spawning rock nest sites during summer 2001, 2002 and 2004. Little Tennessee River discharge from USGS Needmore gage no. 03503000 is presented (Q; m^3/s) for each observation date. Water column mean velocity (i.e. at 0.6 * depth), substrate velocity and water column depth are presented. Embeddedness percentages presented represent the extent to which the spawning rock was surrounded by smaller particles; one of four ranges were assigned. Percent fines represents the percent of surficial coverage of spawning area with particles < 2 mm. Spawning rock dimensions presented are length, width and thickness, respectively. Crevice orientation is relative to main flow direction.

	Temp.	Q	Water	Substrate	Depth	Embed.	Fines (<2mm)	Spawning Rock	Crevice		
Date	(°C)	(m^3/s)	Vel. (m s-1)	Vel. (m s-1)	(m)	(%)	(%)	(m x m x m)	Size (cm)	Orientation	riverweed
7/12/2001	24	11.9	0.93	0.59	0.76	0 - 25	0 - 25	0.7 x 0.4 x 0.4	1	parallel	
7/17/2001	23	9.6	1.01	0.40	0.62	0 - 25	0 - 25	0.8 x 0.6 x 0.3	1	parallel	
7/17/2001	25	9.6	0.70	0.45	0.85	0 - 25	0 - 25	0.5 x 0.3 x 0.3	2	parallel	
7/19/2001	24.5	9.2	0.77	0.53	0.43	0 - 25	0 - 25	1.0 x 0.7 x 0.4	1	parallel	
7/19/2001	24	9.2	1.05	0.41	0.65	0 - 25	0 - 25	0.4 x 0.3 x 0.4	1	parallel	
6/12/2002	28	14.2	0.87	0.67	0.64	0 - 25	0 - 25	0.5 x 0.5 x 0.2	1	parallel	
7/29/2002	29.5	7.9	0.98	0.65	0.46	0 - 25	0 - 25	0.7 x 0.5 x 0.1	1	parallel	
7/31/2002	28.5	6.8	0.75	0.45	0.48	0 - 25	0 - 25	0.3 x 0.3 x 0.2	1	parallel	
8/3/2002	29	7.4	0.79	0.49	0.45	0 - 25	0 - 25	0.4 x 0.2 x 0.1	1	parallel	
8/8/2002	29	5.4	0.83	0.45	0.51	0 - 25	0 - 25	0.6 x 0.6 x 0.2	1	parallel	
8/8/2002	29	5.4	0.95	0.68	0.44	0 - 25	0 - 25	0.9 x 0.5 x 0.2	1	parallel	
8/13/2002	27.0	4.8	0.81	0.48	0.45	0 - 25	0 - 25	0.3 x 0.2 x 0.2	1	parallel	
8/13/2002	27.0	4.8	0.72	0.66	0.38	0 - 25	0 - 25	1.0 x 0.5 x 0.2	2	parallel	
6/8/2004	22.5	16.8	0.91	0.25	0.30	0 - 25	0 - 25	0.3x 0.2 x 0.1	2	parallel	x
6/11/2004	24.0	13.6	0.75	0.56	0.65	0 - 25	0 - 25	0.4 x 0.3 x 0.2	2	parallel	
6/11/2004	24.0	13.6	0.88	0.67	0.30	0 - 25	26 - 50	0.6 x 0.2 x 0.1	1	parallel	
6/20/2004	25.0	12.7	0.80	0.56	0.45	0 - 25	0 - 25	0.3 x 0.7 x 0.3	1	parallel	
7/14/2004	25.5	11.3	0.35	0.15	0.42	0 - 25	26 - 50	0.9 x 0.3 x 0.2	1	perpendicular	x
7/14/2004	26.0	11.3	0.75	0.46	0.90	0 - 25	0 - 25	0.3 x 0.1 x 0.3	2	parallel	
7/15/2004	25.5	11.3	0.80	0.55	0.75	0 - 25	0 - 25	0.4 x 0.2 x 0.4	1	parallel	
7/24/2004	27.0	10.8	0.87	0.65	0.65	0 - 25	0 - 25	0.4x 0.3 x 0.1	1	parallel	

Table 6.2: Habitat characteristics for spotfin chub bedrock crevice, bedrock ledge, and boulder crevice spawning sites during summer 2002 and 2004. Little Tennessee River discharge from USGS Needmore gage no. 03503000 is presented (Q; m^3/s) for each observation date. Water column mean velocity (i.e. at 0.6 * depth), substrate velocity and water column depth are presented. Embeddedness percentages presented represent the extent to which the crevice was surrounded by smaller particles; one of four ranges were assigned. Percent fines represents the percent of surficial coverage of spawning area with particles < 2 mm. Nest type acronyms:'Br Crv' = bedrock crevice; 'Br Ldg' = bedrock ledge; 'Bld Crv' = boulder crevice. Crevice orientation is relative to main flow direction.

Date	Temp. (°C)	Q (m^3/s)	Water Vel. (m s-1)	Substrate Vel. (m s-1)	Depth (m)	Embed. (%)	Fines (<2mm) (%)	Nest type	Crevice Size (cm)	Crevice Orientation	riverweed
7/29/2002	29	7.9	0.94	0.50	0.55	0 - 25	0 - 25	Br Crv	2	parallel	x
7/31/2002	29	6.8	1.03	0.72	0.50	0 - 25	0 - 25	Br Crv	2	parallel	x
8/5/2002	28	6.5	1.08	0.70	0.39	0 - 25	0 - 25	Br Crv	1	parallel	
8/10/2002	27	5.1	0.74	0.53	0.50	0 - 25	0 - 25	Br Crv	1	parallel	x
6/8/2004	25	16.8	0.56	0.39	0.38	0 - 25	26 - 50	Br Crv	1	parallel	x
6/12/2004	26	13.3	0.52	0.31	0.42	0 - 25	0 - 25	Br Crv	2	parallel	
7/15/2004	27	11.3	0.75	0.45	0.67	0 - 25	0 - 25	Br Crv	1	parallel	x
6/19/2002	27	13.0	1.03	0.52	0.58	0 - 25	0 - 25	Br Ldg	2	perpendicular	x
6/11/2004	25	13.6	0.81	0.45	0.61	0 - 25	0 - 25	Br Ldg	1	perpendicular	
7/14/2004	26	11.3	0.38	0.12	0.15	0 - 25	26 - 50	Br Ldg	3	perpendicular	x
5/27/2004	24	19.3	0.65	0.47	0.56	0 - 25	0 - 25	Bld Crv	2	parallel	

Table 6.3: Means and t-test results comparing microhabitat data among survey years 2001, 2002 and 2004. Results for are presented for among-year comparisons of temperature, mean water column velocity, near-bed velocity and water column depth. Comparisons of the latter three measures are presented separately for spawning rock nests and bedrock crevice nests.

		Means			
	Year	Temp. °C	Water Column Vel. (m s^{-1})	Near-bed Vel. (m s^{-1})	Depth (m)
spawning rock	2001	24	0.89	0.48	0.66
	2002	28	0.83	0.56	0.48
	2004	25	0.77	0.48	0.55
Bedrock crevice	2002	28	0.96	0.59	0.49
	2004	25	0.66	0.40	0.49

		T-test P - values			
	Source	Temp °C	Water Column Vel. (m s^{-1})	Near-bed Vel. (m s^{-1})	Depth (m)
Spawning Rock	2001 vs. 2002	<0.0001	0.03	0.22	0.02
	2001 vs. 2004	0.047	0.01	0.19	0.08
	2002 vs. 2004	<0.0001	0.16	0.07	0.19
Bedrock Crevice	2002 vs. 2004	0.047	0.007	0.006	0.42

Figure 6.1: Map of study area within the upper Little Tennessee River (LTR), Macon and Swain Counties, North Carolina. The study area was located just upstream of Fontana Lake between RKM 148 and 160. A USGS gaging station was located at the downstream end of the study area.

Figure 6.2: Upper Little Tennessee River hydrographs for summer (May – September) 2001 – 2004. Discharge data (cms; m^3/s) is from USGS Needmore gaging station 03503000. Dashed line represents periods when water column visibility was poor (river discharge > 20 m^3/s).

Figure 6.3: *Figure A:* Spotfin chub 'spawning rock' nest, showing placement of rock in bedrock chute, spawning crevice and water flow direction (arrows). Spawning rock is shown in cross-section. *Figure B:* View from above a spawning rock oriented perpendicular to water flow (represented by arrows). Spawning rock was sandwiched between bedrock ledges and a large boulder and spawning crevice was located along front edge of spawning rock. The top of spawning rock was level with top of bedrock ledge. *Figure C:* Spotfin chub 'bedrock crevice' nest. Spawning crevice is a crack in the bedrock, located within a 'chute', which are common in local bedrock. As with most bedrock crevice nest, the surrounding bedrock is covered with thick growths of riverweed (*Podostemum ceratophyllum*).

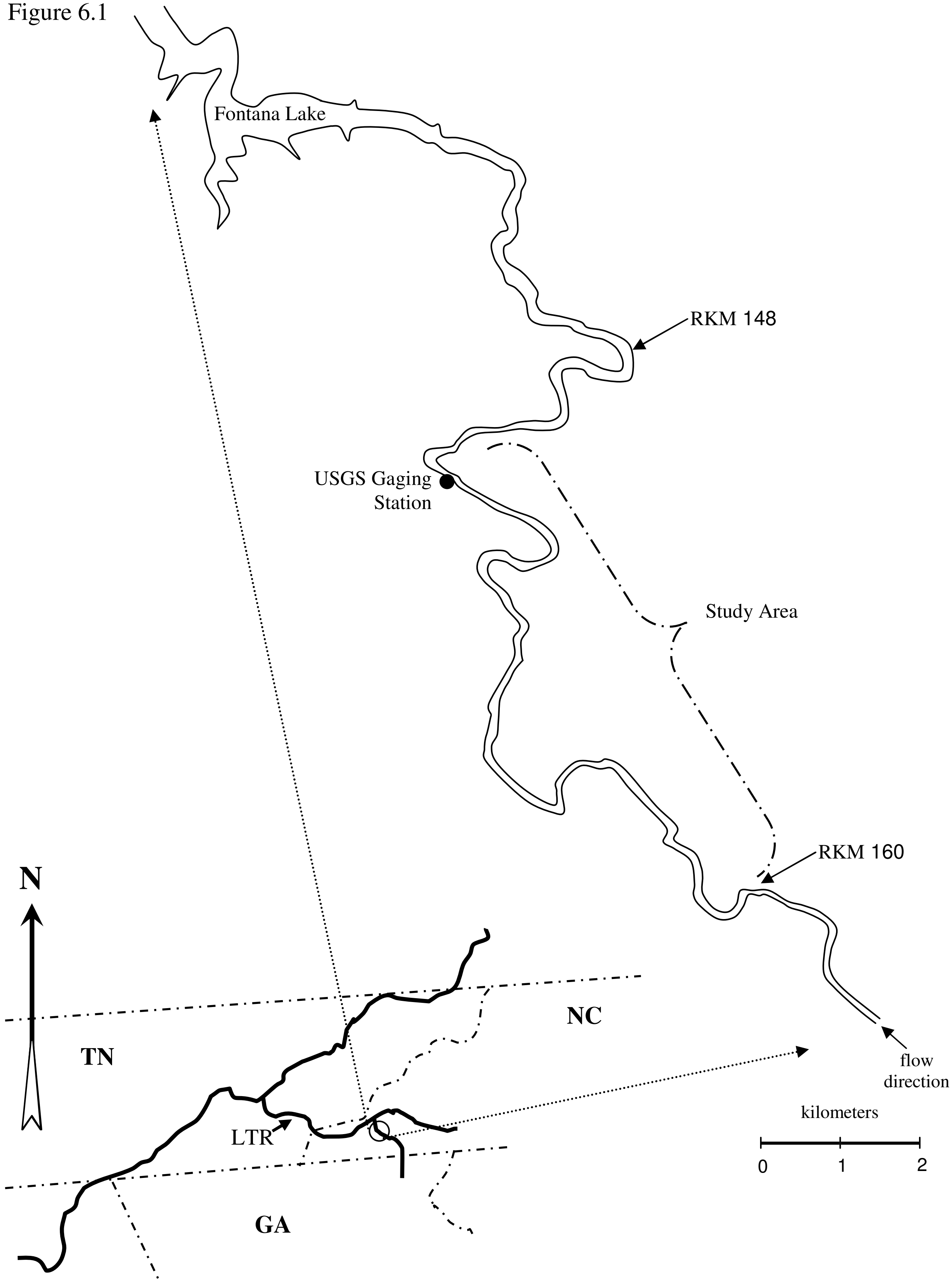
Figure 6.4: *Figure A:* Photograph of a nuptial male guarding a spawning rock nest. *Figure B:* Photograph of a whitetail shiner entering a spotfin chub bedrock ledge nest, just before chasing away male and female spotfin chubs. Number 1 indicates two spotfin chub females; number 2 indicates spotfin chub male; number 3 indicates whitetail shiner male. *Figure C:* Photograph of two spotfin chub males resting between rounds of sparring for

control over bedrock ledge nest. Note abundance of riverweed growing on top and in front of nest ledge.

Figure 6.5: Spotfin chub 'bedrock ledge' nest, showing view from downstream (*Figure A*) and cross-section side view (*Figure B*). Crevice size in this case refers not to size of 'cave' opening, but rather to innermost height, where eggs were laid.

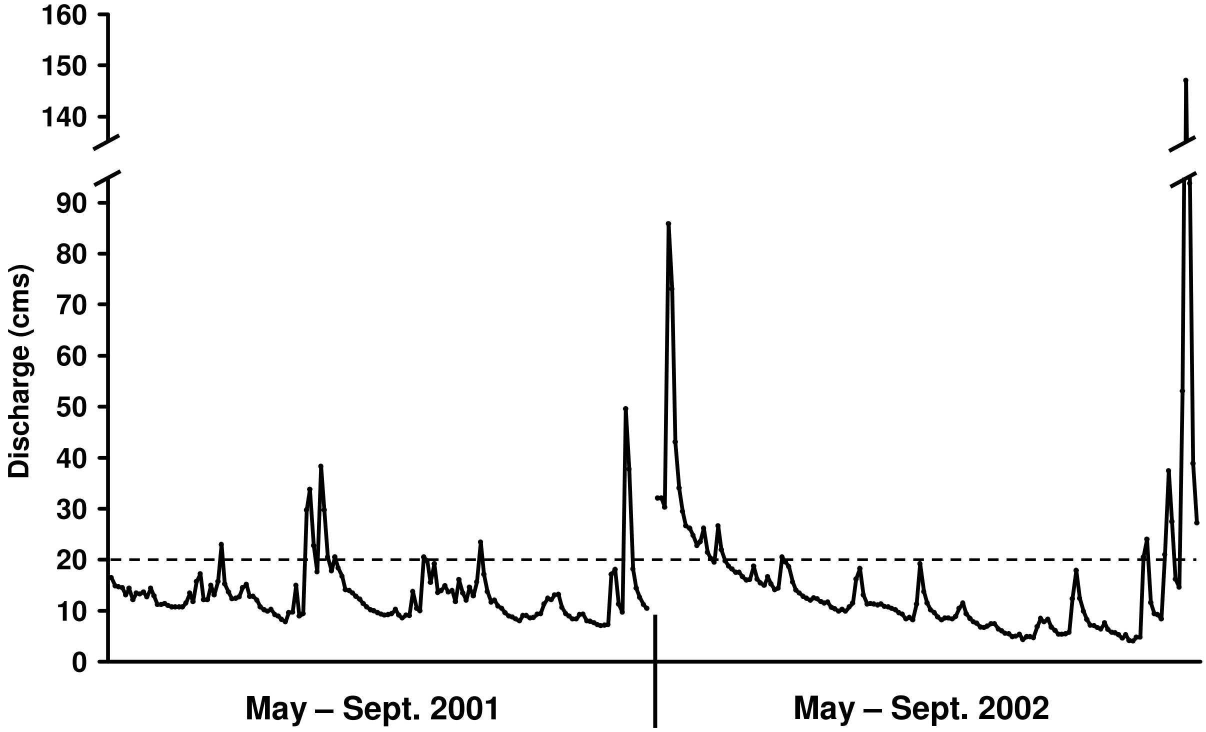
Figure 6.6: Spotfin chub spawning habitat map showing detail of upstream section of study area. Grey polygons represent spawning habitat measured using GPS receiver. Black polygons are islands. Detailed habitat maps of the rest of the study area are in Appendix 2. GPS locations for spawning habitat patches are in Appendix 3.

Figure 6.1



Fontana Lake
RKM 148
USGS Gaging
Station
Study Area
RKM 160
N
TN
NC
GA
LTR
flow
direction
kilometers
0
1
2

Figure 6.2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150
140
90
80
70
60
50
40
30
20
10
0
Discharge (cms)
May – Sept. 2001
May – Sept. 2002

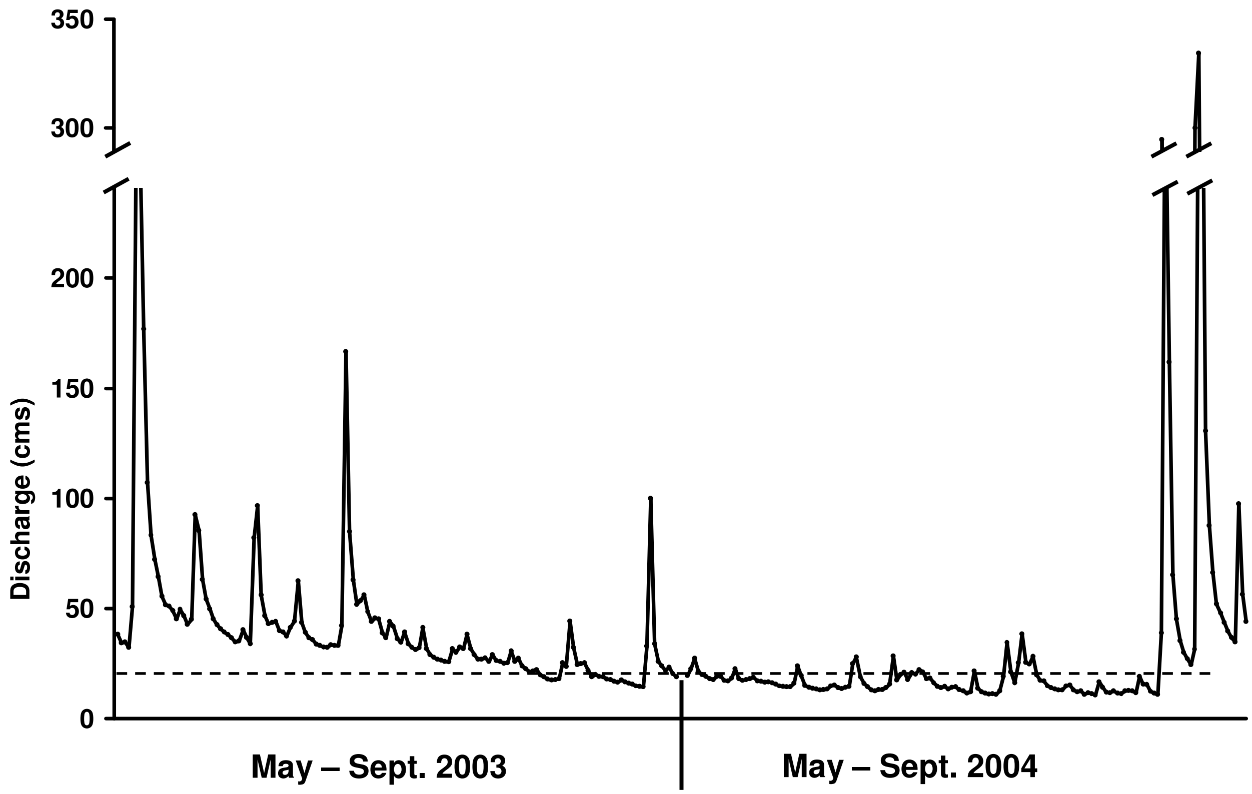

350
300
200
150
100
50
0
Discharge (cms)
May – Sept. 2003
May – Sept. 2004

Figure 6.3

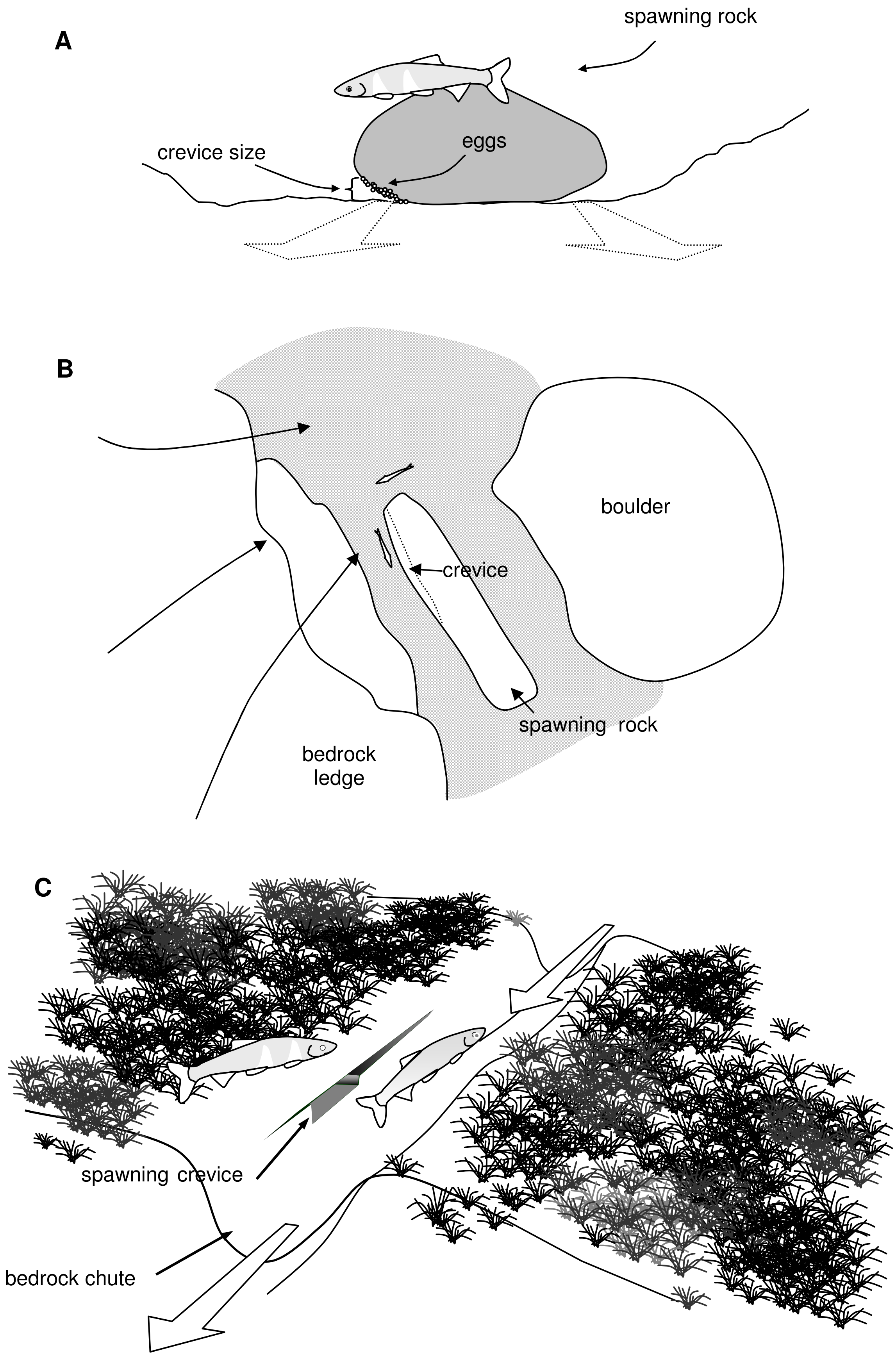

A
spawning rock
eggs
crevice size
B
boulder
crevice
spawning rock
bedrock
ledge
C
spawning crevice
bedrock chute

Figure 6.4

Figure 6.5

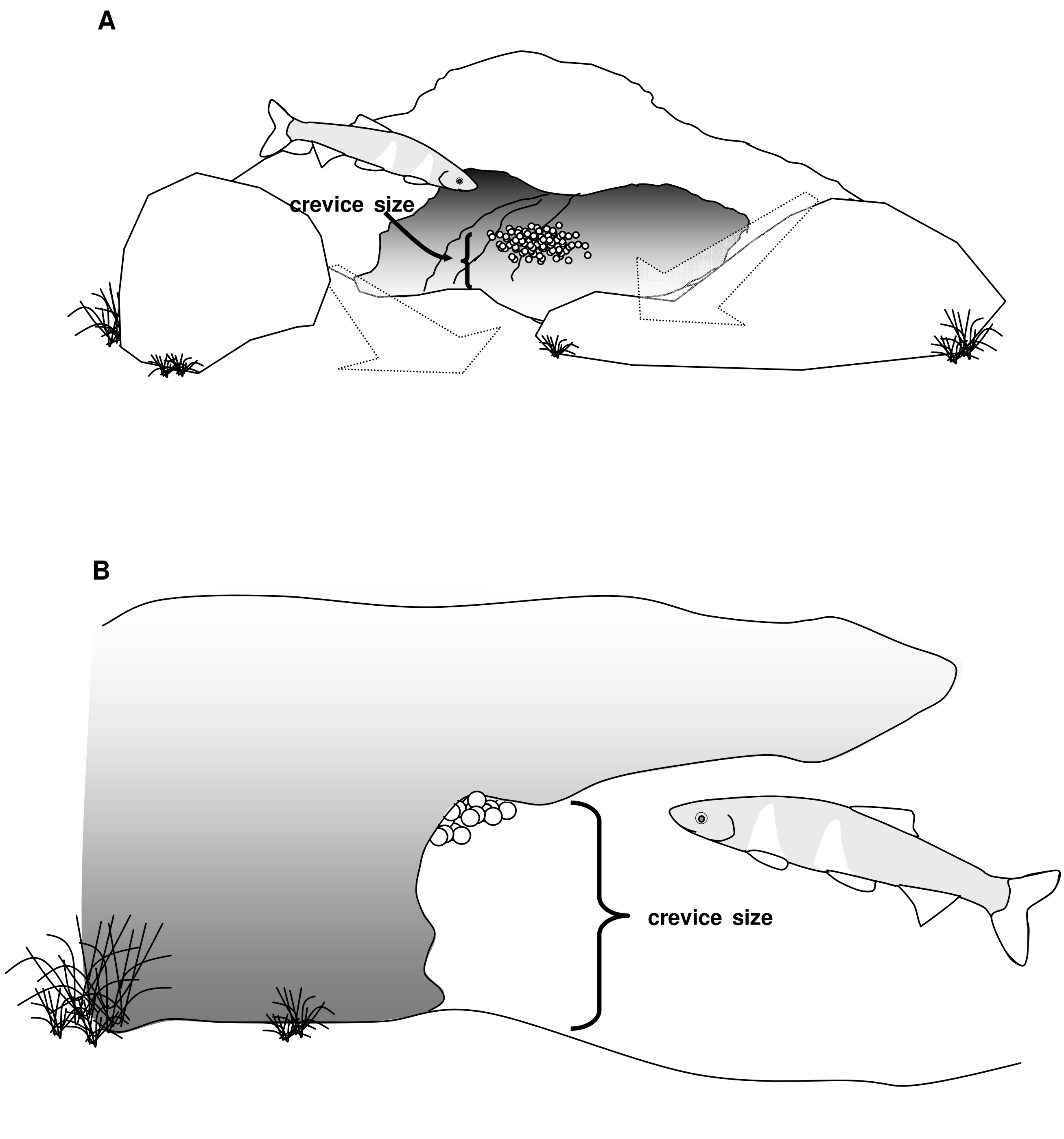
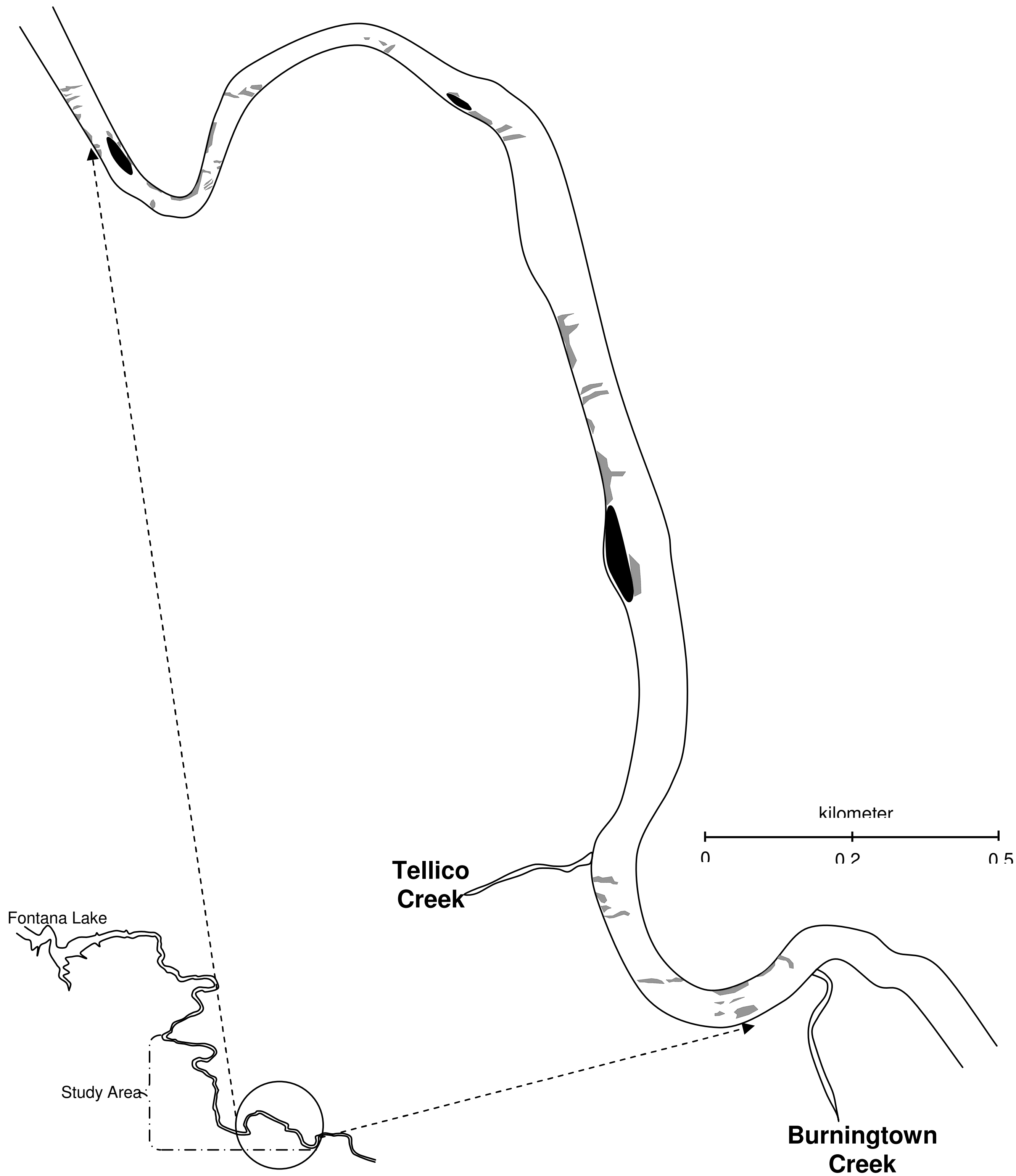


Figure 6.6


kilometer
0
0.2
0.5
Tellico
Creek
Fontana Lake
Study Area
Burningtown
Creek

CHAPTER 7

CONCLUSIONS

The transport and deposition of fine sediment in streams and rivers is a natural occurrence (Gordon et al. 1992). However, human land disturbance often introduces sediment loads well beyond the assimilative capacity of receiving systems (Cairns 1977, Waters 1995, Henley et al. 2000). Current activities such as second home development, urban sprawl and small scale agriculture are the main sources of land disturbance and thus fine sediment in the upper LTR basin (Wear and Bolstad 1998). In addition to increasing land disturbance, historic sediment stored in the substrate, tributary valleys and mainstem valleys may also influence this system for many decades (Harding et al. 1998, Trimble 1999).

The economic cost of water-related erosion from poor land-use practices is ~ $7.4 billion / year (Pimentel et al. 1995). Even more disturbing is the fact that excessive erosion and sedimentation is costing us the irreplaceable fauna of our rivers and streams. The southeastern US harbors an amazing biodiversity of fish species (> 600 freshwater species), and this natural heritage is at risk. Given the estimated future extinction rate of 2.4% per decade (Ricciardi and Rasmussen 1999), 10% of southeastern fish species could be extinct by 2050, and 22% by 2100.

Excessive sedimentation is one of the most pervasive and insidious problems facing aquatic ecosystems. Increasing correlative evidence suggests that excessive sedimentation of streams and rivers results in fish assemblage change and homogenization (Scott and Helfman 2001, Sutherland et al. 2002, Walters et al. 2003). In undisturbed upland rivers, increased substrate heterogeneity is associated with increased habitat quality and habitat availability (Lemly 1982, Berkman and Rabeni 1987, Lenat and Crawford 1994, Waters 1995).

The objective of this dissertation was to increase understanding of the mechanisms driving sediment-related fish assemblage change and decline by quantifying multiple effects of specific suspended sediment concentrations (SSCs) on two upland minnows. An experimental approach was possible with the development of an apparatus (Chapter 2) that maintained SSC without causing excess turbulence, which can be fatal to very young fish. The effects of elevated suspended sediment on the stress response of spotfin chubs (*Erimonax monachus*) and whitetail shiners (*Cyprinella galactura*) was related to fish age (Chapter 3). Spotfin chubs were less sensitive than younger whitetail shiners, and more sensitive than older whitetail shiners. For spotfin chubs, stress increased dramatically between 50 and 100 mg/L SSC, which are sediment concentrations that occur during almost half the year in the upper LTR. Sediment-induced growth reduction was different between species, with spotfin chub exhibiting more sensitivity (Chapter 4). Spotfin chub growth dropped 3- and 15-fold below controls at the 100 and 500 mg/L SSC, respectively. Gill damage to spotfin chubs began to increase at 100 mg/L and was severe at 500 mg/L. Growth rate of spotfin chubs was significantly and inversely related to gill lamellar thickness. Elevated SSC had a moderate effect on whitetail shiner spawning effort, output and timing, suggesting that they may be slightly less sensitive to elevated SSC than other *Cyprinella* species (Chapter 5).

Upper Little Tennessee River (LTR) spotfin chub spawning habitat characteristics were similar to those documented previously (Chapter 6). However, several new discoveries were made, such as: spotfins spawning in different parts of the river or water column, in different flow and sediment conditions and amongst dense growths of riverweed. A relatively small portion of the river was suitable for spotfin chub spawning, but this portion is over twice the area of previous estimates. The distance between spawning habitat patches varied greatly, with some groups of patches isolated by vast areas of substrate smothered with fine sediment. Long distances of heavily silted areas may act as barriers to dispersal and may limit the ability of spotfin chubs to find spawning habitat. Spawning enhancement was modestly successful and suggests that creation of artificial spawning sites may be an effective way to supplement reproduction of imperiled native fishes.

Spotfin chubs begin to be seriously affected when SSC exceeds 100 mg/L. Calculations based on sediment rating curves and annual daily discharge in the upper LTR suggest that in an average year spotfin chubs are exposed to potentially stressful, growth reducing conditions (i.e. SSC $\geq$100 mg/L) for nearly 40% of the time. These SSCs are observed in upland rivers and streams during periods of high discharge. Therefore, it is crucial that those interested in detecting and documenting sediment effects on lotic systems monitor stormflow SSC or turbidity.

The specific relationships observed between fish stress and growth responses to elevated SSC may be useful for the development of science-based turbidity standards. However, turbidity standards alone cannot solve the problems associated with excessive erosion and sedimentation. The effect of erosion on the turbidity of a receiving stream varies due to stream size and mineral composition of the eroded sediment; therefore turbidity may not be a good indicator of erosion control. In addition to continuing to monitor turbidity in streams and rivers that drain developed land, it may also be necessary to focus on regulating the amount of sediment leaving developments.

This research improves our understanding of the effects of excessive sedimentation on stress, growth, gill condition, spawning success and spawning requirements of southeastern upland minnows. However, much is left to be learned about 1) the relative importance to fish populations of sediment-related behavior effects (e.g. spawning behavior) versus lethal effects and habitat alteration; 2) the life-history requirements of at-risk fish species, and how excessive sedimentation affects different life stages; 3) the ecosystem processes that maintain necessary habitats for the entire life-history of vulnerable species; and 4) turbidity and development standards and land management strategies that are protective of vulnerable aquatic fauna.

References

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Pimentel, D., C. Harvey, P. Resosudarmo, K. Sinclair, D. Kurz, M. McNair, S. Crist, L. Shpritz, L. Fitton, R. Saffouri, and R. Blair. 1995. Environmental and economic costs of soil erosion and conservation benefits. Science 267: 1117 – 1123.

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Appendix 1: Results from sediment contaminant tests. Sediment samples collected from the Little Tennessee River basin (Macon Co., NC) were assayed for pesticides and metals by the Soil, Plant and Water Laboratory at the University of Georgia, College of Agricultural and Environmental Science (Athens, GA). 'N.D.' means not detectable.

Appendix 2: Map of study reach in the upper Little Tennessee River, showing habitat patches delineated using GPS receiver. *Appendix 2.a* shows the upstream portion of the study reach. *Appendix 2.b* and *2.c* show the middle and downstream portions of the reach. Habitat patches are numbered, starting from the upstream end of the reach. Habitat patch numbers correspond to those presented in Appendix 3 (GPS coordinates). Large arrows indicate river flow direction.

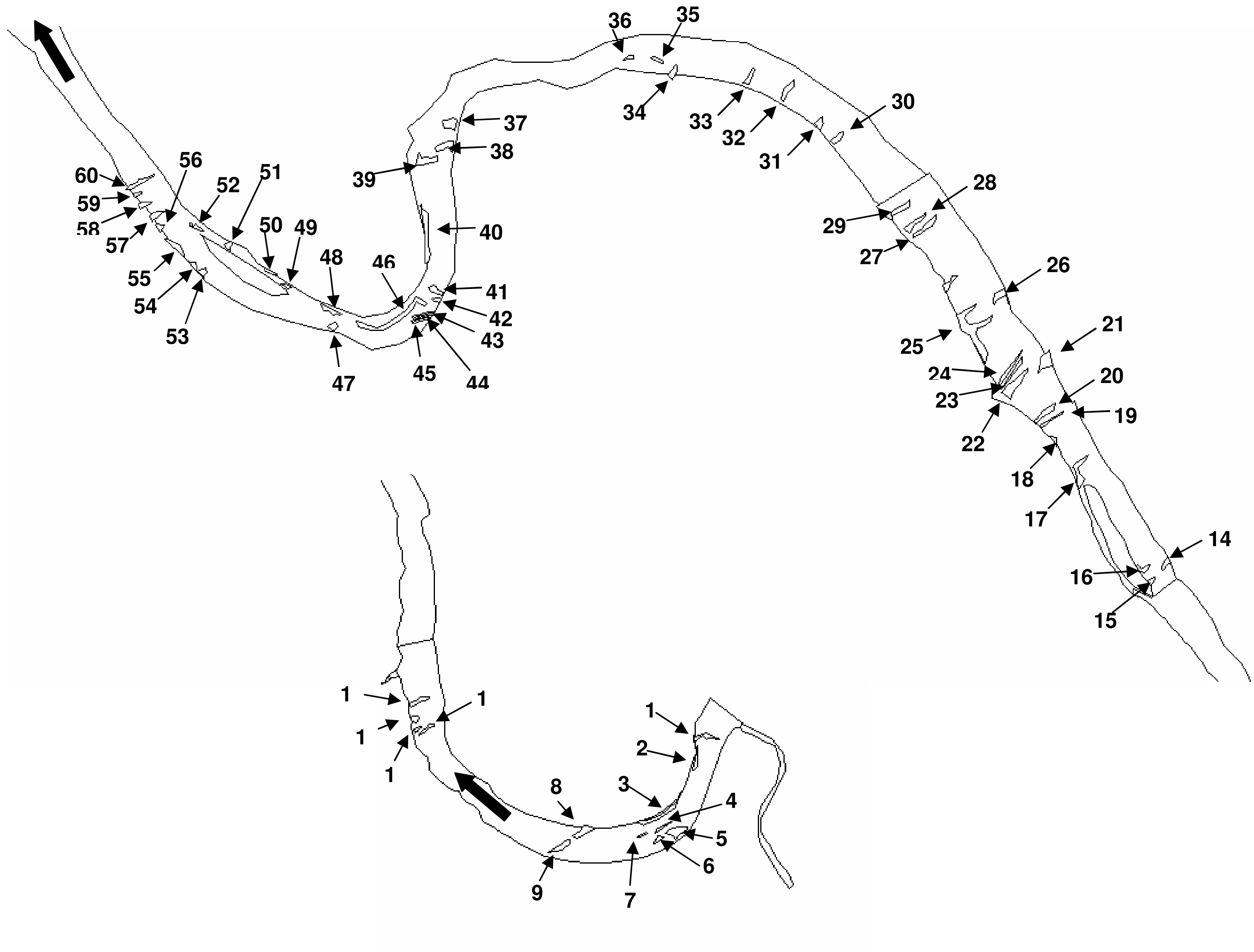
Appendix 3: Habitat patch GPS coordinates. Easting and northing (in degrees and decimal minutes) are presented for each habitat patch and each waypoint within a patch. The number of waypoints varied for each habitat patch, depending on size and shape of patch.

Appendix 1.

Pesticide	concentration (ppm)	delectability limit (ppm)
Aldrin	N.D.	0.003
BHC	N.D.	0.003
Chlordane	N.D.	0.050
Chlorpyrifos	N.D.	0.010
DDD	N.D.	0.005
DDE	N.D.	0.005
DDT	N.D.	0.005
Diazinon	N.D.	0.100
Dieldrin	N.D.	0.010
Dimethoate	N.D.	0.040
Endrin	N.D.	0.010
EPN	N.D.	0.200
Heptachlor	N.D.	0.003
Heptachlor Epoxide	N.D.	0.010
Lindane	N.D.	0.005
Malathion	N.D.	0.050
Methoxychlor	N.D.	0.030
Methyl Parathion	N.D.	0.050
Mirex	N.D.	0.050
PCB 1242	N.D.	0.100
PCB 1248	N.D.	0.100
PCB 1254	N.D.	0.100
PCB 1260	N.D.	0.100
Parathion	N.D.	0.030
Toxaphene	N.D.	0.050

Metal	concentration (ppm)
Al	39.8
B	3.18
Ca	166.9
Cd	6.6
Cr	25.74
Cu	<0.5
Fe	33790
K	7859
Mg	6776
Mn	1156
Mo	<0.5
Na	103.5
Ni	31.06
P	218.5
Pb	<2.5
S	80.04
Zn	91.14

Appendix 2.a



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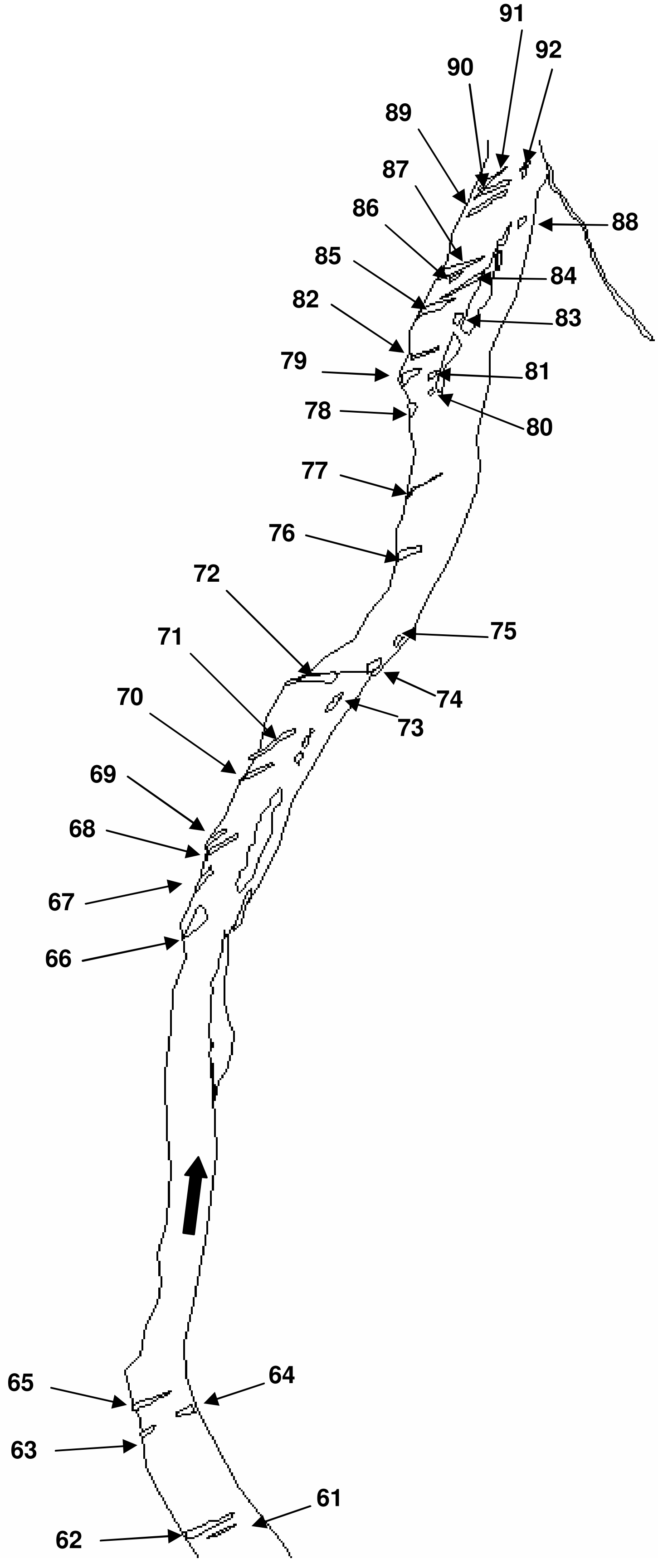
Appendix 2.b



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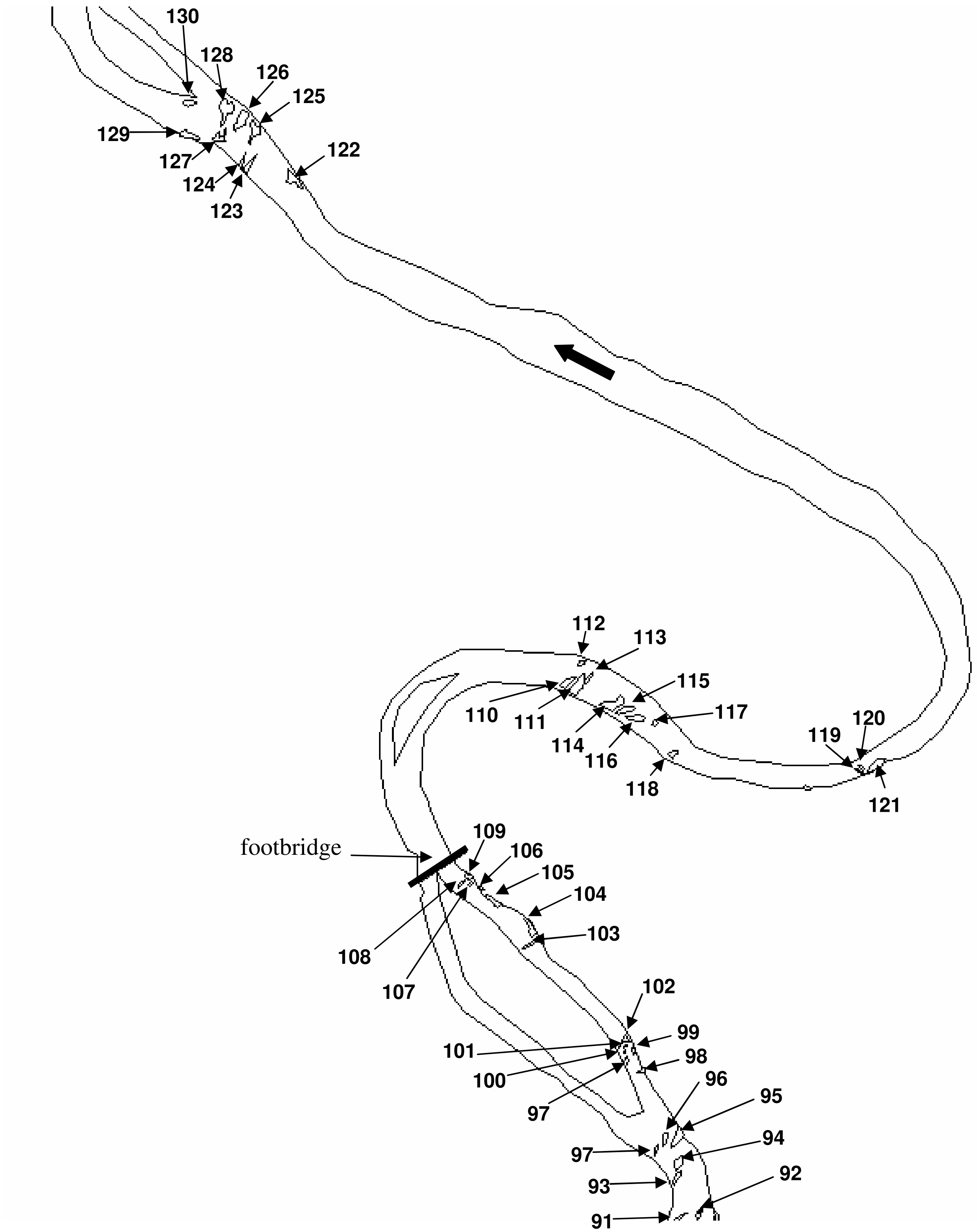
Appendix 2.c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footbridge
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Appendix 3.

Habitat Patch	Waypoint	Easting deg.	Easting min.	Northing deg.	Northing min.
1	001	-83	29.107	35	17.269
	002	-83	29.095	35	17.275
	003	-83	29.083	35	17.268
	004	-83	29.094	35	17.268
	005	-83	29.099	35	17.268
	006	-83	29.107	35	17.263
	007	-83	29.112	35	17.268
2	008	-83	29.106	35	17.257
	009	-83	29.106	35	17.250
	010	-83	29.106	35	17.243
	011	-83	29.112	35	17.233
	012	-83	29.106	35	17.232
	013	-83	29.106	35	17.239
	014	-83	29.099	35	17.244
3	015	-83	29.125	35	17.207
	016	-83	29.131	35	17.195
	017	-83	29.148	35	17.184
	018	-83	29.159	35	17.178
	019	-83	29.167	35	17.172
	020	-83	29.179	35	17.177
	021	-83	29.149	35	17.185
4	022	-83	29.154	35	17.166
	023	-83	29.149	35	17.171
	024	-83	29.142	35	17.177
	025	-83	29.131	35	17.179
5	026	-83	29.112	35	17.173
	027	-83	29.119	35	17.172
	028	-83	29.123	35	17.173
	029	-83	29.131	35	17.167
	030	-83	29.135	35	17.166
	031	-83	29.136	35	17.173
6	032	-83	29.148	35	17.165
	033	-83	29.143	35	17.160
	034	-83	29.148	35	17.160
	035	-83	29.154	35	17.154
	036	-83	29.154	35	17.160
	037	-83	29.148	35	17.161
7	038	-83	29.166	35	17.167
	039	-83	29.159	35	17.167
	040	-83	29.161	35	17.166
	041	-83	29.166	35	17.166
	042	-83	29.172	35	17.159
	043	-83	29.178	35	17.161
8	044	-83	29.239	35	17.172
	045	-83	29.232	35	17.172
	046	-83	29.225	35	17.173
	047	-83	29.231	35	17.165
	048	-83	29.244	35	17.159
	049	-83	29.250	35	17.160
	050	-83	29.255	35	17.160
	051	-83	29.244	35	17.166
	052	-83	29.244	35	17.165
9	053	-83	29.274	35	17.149
	054	-83	29.268	35	17.155
	055	-83	29.261	35	17.154

Habitat Patch	Waypoint	Easting deg.	Easting min.	Northing deg.	Northing min.
9	056	-83	29.257	35	17.155
	057	-83	29.256	35	17.155
	058	-83	29.269	35	17.147
10	059	-83	29.455	35	17.255
	060	-83	29.448	35	17.263
	061	-83	29.443	35	17.262
	062	-83	29.442	35	17.268
	063	-83	29.436	35	17.269
	064	-83	29.442	35	17.262
	065	-83	29.454	35	17.257
11	066	-83	29.454	35	17.263
	067	-83	29.454	35	17.269
	068	-83	29.455	35	17.263
	069	-83	29.461	35	17.262
12	070	-83	29.466	35	17.273
	071	-83	29.455	35	17.273
	072	-83	29.454	35	17.267
	073	-83	29.461	35	17.268
13	074	-83	29.466	35	17.286
	075	-83	29.467	35	17.291
	076	-83	29.455	35	17.299
	077	-83	29.443	35	17.298
	078	-83	29.443	35	17.291
	079	-83	29.447	35	17.291
	080	-83	29.455	35	17.292
	081	-83	29.461	35	17.285
14	082	-83	29.617	35	17.677
	083	-83	29.611	35	17.675
	084	-83	29.617	35	17.671
	085	-83	29.622	35	17.665
	086	-83	29.628	35	17.664
	087	-83	29.623	35	17.671
	088	-83	29.623	35	17.676
15	089	-83	29.646	35	17.651
	090	-83	29.646	35	17.652
	091	-83	29.634	35	17.657
	092	-83	29.633	35	17.651
	093	-83	29.641	35	17.646
16	094	-83	29.657	35	17.664
	095	-83	29.658	35	17.669
	096	-83	29.653	35	17.663
	097	-83	29.646	35	17.669
	098	-83	29.645	35	17.665
	099	-83	29.647	35	17.658
17	100	-83	29.760	35	17.778
	101	-83	29.754	35	17.784
	102	-83	29.737	35	17.789
	103	-83	29.742	35	17.783
	104	-83	29.748	35	17.778
	105	-83	29.748	35	17.771
	106	-83	29.737	35	17.766
	107	-83	29.743	35	17.761
	108	-83	29.747	35	17.753
	109	-83	29.749	35	17.767
	110	-83	29.754	35	17.773

Habitat Patch	Waypoint	Easting deg.	Easting min.	Northing deg.	Northing min.
18	111	-83	29.783	35	17.803
	112	-83	29.790	35	17.807
	113	-83	29.791	35	17.809
	114	-83	29.784	35	17.809
	115	-83	29.778	35	17.803
	116	-83	29.778	35	17.796
19	117	-83	29.790	35	17.832
	118	-83	29.785	35	17.831
	119	-83	29.773	35	17.838
	120	-83	29.778	35	17.837
	121	-83	29.783	35	17.832
	122	-83	29.802	35	17.819
20	123	-83	29.814	35	17.826
	124	-83	29.808	35	17.827
	125	-83	29.802	35	17.838
	126	-83	29.785	35	17.850
	127	-83	2.784	35	17.845
	128	-83	29.802	35	17.831
	129	-83	29.808	35	17.827
21	130	-83	29.790	35	17.893
	131	-83	29.808	35	17.885
	132	-83	29.807	35	17.879
	133	-83	29.808	35	17.892
	134	-83	29.808	35	17.899
	135	-83	29.796	35	17.911
22	136	-83	29.861	35	17.855
	137	-83	29.850	35	17.868
	138	-83	29.845	35	17.874
	139	-83	29.833	35	17.880
	140	-83	29.826	35	17.887
	141	-83	29.826	35	17.879
	142	-83	29.831	35	17.879
	143	-83	29.832	35	17.867
	144	-83	29.845	35	17.863
	145	-83	29.850	35	17.851
23	146	-83	29.833	35	17.899
	147	-83	29.839	35	17.892
	148	-83	29.844	35	17.881
	149	-83	29.851	35	17.873
	150	-83	29.862	35	17.861
24	151	-83	29.868	35	17.867
	152	-83	29.862	35	17.875
	153	-83	29.856	35	17.887
	154	-83	29.839	35	17.897
	155	-83	29.838	35	17.911
	156	-83	29.838	35	17.904
	157	-83	29.844	35	17.892
	158	-83	29.856	35	17.881
	159	-83	29.869	35	17.861
25	160	-83	29.903	35	17.909
	161	-83	29.911	35	17.923
	162	-83	29.921	35	17.929
	163	-83	29.928	35	17.940
	164	-83	29.928	35	17.945
	165	-83	29.922	35	17.945
25	166	-83	29.905	35	17.957
	167	-83	29.905	35	17.953
	168	-83	29.915	35	17.946
	169	-83	29.917	35	17.941
	170	-83	29.910	35	17.934
	171	-83	29.898	35	17.933
26	172	-83	29.862	35	17.971
	173	-83	29.873	35	17.965
	174	-83	29.874	35	17.959
	175	-83	29.880	35	17.957
	176	-83	29.875	35	17.971
	177	-83	29.863	35	17.976
	178	-83	29.856	35	17.959
	179	-83	29.862	35	17.965
	180	-83	29.856	35	17.964
27	181	-83	29.983	35	18.043
	182	-83	29.976	35	18.048
	183	-83	29.965	35	18.061
	184	-83	29.963	35	18.055
	185	-83	29.969	35	18.043
	186	-83	29.975	35	18.043
	187	-83	29.993	35	18.031
	188	-83	29.995	35	18.036
28	189	-83	29.993	35	18.048
	190	-83	29.982	35	18.055
	191	-83	29.976	35	18.061
	192	-83	29.977	35	18.053
	193	-83	29.988	35	18.048
	194	-83	29.994	35	18.041
	195	-83	29.976	35	18.055
	196	-83	29.987	35	18.048
	197	-83	29.994	35	18.042
	198	-83	30.000	35	18.037
	199	-83	30.011	35	18.043
29	200	-83	30.023	35	18.059
	201	-83	30.007	35	18.072
	202	-83	29.999	35	18.071
	203	-83	30.001	35	18.065
	204	-83	30.013	35	18.061
	205	-83	30.024	35	18.054
	206	-83	30.024	35	18.049
	207	-83	30.029	35	18.061
30	208	-83	30.108	35	18.144
	209	-83	30.101	35	18.150
	210	-83	30.103	35	18.145
	211	-83	30.102	35	18.138
	212	-83	30.113	35	18.133
	213	-83	30.121	35	18.138
31	214	-83	30.139	35	18.163
	215	-83	30.133	35	18.163
	216	-83	30.133	35	18.156
	217	-83	30.126	35	18.155
	218	-83	30.133	35	18.149
	219	-83	30.145	35	18.155

Habitat		Easting		Northing	
Patch	Waypoint	deg.	min.	deg.	min.
32	220	-83	30.185	35	18.193
	221	-83	30.173	35	18.205
	222	-83	30.174	35	18.204
	223	-83	30.174	35	18.198
	224	-83	30.180	35	18.191
	225	-83	30.185	35	18.180
	226	-83	30.192	35	18.180
33	227	-83	30.241	35	18.204
	228	-83	30.233	35	18.216
	229	-83	30.227	35	18.216
	230	-83	30.235	35	18.210
	231	-83	30.233	35	18.199
	232	-83	30.246	35	18.197
34	233	-83	30.349	35	18.211
	234	-83	30.342	35	18.216
	235	-83	30.337	35	18.215
	236	-83	30.335	35	18.211
	237	-83	30.342	35	18.204
	238	-83	30.343	35	18.198
35	239	-83	30.373	35	18.223
	240	-83	30.360	35	18.223
	241	-83	30.361	35	18.217
	242	-83	30.360	35	18.215
	243	-83	30.378	35	18.222
36	244	-83	30.409	35	18.221
	245	-83	30.401	35	18.223
	246	-83	30.401	35	18.221
	247	-83	30.408	35	18.223
	248	-83	30.415	35	18.035
37	249	-83	30.648	35	18.132
	250	-83	30.661	35	18.132
	251	-83	30.666	35	18.139
	252	-83	30.661	35	18.138
	253	-83	30.661	35	18.138
	254	-83	30.647	35	18.143
	255	-83	30.642	35	18.144
	256	-83	30.643	35	18.139
	257	-83	30.647	35	18.127
38	258	-83	30.649	35	18.103
	259	-83	30.653	35	18.109
	260	-83	30.661	35	18.107
	261	-83	30.673	35	18.103
	262	-83	30.673	35	18.108
	263	-83	30.666	35	18.109
	264	-83	30.660	35	18.114
	265	-83	30.654	35	18.121
	266	-83	30.649	35	18.120
39	267	-83	30.691	35	18.097
	268	-83	30.684	35	18.096
	269	-83	30.677	35	18.095
	270	-83	30.666	35	18.096
	271	-83	30.667	35	18.091
	272	-83	30.678	35	18.089
	273	-83	30.690	35	18.089
	274	-83	30.697	35	18.091

Habitat		Easting		Northing	
Patch	Waypoint	deg.	min.	deg.	min.
39	275	-83	30.695	35	18.084
	276	-83	30.696	35	18.102
40	277	-83	30.691	35	18.043
	278	-83	30.673	35	17.977
	279	-83	30.679	35	17.977
	280	-83	30.679	35	17.988
	281	-83	30.684	35	18.005
	282	-83	30.683	35	18.037
	283	-83	30.691	35	18.042
	284	-83	30.678	35	18.030
	285	-83	30.679	35	18.023
	286	-83	30.678	35	18.013
	287	-83	30.673	35	17.993
	288	-83	30.673	35	17.989
41	289	-83	30.653	35	17.939
	290	-83	30.659	35	17.940
	291	-83	30.667	35	17.945
	292	-83	30.665	35	17.945
	293	-83	30.672	35	17.946
	294	-83	30.665	35	17.951
	295	-83	30.661	35	17.953
	296	-83	30.661	35	17.945
	297	-83	30.648	35	17.947
42	298	-83	30.654	35	17.935
	299	-83	30.660	35	17.933
	300	-83	30.667	35	17.934
	301	-83	30.660	35	17.934
	302	-83	30.653	35	17.933
43	303	-83	30.667	35	17.922
	304	-83	30.672	35	17.915
	305	-83	30.684	35	17.916
	306	-83	30.691	35	17.911
	307	-83	30.689	35	17.917
	308	-83	30.685	35	17.916
	309	-83	30.677	35	17.916
	310	-83	30.672	35	17.922
	311	-83	30.659	35	17.922
44	312	-83	30.660	35	17.917
	313	-83	30.667	35	17.916
	314	-83	30.671	35	17.916
	315	-83	30.684	35	17.910
	316	-83	30.691	35	17.910
	317	-83	30.696	35	17.909
	318	-83	30.697	35	17.910
	319	-83	30.691	35	17.910
	320	-83	30.684	35	17.917
	321	-83	30.672	35	17.917
	322	-83	30.666	35	17.916
45	323	-83	30.665	35	17.917
	324	-83	30.671	35	17.910
	325	-83	30.685	35	17.910
	326	-83	30.690	35	17.903
	327	-83	30.685	35	17.910
	328	-83	30.672	35	17.915
	329	-83	30.665	35	17.917

Habitat Patch	Waypoint	*Easting* deg.	*Easting* min.	*Northing* deg.	*Northing* min.
46	330	-83	30.762	35	17.904
	331	-83	30.757	35	17.904
	332	-83	30.743	35	17.899
	333	-83	30.732	35	17.904
	334	-83	30.726	35	17.911
	335	-83	30.708	35	17.917
	336	-83	30.703	35	17.922
	337	-83	30.696	35	17.928
	338	-83	30.691	35	17.934
	339	-83	30.678	35	17.934
	340	-83	30.671	35	17.928
	341	-83	30.679	35	17.928
	342	-83	30.685	35	17.929
	343	-83	30.691	35	17.934
	344	-83	30.697	35	17.922
	345	-83	30.701	35	17.916
	346	-83	30.707	35	17.909
	347	-83	30.721	35	17.904
	348	-83	30.750	35	17.899
	349	-83	30.756	35	17.899
	350	-83	30.767	35	17.898
	351	-83	30.775	35	17.903
	352	-83	30.761	35	17.904
	353	-83	30.755	35	17.904
47	354	-83	30.804	35	17.905
	355	-83	30.797	35	17.905
	356	-83	30.799	35	17.903
	357	-83	30.799	35	17.899
	358	-83	30.803	35	17.892
	359	-83	30.811	35	17.899
48	360	-83	30.793	35	17.916
	361	-83	30.792	35	17.911
	362	-83	30.799	35	17.911
	363	-83	30.805	35	17.911
	364	-83	30.810	35	17.915
	365	-83	30.815	35	17.917
49	366	-83	30.864	35	17.940
	367	-83	30.869	35	17.939
	368	-83	30.876	35	17.941
	369	-83	30.883	35	17.946
	370	-83	30.876	35	17.946
50	371	-83	30.888	35	17.958
	372	-83	30.888	35	17.952
	373	-83	30.888	35	17.951
	374	-83	30.899	35	17.958
	375	-83	30.905	35	17.958
	376	-83	30.907	35	17.965
51	377	-83	30.948	35	17.989
	378	-83	30.954	35	17.988
	379	-83	30.953	35	17.982
	380	-83	30.954	35	17.983
	381	-83	30.961	35	17.982
	382	-83	30.961	35	17.988
	383	-83	30.953	35	17.988

Habitat Patch	Waypoint	*Easting* deg.	*Easting* min.	*Northing* deg.	*Northing* min.
52	384	-83	30.990	35	18.000
	385	-83	30.996	35	18.001
	386	-83	31.002	35	18.006
	387	-83	31.008	35	18.006
	388	-83	31.008	35	18.007
	389	-83	31.002	35	18.006
53	390	-83	30.989	35	17.959
	391	-83	30.983	35	17.958
	392	-83	30.983	35	17.952
	393	-83	30.991	35	17.951
	394	-83	30.991	35	17.946
	395	-83	30.990	35	17.951
	396	-83	31.003	35	17.951
54	397	-83	31.008	35	17.965
	398	-83	31.003	35	17.965
	399	-83	30.997	35	17.958
	400	-83	30.997	35	17.959
	401	-83	31.003	35	17.959
	402	-83	31.013	35	17.965
55	403	-83	31.044	35	17.988
	404	-83	31.032	35	17.987
	405	-83	31.021	35	17.982
	406	-83	31.020	35	17.976
	407	-83	31.013	35	17.969
	408	-83	31.020	35	17.970
	409	-83	31.045	35	17.988
56	410	-83	31.056	35	18.007
	411	-83	31.049	35	18.005
	412	-83	31.045	35	18.006
	413	-83	31.050	35	18.000
	414	-83	31.050	35	17.994
	415	-83	31.062	35	18.005
57	416	-83	31.063	35	18.018
	417	-83	31.057	35	18.019
	418	-83	31.049	35	18.024
	419	-83	31.043	35	18.025
	420	-83	31.049	35	18.019
	421	-83	31.057	35	18.013
	422	-83	31.063	35	18.013
	423	-83	31.061	35	18.006
	424	-83	31.069	35	18.017
58	425	-83	31.079	35	18.031
	426	-83	31.074	35	18.030
	427	-83	31.069	35	18.031
	428	-83	31.068	35	18.031
	429	-83	31.074	35	18.030
	430	-83	31.081	35	18.023
	431	-83	31.087	35	18.031
59	432	-83	31.085	35	18.042
	433	-83	31.080	35	18.041
	434	-83	31.079	35	18.043
	435	-83	31.080	35	18.042
	436	-83	31.087	35	18.036
	437	-83	31.091	35	18.036
	438	-83	31.093	35	18.043

Habitat Patch	Waypoint	*Easting* deg.	*Easting* min.	*Northing* deg.	*Northing* min.
60	439	-83	31.105	35	18.043
	440	-83	31.097	35	18.049
	441	-83	31.097	35	18.049
	442	-83	31.087	35	18.054
	443	-83	31.087	35	18.054
	444	-83	31.080	35	18.061
	445	-83	31.073	35	18.061
	446	-83	31.063	35	18.066
	447	-83	31.063	35	18.060
	448	-83	31.074	35	18.060
	449	-83	31.074	35	18.055
	450	-83	31.086	35	18.049
	451	-83	31.092	35	18.047
	452	-83	31.099	35	18.041
61	453	-83	31.278	35	18.241
	454	-83	31.254	35	18.252
	455	-83	31.259	35	18.246
	456	-83	31.267	35	18.241
	457	-83	31.279	35	18.240
	458	-83	31.278	35	18.240
	459	-83	31.265	35	18.246
	460	-83	31.260	35	18.246
62	461	-83	31.285	35	18.247
	462	-83	31.278	35	18.253
	463	-83	31.271	35	18.253
	464	-83	31.266	35	18.253
	465	-83	31.266	35	18.259
	466	-83	31.260	35	18.259
	467	-83	31.260	35	18.252
	468	-83	31.265	35	18.251
	469	-83	31.271	35	18.247
	470	-83	31.285	35	18.245
	471	-83	31.285	35	18.241
	472	-83	31.291	35	18.241
	473	-83	31.296	35	18.241
	474	-83	31.303	35	18.241
63	475	-83	31.331	35	18.312
	476	-83	31.332	35	18.318
	477	-83	31.325	35	18.312
	478	-83	31.333	35	18.312
	479	-83	31.332	35	18.305
	480	-83	31.339	35	18.306
	481	-83	31.339	35	18.312
64	482	-83	31.303	35	18.323
	483	-83	31.308	35	18.325
	484	-83	31.307	35	18.325
	485	-83	31.303	35	18.330
	486	-83	31.297	35	18.331
	487	-83	31.297	35	18.331
	488	-83	31.297	35	18.324
65	489	-83	31.339	35	18.330
	490	-83	31.333	35	18.336
	491	-83	31.326	35	18.336
	492	-83	31.320	35	18.337
	493	-83	31.315	35	18.341

Habitat Patch	Waypoint	*Easting* deg.	*Easting* min.	*Northing* deg.	*Northing* min.
65	494	-83	31.321	35	18.337
66	495	-83	31.315	35	18.673
	496	-83	31.315	35	18.677
	497	-83	31.308	35	18.678
	498	-83	31.308	35	18.671
	499	-83	31.302	35	18.667
	500	-83	31.309	35	18.667
	501	-83	31.320	35	18.655
67	502	-83	31.303	35	18.701
	503	-83	31.303	35	18.696
	504	-83	31.308	35	18.691
	505	-83	31.314	35	18.684
	506	-83	31.314	35	18.690
	507	-83	31.314	35	18.697
68	508	-83	31.301	35	18.714
	509	-83	31.291	35	18.721
	510	-83	31.285	35	18.726
	511	-83	31.279	35	18.727
	512	-83	31.277	35	18.725
	513	-83	31.290	35	18.721
	514	-83	31.296	35	18.713
	515	-83	31.301	35	18.708
	516	-83	31.308	35	18.709
	517	-83	31.309	35	18.714
69	518	-83	31.296	35	18.726
	519	-83	31.290	35	18.733
	520	-83	31.289	35	18.732
	521	-83	31.290	35	18.726
	522	-83	31.295	35	18.725
	523	-83	31.303	35	18.721
	524	-83	31.308	35	18.714
	525	-83	31.309	35	18.720
70	526	-83	31.273	35	18.775
	527	-83	31.261	35	18.774
	528	-83	31.253	35	18.780
	529	-83	31.254	35	18.774
	530	-83	31.253	35	18.775
	531	-83	31.259	35	18.774
71	532	-83	31.260	35	18.785
	533	-83	31.247	35	18.797
	534	-83	31.237	35	18.803
	535	-83	31.236	35	18.797
	536	-83	31.242	35	18.797
	537	-83	31.247	35	18.793
	538	-83	31.254	35	18.787
	539	-83	31.266	35	18.780
	540	-83	31.271	35	18.781
72	541	-83	31.230	35	18.834
	542	-83	31.231	35	18.840
	543	-83	31.218	35	18.841
	544	-83	31.212	35	18.841
	545	-83	31.205	35	18.841
	546	-83	31.201	35	18.841
	547	-83	31.206	35	18.835
	548	-83	31.218	35	18.834

Habitat Patch	Waypoint	*Easting* deg.	*Easting* min.	*Northing* deg.	*Northing* min.
72	549	-83	31.236	35	18.833
	550	-83	31.241	35	18.833
	551	-83	31.248	35	18.833
	552	-83	31.242	35	18.833
73	553	-83	31.200	35	18.829
	554	-83	31.200	35	18.822
	555	-83	31.206	35	18.822
	556	-83	31.199	35	18.828
74	557	-83	31.164	35	18.852
	558	-83	31.163	35	18.846
	559	-83	31.177	35	18.847
	560	-83	31.175	35	18.840
	561	-83	31.177	35	18.851
	562	-83	31.165	35	18.853
75	563	-83	31.153	35	18.870
	564	-83	31.147	35	18.871
	565	-83	31.152	35	18.864
	566	-83	31.158	35	18.863
	567	-83	31.151	35	18.871
76	568	-83	31.153	35	18.931
	569	-83	31.134	35	18.931
	570	-83	31.134	35	18.930
	571	-83	31.145	35	18.923
	572	-83	31.158	35	18.918
	573	-83	31.158	35	18.925
77	574	-83	31.140	35	18.977
	575	-83	31.127	35	18.985
	576	-83	31.123	35	18.983
	577	-83	31.127	35	18.978
	578	-83	31.145	35	18.972
	579	-83	31.151	35	18.966
	580	-83	31.147	35	18.973
78	581	-83	31.152	35	19.019
	582	-83	31.152	35	19.027
	583	-83	31.152	35	19.032
	584	-83	31.151	35	19.032
	585	-83	31.147	35	19.032
	586	-83	31.146	35	19.025
	587	-83	31.153	35	19.020
79	588	-83	31.146	35	19.055
	589	-83	31.145	35	19.051
	590	-83	31.151	35	19.049
	591	-83	31.159	35	19.044
	592	-83	31.159	35	19.045
	593	-83	31.159	35	19.050
	594	-83	31.151	35	19.056
	595	-83	31.147	35	19.057
80	596	-83	31.135	35	19.045
	597	-83	31.134	35	19.039
	598	-83	31.135	35	19.044
81	599	-83	31.135	35	19.056
	600	-83	31.128	35	19.057
	601	-83	31.133	35	19.051
	602	-83	31.134	35	19.057

Habitat Patch	Waypoint	*Easting* deg.	*Easting* min.	*Northing* deg.	*Northing* min.
82	603	-83	31.151	35	19.061
	604	-83	31.146	35	19.067
	605	-83	31.135	35	19.068
	606	-83	31.129	35	19.074
	607	-83	31.128	35	19.069
	608	-83	31.141	35	19.069
	609	-83	31.151	35	19.063
	610	-83	31.153	35	19.069
83	611	-83	31.110	35	19.092
	612	-83	31.110	35	19.098
	613	-83	31.110	35	19.093
	614	-83	31.117	35	19.086
	615	-83	31.116	35	19.086
	616	-83	31.117	35	19.092
84	617	-83	31.093	35	19.128
	618	-83	31.098	35	19.116
	619	-83	31.104	35	19.116
	620	-83	31.111	35	19.116
	621	-83	31.123	35	19.110
	622	-83	31.129	35	19.103
	623	-83	31.115	35	19.116
	624	-83	31.104	35	19.122
	625	-83	31.092	35	19.123
85	626	-83	31.135	35	19.098
	627	-83	31.123	35	19.105
	628	-83	31.115	35	19.105
	629	-83	31.127	35	19.098
	630	-83	31.128	35	19.098
	631	-83	31.147	35	19.093
	632	-83	31.146	35	19.098
86	633	-83	31.111	35	19.121
	634	-83	31.115	35	19.123
	635	-83	31.122	35	19.115
	636	-83	31.123	35	19.121
87	637	-83	31.122	35	19.128
	638	-83	31.111	35	19.129
	639	-83	31.098	35	19.133
	640	-83	31.098	35	19.133
	641	-83	31.110	35	19.127
	642	-83	31.123	35	19.122
	643	-83	31.133	35	19.115
	644	-83	31.128	35	19.122
88	645	-83	31.068	35	19.164
	646	-83	31.061	35	19.165
	647	-83	31.061	35	19.164
	648	-83	31.063	35	19.159
	649	-83	31.068	35	19.159
	650	-83	31.068	35	19.165
89	651	-83	31.099	35	19.170
	652	-83	31.087	35	19.183
	653	-83	31.079	35	19.183
	654	-83	31.080	35	19.177
	655	-83	31.093	35	19.177
	656	-83	31.092	35	19.169

Habitat		*Easting*		*Northing*	
Patch	Waypoint	deg.	min.	deg.	min.
90	657	-83	31.098	35	19.182
	658	-83	31.086	35	19.182
	659	-83	31.081	35	19.188
	660	-83	31.073	35	19.189
	661	-83	31.081	35	19.188
	662	-83	31.081	35	19.182
91	663	-83	31.097	35	19.189
	664	-83	31.091	35	19.189
	665	-83	31.091	35	19.194
	666	-83	31.080	35	19.194
	667	-83	31.081	35	19.200
	668	-83	31.080	35	19.194
	669	-83	31.092	35	19.188
	670	-83	31.098	35	19.183
	671	-83	31.104	35	19.182
	672	-83	31.105	35	19.182
	673	-83	31.099	35	19.181
92	674	-83	31.062	35	19.206
	675	-83	31.063	35	19.201
	676	-83	31.063	35	19.195
	677	-83	31.061	35	19.199
93	678	-83	31.093	35	19.236
	679	-83	31.092	35	19.243
	680	-83	31.087	35	19.235
	681	-83	31.086	35	19.236
	682	-83	31.092	35	19.230
	683	-83	31.099	35	19.223
	684	-83	31.099	35	19.229
94	685	-83	31.092	35	19.254
	686	-83	31.087	35	19.253
	687	-83	31.086	35	19.249
	688	-83	31.097	35	19.243
	689	-83	31.098	35	19.241
	690	-83	31.098	35	19.248
	691	-83	31.092	35	19.255
95	692	-83	31.087	35	19.278
	693	-83	31.092	35	19.273
	694	-83	31.097	35	19.267
	695	-83	31.103	35	19.259
	696	-83	31.105	35	19.266
	697	-83	31.098	35	19.272
	698	-83	31.099	35	19.278
	699	-83	31.092	35	19.284
96	700	-83	31.111	35	19.279
	701	-83	31.110	35	19.273
	702	-83	31.110	35	19.266
	703	-83	31.116	35	19.273
	704	-83	31.110	35	19.278
97	705	-83	31.116	35	19.266
	706	-83	31.116	35	19.261
	707	-83	31.122	35	19.254
	708	-83	31.123	35	19.260
	709	-83	31.122	35	19.260
	710	-83	31.117	35	19.265

Habitat		*Easting*		*Northing*	
Patch	Waypoint	deg.	min.	deg.	min.
98	711	-83	31.141	35	19.338
	712	-83	31.145	35	19.338
	713	-83	31.139	35	19.338
	714	-83	31.139	35	19.344
99	715	-83	31.151	35	19.356
	716	-83	31.159	35	19.362
	717	-83	31.151	35	19.362
100	718	-83	31.163	35	19.344
	719	-83	31.165	35	19.351
	720	-83	31.163	35	19.355
	721	-83	31.158	35	19.351
	722	-83	31.158	35	19.349
	723	-83	31.163	35	19.343
101	724	-83	31.164	35	19.362
102	725	-83	31.158	35	19.369
	726	-83	31.164	35	19.368
	727	-83	31.169	35	19.362
	728	-83	31.165	35	19.373
103	729	-83	31.279	35	19.471
	730	-83	31.279	35	19.464
	731	-83	31.285	35	19.457
	732	-83	31.297	35	19.459
	733	-83	31.284	35	19.464
	734	-83	31.278	35	19.469
104	735	-83	31.284	35	19.470
	736	-83	31.290	35	19.475
	737	-83	31.291	35	19.483
	738	-83	31.296	35	19.489
	739	-83	31.291	35	19.489
	740	-83	31.284	35	19.483
	741	-83	31.285	35	19.475
	742	-83	31.277	35	19.477
	743	-83	31.285	35	19.470
105	744	-83	31.343	35	19.512
	745	-83	31.339	35	19.507
	746	-83	31.325	35	19.506
	747	-83	31.325	35	19.501
	748	-83	31.338	35	19.506
	749	-83	31.343	35	19.505
	750	-83	31.344	35	19.513
106	751	-83	31.344	35	19.518
	752	-83	31.351	35	19.512
	753	-83	31.351	35	19.518
107	754	-83	31.362	35	19.525
	755	-83	31.356	35	19.524
	756	-83	31.363	35	19.518
	757	-83	31.363	35	31.524
108	758	-83	31.369	35	19.523
	759	-83	31.369	35	19.518
	760	-83	31.375	35	19.512
	761	-83	31.374	35	19.518
	762	-83	31.368	35	19.523
109	763	-83	31.368	35	19.529
	764	-83	31.363	35	19.529
	765	-83	31.357	35	19.524

Habitat Patch	Waypoint	Easting deg.	Easting min.	Northing deg.	Northing min.
109	766	-83	31.362	35	19.525
	767	-83	31.369	35	19.525
	768	-83	31.369	35	19.530
110	769	-83	31.259	35	19.734
	770	-83	31.247	35	19.735
	771	-83	31.260	35	19.722
	772	-83	31.265	35	19.722
111	773	-83	31.248	35	19.729
	774	-83	31.236	35	19.733
	775	-83	31.237	35	19.739
	776	-83	31.236	35	19.728
	777	-83	31.237	35	19.721
	778	-83	31.243	35	19.722
	779	-83	31.243	35	19.722
	780	-83	31.241	35	19.717
	781	-83	31.254	35	19.716
	782	-83	31.253	35	19.721
112	783	-83	31.237	35	19.753
	784	-83	31.235	35	19.746
	785	-83	31.243	35	19.745
	786	-83	31.243	35	19.752
113	787	-83	31.223	35	19.735
	788	-83	31.237	35	19.728
	789	-83	31.225	35	19.741
114	790	-83	31.206	35	19.711
	791	-83	31.193	35	19.709
	792	-83	31.189	35	19.716
	793	-83	31.188	35	19.711
	794	-83	31.194	35	19.705
	795	-83	31.199	35	19.699
	796	-83	31.219	35	19.703
115	797	-83	31.183	35	19.703
	798	-83	31.169	35	19.703
	799	-83	31.176	35	19.705
	800	-83	31.188	35	19.699
	801	-83	31.194	35	19.699
116	802	-83	31.175	35	19.699
	803	-83	31.165	35	19.697
	804	-83	31.158	35	19.699
	805	-83	31.157	35	19.692
	806	-83	31.170	35	19.692
	807	-83	31.177	35	19.693
	808	-83	31.183	35	19.697
117	809	-83	31.146	35	19.691
	810	-83	31.140	35	19.693
	811	-83	31.146	35	19.686
	812	-83	31.151	35	19.693
	813	-83	31.145	35	19.692
118	814	-83	31.123	35	19.662
	815	-83	31.117	35	19.662
	816	-83	31.116	35	19.662
	817	-83	31.123	35	19.656
	818	-83	31.123	35	19.656
	819	-83	31.128	35	19.663

Habitat Patch	Waypoint	Easting deg.	Easting min.	Northing deg.	Northing min.
119	820	-83	30.894	35	19.657
	821	-83	30.894	35	19.649
	822	-83	30.889	35	19.649
	823	-83	30.895	35	19.651
	824	-83	30.893	35	19.657
120	825	-83	30.889	35	19.656
	826	-83	30.882	35	19.656
	827	-83	30.888	35	19.649
	828	-83	30.894	35	19.657
121	829	-83	30.875	35	19.657
	830	-83	30.871	35	19.662
	831	-83	30.859	35	19.662
	832	-83	30.864	35	19.656
	833	-83	30.876	35	19.651
	834	-83	30.882	35	19.650
122	835	-83	31.615	35	20.221
	836	-83	31.609	35	20.215
	837	-83	31.614	35	20.215
	838	-83	31.614	35	20.221
	839	-83	31.627	35	20.213
	840	-83	31.625	35	20.221
	841	-83	31.626	35	20.225
	842	-83	31.625	35	20.233
123	843	-83	31.680	35	20.232
	844	-83	31.675	35	20.238
	845	-83	31.669	35	20.245
	846	-83	31.668	35	20.238
	847	-83	31.673	35	20.231
	848	-83	31.674	35	20.226
	849	-83	31.681	35	20.226
	850	-83	31.680	35	20.233
124	851	-83	31.680	35	20.244
	852	-83	31.680	35	20.239
	853	-83	31.687	35	20.233
	854	-83	31.680	35	20.233
	855	-83	31.686	35	20.232
125	856	-83	31.673	35	20.280
	857	-83	31.668	35	20.281
	858	-83	31.669	35	20.273
	859	-83	31.662	35	20.274
	860	-83	31.663	35	20.268
	861	-83	31.669	35	20.263
	862	-83	31.674	35	20.261
	863	-83	31.674	35	20.256
	864	-83	31.675	35	20.256
	865	-83	31.674	35	20.261
	866	-83	31.673	35	20.268
	867	-83	31.673	35	20.274
	868	-83	31.674	35	20.281
126	869	-83	31.679	35	20.285
	870	-83	31.679	35	20.279
	871	-83	31.681	35	20.273
	872	-83	31.692	35	20.274
	873	-83	31.692	35	20.268
	874	-83	31.693	35	20.275

Habitat		Easting		Northing	
Patch	Waypoint	deg.	min.	deg.	min.
126	875	-83	31.691	35	20.280
	876	-83	31.687	35	20.285
	877	-83	31.687	35	20.292
127	878	-83	31.723	35	20.262
	879	-83	31.715	35	20.262
	880	-83	31.717	35	20.269
	881	-83	31.717	35	20.262
	882	-83	31.717	35	20.262
	883	-83	31.710	35	20.263
	884	-83	31.710	35	20.269
	885	-83	31.705	35	20.267
	886	-83	31.703	35	20.257
	887	-83	31.710	35	20.256
	888	-83	31.716	35	20.256
	889	-83	31.723	35	20.262
128	890	-83	31.704	35	20.298
	891	-83	31.697	35	20.298
	892	-83	31.697	35	20.293
	893	-83	31.698	35	20.287
	894	-83	31.703	35	20.286
	895	-83	31.703	35	20.280
	896	-83	31.705	35	20.273
	897	-83	31.710	35	20.274
	898	-83	31.711	35	20.287
	899	-83	31.717	35	20.293
	900	-83	31.710	35	20.298
129	901	-83	31.758	35	20.262
	902	-83	31.758	35	20.268
	903	-83	31.751	35	20.269
	904	-83	31.751	35	20.262
	905	-83	31.746	35	20.263
	906	-83	31.740	35	20.262
	907	-83	31.740	35	20.256
	908	-83	31.747	35	20.256
	909	-83	31.758	35	20.262
130	910	-83	31.753	35	20.298
	911	-83	31.746	35	20.298
	912	-83	31.746	35	20.292
	913	-83	31.752	35	20.291
	914	-83	31.757	35	20.293
	915	-83	31.759	35	20.299