DRAFT

Submitted to American Fisheries Society Monograph on
Connecticut River Shortnose Sturgeon, December 2003

Spawning of Shortnose Sturgeon in an Artificial Stream: Adult Behavior and Rearing and Dispersal of Early Life Stages

Boyd Kynard, Don Pugh, Micah Kieffer, and Tim Parker

S.O. Conte Anadromous Fish Research Center,

Leetown Science Center (U.S. Geological Survey),

One Migratory Way, Turners Falls, Massachusetts 01376, USA

Abstract.- In the spring of 2002 and 2003, we conducted tests with adult pre-spawning Connecticut River shortnose sturgeon Acipenser brevirostrum in a large artificial, endless stream (1.5 m wide x 23.2 m outside circumference) to study adult spawning and rearing of early life stages (ELS). We tested five females and 14 males in 2002 and six females and 15 males in 2003. Photoperiod, not water temperature, controlled endogenous readiness of females to spawn, so this control mechanism evolved early in Teleost fishes. Females began spawning within 24–36 h of introduction, showing an ability to quickly spawn when the physiological spawning window is open, stream conditions are acceptable to innate habitat preference, and ripe males are present. Female spawning style was a long-duration style, i.e., spawning small batches of eggs at 4 bouts/h in discrete locations during 24–48 h. Females spawned at a mean water depth of 61 cm, on rubble-boulder substrate, and mostly in 30-60 cm/s water velocity. Shortnose sturgeon have a polygamous mating system with males mating with multiple females and females mating with multiple males. Males competed for females and differed greatly in competitive ability, i.e., some males mated many times, while others did not. Mating bouts that involved one female and multiple males were rare on day 1 of spawning, but increased significantly in frequency on day 2, suggesting a change in male behavior. Male size was not related to mating success, which seemed to rely on behavior. We did not detect any acoustic call by adults during spawning, so the signal synchronizing fertilization is not known. After 240–250 cumulative temperature units post-egg fertilization, larvae initiated a nocturnal downstream dispersal lasting 8 d (3–4 d peak). Larval production was 165 in 2002 (2.2% of live eggs) and 7,935 in 2003 (11.61% of live eggs). The artificial stream seems capable of producing 8,000–10,000 larvae from natural spawning and semi-natural rearing. Larvae produced in an artificial stream by maintaining natural selection on phenotypes are superior to cultured larvae for conservation purposes.

Field research on spawning of shortnose sturgeon Acipenser brevirostrum in the Connecticut and Merrimack Rivers has led to a good understanding of many basic aspects of spawning, i.e., spawning timing (Taubert 1980, Buckley and Kynard 1985, Kieffer and Kynard present volume-a); spawning periodicity, particularly for males (Kieffer and Kynard present volume-a); number of males aggregated with a female (Buckley and Kynard 1985, Kieffer and Kynard present volume-a, Kynard et al. present volume-a); spawning microhabitat (Buckley and Kynard 1985, Kieffer and Kynard 1996, Kieffer and Kynard present volume-a, Kynard et al. present volume-a); day length, water temperature, and river discharge suitability windows for spawning (Kieffer and Kynard present volume-a), and annual variation in spawning success (Kieffer and Kynard present volume-a). However, some aspects of spawning have not been studied, particularly those aspects requiring direct observation of individuals, i.e., like mate choice, mating success, mating position, etc. (Kynard 1997).

Techniques for culturing sturgeons in hatcheries for commercial uses have been well-developed for many years (Conte et al. 1988); but we can find no literature on spawning and rearing of any sturgeon species in an artificial stream that simulates natural conditions. Yet, for conservation stocking and population restoration, most biologist would agree that sturgeon produced naturally are far superior to cultured sturgeon. We conduced the present research to develop techniques to spawn and rear shortnose sturgeon in an artificial stream, and to study behavior of adults and the production and behavior of early life stages (ELS).

The successful creation of an artificial stream that sturgeon will spawn in depends on creating an environment that is acceptable to all the innate habitat preferences of females. Field studies indicate the relative importance of three physical factors to spawning site preference of female shortnose sturgeon. Water depth used by spawning females is highly variable (1–5 m) at the

Montague spawning site, Connecticut River (Kieffer and Kynard present volume-a). The wide range of water depths suggests that depth is not an important factor for spawning site preference. However, substrate type at spawning sites is always rocky, with diameter of substrate varying from gravel to boulder. Most spawning is in rubble (64-cm diameter) to boulder (265-cm diameter) depending on the spawning site (Buckley and Kynard 1985, Kieffer and Kynard present volume-a, Kynard et al. present volume-a). Coarse substrate provides crevices, which give the sticky eggs attachment sites, and also, provides a secure anchor during swift flows and concealment from predators (Kynard and Horgan 2002). Spawning also occurs at moderate bottom water velocities of 25–130 cm/s (mean, 70 cm/s; Kieffer and Kynard present volume-a, Kynard et al. present volume-a). Thus, the available field data identify characteristics of water depth, substrate type and bottom velocity that are likely innate habitat preferences of females during spawning. The most rigorous test of the acceptance of habitat features by females is done under controlled conditions, i.e., in the laboratory, where spawning can be directly observed.

Spawning movements of adult shortnose sturgeon have been generally inferred from gillnetting or telemetry tracking, but many aspects of spawning behavior are poorly understood because individuals have not been directly observed. Gill net captures of pre-spawning adults indicates that a spawning cohort consists of several males (3–7 males) that chase each female (Buckley and Kynard 1985, Kieffer and Kynard present volume-a, Kynard et al. present volume-a). The movement tracks of radio or sonic-tagged males and females revealed movements in response to habitats and other tagged adults; however, tracking does not reveal behavior of individuals as number of matings, spawning position, etc. Direct observation of sturgeon spawning is rare because it is very difficult. Thus, we have a poor understanding of any aspect of mating behavior or egg fertilization. Placement of eggs by female shortnose sturgeon is

believed to be close to the substrate, but this behavior has not been observed. Bruch and Binkowski (2002) observed spawning by lake sturgeon A. fulvescens in a shallow river. Males repeatedly struck the abdomen of a female during spawning bouts and males appeared to make a thud sound, possibly internally, which may be a signal during spawning. Females completed spawning hundreds of thousands of eggs in 8–12 h.

Behavior and migration of shortnose sturgeon early life stages (ELS) have been studied in the field (Taubert 1980, Taubert and Dadswell 1980), in small artificial tanks in the laboratory (Richmond and Kynard 1995), and in a small laboratory stream channel (Kynard and Horgan 2002). During the laboratory stream study, free embryos (Balon 1975, hereafter, embryos) remained under cover and larvae initiated a downstream dispersal migration that lasted 5 d (90% during 2 d; Kynard and Horgan 2002). These results suggest that in the river, embryos would remain at a spawning site and larvae would initiate the dispersal migration. Drift-net studies in the Connecticut River show some embryos also drift downstream and embryos and larvae move less than 15 km downstream from the spawning site (Taubert and Dadswell 1980, Kynard and Horgan 2002). The laboratory stream studies have been criticized by some researchers because the stream was small, had a few simple habitats, and low velocity, which is unlike rivers. Spawning of adults in a large artificial stream presents the opportunity to study the dispersal migration of early life stages under more natural conditions and examine the criticisms.

Factors that affect survival of sturgeon (ELS) are not understood for any species of sturgeon (Parsley et al. 2002, Gross et al. 2002). While an artificial stream does not duplicate all the potential sources of mortality for ELS, particularly predation, that are present in a natural spawning area, an artificial stream provides the best opportunity to study ELS survival. The absence of predators in an artificial stream is not likely different from the natural situation for

Connecticut River shortnose sturgeon because predation at the spawning site on ELS is very low (Kynard and Horgan 2002).

While traditional sturgeon culture uses highly artificial methods for commercial production goals, some culturists with conservation goals of enhancing or restoring populations have, for many years, used semi-natural methods to avoid relaxing natural selection during spawning and early rearing and producing maladapted fish (Calaprice 1969). Salmonid culturists use stream boxes to rear eggs and alevins and artificial streams are used for salmonid spawning and rearing of ELS. These semi-natural environments avoid extreme artificial selection, and sometimes, replace lost spawning and rearing habitats (Johansson 1981, Bams 1983). Recently, several riverine species of endangered fish in the southern United States were reared in semi-natural environments to develop rearing techniques for culture and restocking and to study spawning (Rakes et al. 1999).

We created an artificial stream with substrate and bottom water velocity that duplicated spawning conditions females use in the Connecticut River. For 2 years, we introduced pre-spawning females and males into the stream and visually observed mating. After removing adults, we studied the spatial distribution and abundance of eggs (live and dead) and monitored the dispersal of embryos and larvae to compare with previous laboratory studies.

Methods

Artificial Stream

The endless artificial stream (1.5 m wide, 23.2 m circumference) was created inside a rectangle 8.3 m long H 3.3 m wide with a center wall (Figure 1). Water from the Connecticut River was supplied at ambient temperature to the stream by two inlets. Water depth varied according to the life stage of sturgeon being studied. During both years, water depths were the

following: adult spawning (mean, 60.6 cm; range, 56–65 cm), egg-embryo rearing (mean, 37.4 cm; range, 34–41 cm), and larva dispersal (mean, 10.8 cm; range, 8–16 cm).

Water temperature was monitored every 2 h in 2002 and hourly in 2003 using a data logger. We used the temperature data to calculate the daily mean temperature and the cumulative temperature degree units (CTU) for each day during rearing of ELS. Cumulative degree-days were calculated for each day (beginning at 1200 hours) and used the mean hourly temperature (°C) for each day. For example, on day-0 eggs were spawned and accumulated 0 degree days, on day-1 eggs accumulated the mean temperature for day 1, and eggs on day 2 accumulated the degree days of day 1 plus the mean temperature of day 2. We calculated CTU for each day and were interested in the CTU at the initiation, peak, and cessation of larval dispersal.

We obtained substrate from a commercial quarry, so rocks had not been exposed to a fluvial environment for many thousands of years and were free from stream odors. Substrate diameter was 64–256 cm (rubble and boulder). We piled substrate deeper in 2002 than in 2003 to test the hypothesis that depth of eggs in the substrate affects egg survival. Mean substrate depth, as determined from three sample sites along each transect (same as egg sampling stations), was 15.1 cm in 2002 and 7.5 cm in 2003.

We installed an electric propeller system on the East and West sides of the stream to control water velocity (Figure 1). The electric propellers were shielded from contact with fish by a covering of 2.5 cm plastic mesh. The two propeller systems, the water jet on the East and West sides, and the vertical louvers at each corner of the rectangular tank kept the water moving around the stream. We covered the louver's sharp edges with foam pipe insulation to protect fish from damage. As previously noted, water depth was highest during spawning, then reduced during egg-embryo rearing, and reduced further during larval dispersal.

Water velocities were different during the three water level stages (Figure 2). During the highest water stage at spawning, water velocity at 0.6 depth was 48 cm/s mean (range, 17–126 cm/s), and velocity 5 cm above the bottom was 36 cm/s mean (range, 7–79 cm/s). Water velocity during egg-embryo rearing at 0.6 depth was 30 cm/s mean (range, 13–87 cm/s), and at 5 cm above the bottom, velocity was 19 cm/s mean (range, 0–44 cm/s). During larval dispersal, water velocity 5 cm above the bottom was 4 cm/s (range, 0–12 cm/s).

In 2002, we noted that movements of adults holding position at the same location dislodged many eggs from their attachment sites on rocks. This would not likely occur as frequently in an open, natural, spawning site, so we attempted to protect attached eggs in 2003. We laid a barrier of 2.5-cm mesh plastic screen on top of the rocks in the transect areas where active spawning did not occur in 2002 (see Figure 1 for location of the protective screen).

Test Adults

We captured females with gill nets in the Connecticut River during the fall preceding spring spawning tests. Each sturgeon was PIT-tagged immediately in the musculature at the base of the dorsal fin for individual identification. In 2002, two females were from the upstream population segment (upstream of Holyoke Dam at river km 140), and four were from the downstream segment (downstream of Holyoke Dam). In 2003, all females were from the downstream population segment.

Females captured in fall were held during the winter in river water at either ambient temperature (0–11°C) or in warm temperature (mean, 9.0°C; range, 6.8–10.0°C). All 2002 females were held in heated water to enable incisions from internal tagging or control incisions in late fall to heal (mean, 9.4; range, 6.5–11.0°C). In 2003, four females were held in heated water and two were not internally tagged and were held in ambient water river temperature. We

held some females on ambient and some on heated water to test the hypothesis that water temperature during the winter has no effect on readiness of females to spawn. We tagged some test females internally with radio tags each fall to determine if internal tags interfered with successful spawning, possibly by blocking eggs from egress through the oviduct valve (Kynard and Kieffer 2002). Females in both years experienced a natural photoperiod. Finally, just before placing females into the artificial stream each year, we weighed each fish, drilled a small hole through a dorsal scute and tied a different colored ribbon to each sturgeon. This enabled us to visually identify individual females and males during all aspects of spawning.

We gillnetted males for tests during the pre-spawning period in April of each year. We PIT-tagged each sturgeon for individual identification. Thus, test males had not spawned previously that year or been exposed to courtship or ripe females at a spawning site. We transported males to the laboratory and held them in circular 1,870-L flow through tanks supplied with ambient Connecticut River water until placing them into the artificial stream. Males were held in separate tanks from females, and since no shortnose sturgeon are present in the river upstream of the water intake, neither sex could receive olfactory cues from wild sturgeon (Kynard and Horgan 2002). We handled males similarly and held all for several days after capture to insure that none were injured during capture and handling. All males were running ripe when used in tests and should be of similar motivation and readiness to spawn. In 2003, eleven males were tested in an experimental fish ladder prior to being placed in the artificial stream.

Each spring, we waited until wild adults moved onto spawning sites or began to spawn before we began artificial stream studies. Males were weighed and tagged in a similar manner to females with a unique colored ribbon prior to introduction into the artificial stream. After adults

ceased spawning, we removed the adults, reweighed each to determine weight loss during spawning, and noted any external damage from the tests. We released adults at their capture site.

We estimated the number of eggs of each pre-spawning female using the relationship of 11,500 eggs/kg body weight and the number of eggs represented by the gram weight loss of females during spawning (45.5 eggs/g) measured on Saint John River shortnose sturgeon (Dadswell 1979). Using these two measurements, we estimated (1) the number of eggs for each pre-spawned female (body weight x 11,500 eggs/kg body weight), and (2) the number of eggs actually spawned (weight loss in grams x 45.5 eggs/g). We also weighed pre-spawning and post-spawning males to determine their weight loss during spawning.

We visually observed spawning behavior and also viewed spawning using underwater video. For each mating bout, we noted the, location along the transect and the ribbon color of the female and male (or males) that participated. Using this data, we determined the mating frequency and mating locations for each female and male, the pairing of females with individual males, the duration of mating bouts, and the total number of bouts for each female and male.

In 2003 we monitored adults during several mating bouts for the occurrence of an acoustic call. We used a passive acoustic detection system that used a low frequency hydrophone (20–20,000 Hz) to detect and record acoustic signals to a digital recorder. This same system has been used previously to record fish calls (B. Kynard unpublished data).

ELS Sampling

Each year after removing adults, we reduced water depth to the egg–embryo rearing level and just prior to egg hatching, we surveyed stream transects for the number of live and dead eggs attached to rocks. We sampled all transects in 2002, but in 2003, the screens prevented sampling all transects (Figure 1).

We sampled eggs by counting the number of eggs at three stations on each transect, i.e., in the transect center, and at each end of the transect, 30 cm from the wall. To select rocks for sampling at each station, we placed a 19.2 cm diameter plastic ring on top of the rocks and removed each rock within the ring one at a time, top to bottom. Rocks had to extend one-half or more of their greatest diameter into the imaginary cylinder created by the ring to be included in the sample. On each rock, we counted the number of live and dead single eggs, and we also estimated the number of clumped live and dead eggs. We classified eggs as dead if they contained fungus or if they had a marbleized appearance of light tan to dark brown. We used regression analysis to examine the relationship between numbers of live and dead eggs and rock depths (2002 only).

In 2002, we removed 100 of the marbleized eggs we classified as dead, and after giving them a prophylactic treatment to control fungus, we reared them in a hatching jar at the laboratory. Three eggs hatched, but the embryos later died within a day, so our characterization of the marbleized eggs as dead eggs was correct.

We used one or more rectangular nets (each, 19 cm x 15 cm, 285 cm^2) with 1-mm clear mesh to capture drifting eggs and embryos and determine the number of these life intervals that drifted. In 2002, we placed one drift net, and in 2003 we placed three drift nets (855 cm^2) side-by-side in the center of transect W4. We positioned net(s) in mid-column to capture fish from just above the substrate to the water's surface. We sampled with the net(s) from morning to late afternoon and checked the catch (day sample); then, we reset the net to sample overnight and checked it the next morning (night sample).

Before embryos developed into larvae, we began operating the box sampler to capture downstream migrants (Figure 1). The sampler had a porous mesh of 2-mm clear openings in

2002. Screen mesh was on three sides and the bottom of the removable trap box. This allowed water to exit the trap, but retain larvae. In 2003, a finer mesh (0.25 mm) was used on the bottom and downstream side of the trap. We reduced water level during trap operation so that one drain pipe passed all water through the trap (Figure 1). The trap was checked twice each day, at the same time as drift nets.

We used the number of eggs and embryos captured during the day and night in the small drift nets to determine the diel pattern of drift of these two life intervals. Similarly, we used the day and night samples of larvae in the trap to show the diel pattern of dispersion, the initiation, peak, and end of dispersion, and the total number of larvae produced.

Results

Water temperature and velocity

Water temperature during spawning in 2002 was 12.6°C (Figure 3). Water temperature during the 2 d of spawning in 2003 was 11.2°C.

During 2002, water velocity in all transects was always highest in the center and outside wall stations and lowest at the inside wall stations (Figure 2). Females had a choice of velocities at 0.6 depth that ranged from 17 to 126 cm/s, and at 5 cm above the bottom, a choice of velocities that ranged from 7 to 79 cm/s. The highest velocities were in the three transects directly downstream from the propeller systems on both sides of the stream. Velocity was not measured in 2003, but was similar to 2002 values because water levels were the same, propellers were set to the same settings, and water input was the same.

Adult Characteristics and Weight Loss

Characteristics of test females and males are shown in Table 1. Body weight of 2002 females was a mean of 6.5 kg (range, 2.5–10.2 kg) and mean body weight of 2003 females was also 6.5

kg (range, 3.1–9.6 kg). Mean body weight of 7 of 14 males in 2002 (seven were not measured) was 3.3 kg (range, 3.0–4.7 kg); mean body weight of 2003 males was 4.3 kg (range, 1.8–7.9 kg).

Percent of body weight lost by females during spawning ranged from a low of zero (female did not spawn) to a high of 40.2% (Figure 4). Female body weight in 2002 decreased a mean of 2.0 kg (range, 0.8–3.3 kg) during spawning, and body weight of 2003 females decreased a mean of 1.3 kg (range, 0.0–2.6 kg). Male body weight in 2002 decreased a mean of 0.1 kg (range, 0.0–0.3 kg); male body weight in 2003 decreased a mean of 0.3 kg (range, 0.1–0.7 kg).

Spawning Behavior

On 8 May 2002, we introduced five females and 14 males into the stream at 1600 hours. Adults were spawning 2 d later (10 May) at 0800 hours when observations of spawning behavior began. We observed spawning until 1700 hours. We resumed observations at 0800 on 11 May, but spawning had ceased. Spawning likely began at night on 9 May and ended at night on 10 May for a total estimated spawning duration of 24–30 h. We observed females spawning for a mean of 4 h 44 min (range, 2 h 27 min–6 h 32 min). We left adults in the artificial stream until 13 May to insure spawning had ceased, then removed them, weighed, and released them.

On 7 May 2003, we placed six females and 15 males in the stream at 1500 hours. Spawning had not begun by 1820 hours on 8 May, when observations ceased. On 8 May, males were observed prodding females with their heads along the body and sidled alongside females in likely courtship behavior. At 0610 hours on 9 May, females were spawning; thus, spawning began during the night of 8 May (about 36 h after introduction). We observed spawning until 1700 hours on 9 May. Observations resumed on 10 May at 0605 hours and continued until 1600 hours. Spawning ceased by 0615 hours on May 11 and we estimate spawning lasted a total of 48 h. We observed spawning in 2003 a mean of 9 h 06 min (range, 7 h 04 min–10 h 28 min on 9

May and a mean of 7 h 46 min (range, 6 h 41–9 h 18 min) on 10 May. We removed adults at 1500 hours on 12 May.

The mean number of mating bouts/female in 2002 was 22.4 (range, 5–44) and the mean number bouts/female in 2003 was 85.2 (range, 17–269; Figure 5). Female R had the highest number of matings in 2002 and the highest mean number of mating bouts/h. Female W in 2003 clearly had the highest number of matings and number of mating bouts/h (Figure 5). Other than female W in 2003, females in the 2 years were similar for number of mating bouts/h.

Males in 2002 mated a mean of 8 times and at a mean mating rate of 2.3 bouts/h (range, 1.1–4.5 bouts/h; Figure 5). Males in 2003, which were observed longer than 2002 males, had similar mean number of matings/h (2003 mean, 2.5; range, 0.7–4.1 bouts/h). Within each year, some males clearly mated more than others, i.e., in 2002 the number of matings varied from 1 to 21, and in 2003, matings varied from 0 to 77 (Figure 5).

Males participated in some matings in which more than one male quivered, an indication of sperm release, i.e., a multiple mating bout. In 2002, when all spawning occurred during 1 d, male OW, YO, and YOB participated in 3–4 of these multiple mating bouts, but most males only participated in one of these bouts (Table 2). In 2003, where spawning occurred during 2 d, a few males participated in 1–2 multiple mating bouts on day 1, but multiple mating bouts were common on day 2 (Table 2). The number of multiple mating bouts was different on day 1 and day 2 (Mann-Whitney Rank Sum Test, $P = 0.002$). Multiple mating bouts were a small proportion of the total number of mating bouts of males. For example, male RW participated in the most multiple matings ($N = 10$) of any male in 2003, but this number was only 25.6% of the total number of his mating bouts (Table 2, Figure 5).

In both years, males were highly variable for spawning frequency with some males spawning much more frequently than others (Figure 5). In 2002, three males (OW, YO, and YW) dominated spawning with 17 or more matings. Eight males mated five or less times, but all 14 males spawned at least once. No males abandoned chasing females and swam slowly alone upstream around the stream. In 2003, male BW had the most matings (78), and five other males spawned 50 or more times (Figure 5). Three males mated less than 17 times. As noted previously, small male RBRBR did not participate in spawning.

In 2002, most females swam slowly upstream being chased by males and making loops around the stream. After many loops, a female would pause, spawn, then either pause nearby or continue to swim loops until she spawned again. Loop time and swimming ground speed for four females during non-spawning loops around the stream (assuming fish swam in the center of the stream for an 18,200 cm loop distance) was (n, mean loop time; mean ground speed): female R (n = 2, 145 s/loop, 125 cm/s ground speed); female W (n = 9; 177 s/loop, 103 cm/6 s ground speed); female B (n = 2, 247 s/loop, 74 cm/s ground speed); and female Y (n = 7, 216 s/loop, 84 cm/s ground speed). Some females held position in slow velocity areas just downstream of the main spawning location on the East or West sides and when ready to spawn, moved upstream a short distance, spawned, and returned to the resting site. In 2003, females did not swim loops. Instead, females held position in transects with slow velocity just downstream of transects with fast velocity used for spawning, then moved back and forth to spawn and rest.

Spawning behavior in 2002 follows. Females paused only briefly to spawn, a male would sidle quickly alongside and quiver, beating his tail side-to-side on the female as gametes were released, then both quickly continued upstream. Female O in 2002 completed spawning by 1200 hours and quit swimming loops around the stream. Females like O that stopped swimming loops

did not attract males, so stopping may be a signal to males that the female is not receptive. Some males swam underneath females, possibly to stimulate her vent or to physically push her, disrupt swimming, and cause her to stop. Some males were very skilled at swimming just in front of a female and guiding or pushing her to stop. Spawning sometimes resulted from this male behavior. A cluster of males was always following just downstream of some females making loops, but some females making loops were not followed by a cluster of males. Although it appeared that many males could spawn with a paused female, usually one male in the cluster spawned. When spawning, the female's body was horizontal, not tilted side-to-side, with her ventral side and tail touching or just above the substrate, so eggs were deposited directly into the substrate. The typical male behavioral sequence was a chase position just downstream of the female; a move under and in front of or alongside the female; a push and guide female to bottom; a sidle alongside; then quiver tail-beat during spawning; then resume swimming. A female did not have to pause for a male to initiate an active pushing movement and skilled males took the initiative. At our gross level of observation, we could not detect any movements from a female that signaled a male when she released eggs.

During 2003, spawning occurred during 2 days and there were differences between the frequency of mating on days 1 and 2 (Figure 6). The mean number of mating bouts for the six females on day 1 (9 May) was 37.7 (range, 9–69 bouts) and the mean number of mating bouts for the three spawning females on day 2 (10 May) was 95 (range, 8–231 bouts). Female W did most of the spawning on 10 May. The mean rate of spawning bouts for females on day 1 was 4.0 bouts/h (range, 1.1–6.9 bouts/h) and the mean rate on day 2 was more than twice that of day 1, 10.9 bouts/h (range, 1.1–24.8 bouts/h). Males that spawned on day 1 mated a mean of 17.4 times (range, 1–31 bouts), and on day 2, males mated a mean of 20.2 times (range, 2–46 bouts). The

mean mating rate for males that spawned on day 1 was 3.7 bouts/h (range, 1.0–20.0 bouts/h), and on day 2, mating rate was 2.7 (range, 0.4–5.0 bouts/h). Male BRB spawned only two times within six minutes resulting in a spawning rate of 20 bouts/h that is notably higher than other males. One male, male RBRBR did not spawn on either day, circling the artificial stream both days.

During our observation periods, females differed for the number of mates, particularly in 2002. In 2002, females R, B, and W mated with 11 or 12 males; but female Y mated with only four males and female O mated with only six males (Figure 7). Female O ceased spawning by 1200 hours, so she could have spawned with other males at night, before our observations began. We did not collect information on nocturnal mating due to the difficulty of correctly identifying adults. In 2003, the mating differences among females was slight, with all females mating with eight or more males and female B mating with 14 males.

Males differed greatly for the number of females with which they mated (Figure 8). In 2002 only one male (OW) mated with all five females and three males (BR, WRB, and YRW) mated with one or two females. In 2003, five males mated with all six females, and only two males (BRB and RBR) mated with one or two females. Males YB, RBR, WBWBW, and BWB, which were not tested in a spiral fishway prior to placing in the artificial stream, appeared to obtain mates at a frequency similar to other males (Figure 8).

Male mating success was only weakly positively related to either TL or body weight (Figure 9). The analyses examined males with a large range of body size (73–110 cm TL), but only a few very small or large males were tested (only three were ≤ 80 cm TL and only two were >100 cm TL).

In 2002, mating bouts were about evenly distributed on the East and West sides, but in 2003, most mating occurred on the East side (Figure 10). Mating bouts were most frequent in E1 and E2 (East side), and in W1 and W2 (West side), transects close to the propellors. The transects most distant downstream from the propeller systems were used the least for spawning (no spawning in W5 either year and only one mating bout in E5 in 2002.

Some females and males showed a preference for spawning on the East or West sides (Figure 11). In 2002, females spawned about evenly (40–52%) on the East side, but some males showed a strong side bias, with some spawning mostly on the East side, and others spawning mostly on the West side. In 2003, four of six females showed strong side preference, i.e., three females (R, RB, and W) spawned 99–100% on the East side, RW spawned 4% on the East side, and BW and B spawned about evenly on both sides (Figure 11). Males in 2003 spawned mostly on the East side, with only three males (BW, BWB, and WRB) spawning 25% or more on the West side.

Transect locations of the 112 mating bouts in 2002 and 512 bouts in 2003 show females spawned mostly at a few stations of moderate to high velocity (Figure 12). In 2002 and 2003, females spawned most in E1, E2, E3 and W1, W2, W3. Few females spawned on the inside transect locations, instead most spawned in the center or along the outside wall.

The frequency distribution of the number of mating bouts at each water velocity station for 2002 and 2003 shows, at 0.6 water depth, most spawning occurred at 31-60 cm/s velocity Figures 2, 12). Selection of water velocities by females was also in the proportion the velocities were available, i.e., there was no significant difference between frequency of mating bouts in selected or available water velocities (Figure 13). The frequency of bouts in selected and available water velocities 5 cm above the bottom were also similar (Chi-square, $P>0.05$).

To detect fish calls, we suspended the hydrophone in the water column just upstream of Transect E1 where much spawning occurred. We monitored constantly for fish calls on 9 May from 1140 to 1200 hours and from 1535 to 1700 hours (total spawning bouts = 22) by three females and several males. While we heard sounds of fish scraping on rocks during spawning, we did not detect any fish calls.

The incidence of spawning by internally tagged females was similar to the incidence of spawning of non-tagged females indicating that internal tagging did not interfere with spawning. In 2002, the three females that were internally tagged and the number of mating bouts (in parenthesis) were B (25), O (10), and R (44) and the two females without tags were W (28) and Y (5). In 2003, the females with internal tags (number of mating bouts in parenthesis) were R (17), W (269), and BW (62). The non-tagged 2003 females and number of mating bouts (in parenthesis) was RB (69) and RW (27), and one female B, with only an incision (67). After tests were complete and sturgeon were released in the river, some internally tagged females resumed feeding quickly. Female R added 1.5 kg in 3 months and female O added 1.3 kg in 1 month. However, female RW was recaptured after 1 month and weighed the same as when released.

Early Life Stages

Water temperature during rearing of eggs and embryos, and dispersal of larvae is shown in Figure 3. The day that eggs began to hatch in 2002 was not recorded; however; in 2003, eggs began hatching on 19 May, so eggs took 9 d to begin hatching after the earliest spawning date. In 2002 and 2003, the artificial stream contained ELS for 32 d.

On 19–20 May 2002, we counted eggs at the five East and five West transects. We found a total of 1,673 eggs (403 live and 1,270 dead). The greatest number of live eggs on the East side was at Transect E 5 and the greatest number of live eggs on the West side was at Transect W5

(Figure 14). Similarly, the greatest numbers of dead eggs on the East side were in Transects E 4 and E5 and the greatest numbers of dead eggs on the West side were in Transects W 3, W 4, and W 5. Few mating bouts occurred in any of these transects. Thus, although 18% of the mating bouts occurred at Transect E 1 and 18% occurred at W1, low numbers of live or dead eggs were at these transects, showing spawned eggs drifted 2–3 m or more before attaching. No spawning occurred in W5 where the greatest number of live eggs occurred on the West side. Almost all live eggs were single eggs (n = 349; 86.6%), not egg clumps, and most dead eggs (n = 817; 64.3% of total) were clumped, not single eggs.

In 2002, we counted the number of live and dead eggs over a range of rock depths (0–19 cm deep). Regression analysis found no significant relationship between depth of rocks and several variables (number of single live eggs, number of single dead eggs, or clump size of dead eggs).

On 20 May 2003, we counted eggs at two East transects and four West transects that were not covered by screening and one East transect covered by screening (Figures 1 and 14). We found a total of 5,964 eggs (2,143 live) downstream of Transect E1 where many spawning bouts occurred. Large clumps of live eggs were abundant at Transect 3.5 under the bottom screen. The number of eggs in live clumps (n = 1,065) was similar to the number of single live eggs (n = 1,078). However, the number of clumped dead eggs (n = 3,237) was six times greater than the number of single dead eggs (n = 584). Live egg numbers were 23.4% of all eggs in 2002, and 35.7% of all eggs in 2003. During both years, an unknown number of eggs left the stream in drain water, so the number of eggs that remained are an unknown subset of the total number of eggs spawned.

Using the pre-spawning body weight of females and the weight loss of females, we estimated the potential number of eggs that females spawned. In 2002, the number of eggs estimated using

female weight was 373,750, and the number estimated from weight loss was 450,450; for 2003, the number estimated from female weight was 370,300 and from weight loss was 359,450 (Table 3). The agreement between the two methods was quite good for 2003. The discrepancy of 76,700 eggs between the two estimates in 2002 was mostly due to one female (B) that was estimated to have 94,300 eggs based on her body weight, but 150,150 eggs based on weight loss (150,150 - 94,300 = 55,850).

In 2002, we set the small drift net to capture downstream moving eggs and embryos from 10 May (adult spawning) to 30 May; then we fished the box sampler for larvae until 10 June. The drift net captured 149 live eggs on 10 May, 12 eggs (10 live) on 11 May, 13 eggs (1 live) on 12 May, and 1 dead egg on 13 May. Thus, we captured drifting eggs for only 3 d post-spawning. Although eggs were present for an additional 6–7 d, they did not enter the drift at the slow water velocity. Although the drift net was set day and night until 30 May, we captured only four embryos in a daytime set on 24 May.

In 2003, we set three drift nets from 17 May to 2 June to monitor egg and embryo dispersal. We only captured five eggs (1 live) on 21 May. We did not capture drifting eggs because nets began fishing too late (based on the 3 d post-spawning drift of eggs in 2002). We captured 374 embryos from 20 May to 31 May (mean daily catch, 23.3 embryos; Figure 15). About one-half (55%) of the embryos were alive when examined in the net. Mortality of some embryos was likely due to the long time spent in the net. On the days when we had day and night samples, there was no indication of a differential day vs. night drift of embryos.

In 2002, we captured the first larvae in the box trap on 31 May (21 d after spawning). We captured larvae until 7 June (8 d; Figure 16). Ninety percent of the 156 larvae (133 live) were captured on 3–6 June (4 d). In 2003, we captured larvae in the box trap from 27 May to 4 June

(8 d) and 90% of the larvae were captured on 29 May–1 June (3 d). In 2003, we captured 7,935 larvae and 6,468 (81.5%) were alive. Some of the larvae likely died in the box trap before we altered screen size. Catch of larvae was highest in the overnight sets (mean catch/h in day samples = 8.0, and mean catch/h in overnight samples = 46.2).

In 2002, the cumulative degree units from the first day of egg fertilization to day of initial capture of larvae was 250 CTU, and in 2003, the CTU for the same period was 240 (Figure 16). In 2002, dispersal of larvae peaked at 340 CTU and ceased at 375 CTU. In 2003, dispersal peaked at 300 CTU and ceased at 375 CTU, like in 2002.

We trapped 156 larvae in 2002 and 7,935 in 2003 (Table 4). Egg sampling estimated there were a total of 7,070 live eggs present in 2002 and 68,339 live eggs present in 2003. Larval survival based on this estimated number of live eggs was 2.21% for 2002 and 11.61% for 2003.

Discussion

In north temperate fish species, photoperiod mediates the physiological (endocrine) readiness for spawning of females (Lucas and Baras 2001). The simultaneous spawning in 2003 of six females, two held in warm water and four held at cold ambient temperature during winter, show clearly that water temperature has little effect on spawning timing of shortnose sturgeon. Thus, the ancient sturgeon appears to have the same endogenous timing mechanism as modern teleosts, suggesting that the photoperiod-endocrine control mechanism evolved early in the evolution of teleost fishes.

Based on years of field observations on female spawning habitat selection (Buckley and Kynard 1985, Kieffer and Kynard present volume-a, Kynard et al. present volume–a), we predicted the physical environment in the artificial stream would be within the acceptable range of a female's innate habitat preference for water depth, water velocity, and substrate type. Water

depth in the artificial stream was shallower than any spawning depth we have ever found used by wild females. However, because water depth is so variable during spawning of wild females, we predicted females would accept a shallow water depth. The spawning of 11 females in the shallow water in the artificial stream supports this hypothesis. We conclude that the acceptable range of spawning depth for shortnose sturgeon includes water only 60 cm deep. Females did not have a choice of variable substrate types, so the range of substrate that is acceptable is not known. The present results with 11 females clearly show that a female's habitat preference includes rubble–boulder substrate. Most spawning in the wild is over rubble-boulder substrate, but some spawning also occurs over gravel (Kieffer and Kynard 1996, Kynard et al. present volume–a). For water velocity selection, females had a wide range of water velocities available (15–135 cm/s) and about 70% of the mating bouts each year were in velocities of 30–60 cm/s at 0.6 depth. While this result supports the field data that shows spawning females select a mean of 70 cm/s velocity, the frequencies of velocities utilized by spawning females and those available were similar, so the data do not show female preference for velocity.

The results of studying spawning of Connecticut River shortnose sturgeon during 11 years in the field (Kieffer and Kynard present volume-a) and manipulation of wintering water temperatures in the present study suggest a conceptual model for spawning initiation. Photoperiod (day length) causes females to be physiologically ready to spawn for 24 d (27 April–20 May). The endogenous factors also stimulate pre-spawning adults to migrate to their natal area, which has substrate acceptable to the innate preference of females. Then, females wait until ripe males are present and river discharge (bottom water velocity) is in the acceptable range for their innate velocity habitat preference. When the three environmental windows (day length, substrate type, and bottom velocity) are open (acceptable), spawning occurs. The

successful spawning of females in the artificial stream during 2 years without any testing of their endocrine state or readiness to spawn, strongly suggests the conceptual model is correct. Complicated monitoring of physiological state, which is needed for precise timing of egg fertilization during artificial culture, is not needed to for successful spawning of sturgeon in an artificial spawning stream.

The short spawning time of females in the artificial stream confirmed predictions on spawning duration from field observations on individual females. During field tracking, females remained on a spawning site for 24–48 h (Kieffer and Kynard present volume-a, Kynard et al. present volume-a). Spawning of small and large size females was completed in 24–48 h in the artificial stream, showing that even large females can spawn their entire egg complement in this time period. Sturgeon release ovulated eggs free into the body cavity and the eggs exit the cavity through the small oviduct valve (Kynard and Kieffer 2002). Some sturgeon species require only a short time period to spawn great numbers of eggs (Chinese sturgeon spawn 3-6 million eggs in <12 h (Wei 2003), which is similar to lake sturgeon (Bruch and Binkowski 2002), except for fewer eggs.

The present information suggest there are at least two spawning styles of sturgeons: species with a long-duration style and species with a short-duration style. The long-duration style is represented by shortnose sturgeon which spawns eggs in discrete batches multiple times during 24–48 h. The short-duration spawning style is represented by the Chinese sturgeon (Wei 2003). This species spawns a large number of eggs (3–6 million) during 8–12 h at night. The available information suggests that predation intensity may be the selective factor responsible for the two spawning styles. Predation is very low on shortnose sturgeon (Kynard and Horgan 2002), so spawning slowly during the day and night in small batches in many places likely has advantages

for egg survival and dispersal, but no disadvantages from predation on eggs. However, many years of data show clearly that several species of predatory fish intensively forage on Chinese sturgeon eggs consuming millions of eggs each spawning season. Possibly, females spawn all their eggs during a short period to swamp predators with great numbers of eggs (Krebs and Davies 1978). Lake sturgeon also spawn large numbers of eggs in 8–12 h, but they spawn during the day and night (Bruch and Binkowski 2002). No information is available on predation intensity on lake sturgeon eggs and their spawning strategy needs further study.

The body position of female lake sturgeon during egg release was recently observed by Bruch and Binkowsky (2002). Lake sturgeon spawned with their body close to the bottom, so that eggs are released close to the substrate. Female shortnose sturgeon also released eggs with their bodies on or near the bottom. Because sticky eggs are released close to the substrate and females prefer rocky substrate, females are likely placing eggs directly into rock crevices. However, the present study shows that most eggs drift downstream 2–3 m or more before attaching. Some of this egg drift from the spawning area in the artificial stream was likely due to multiple spawnings over the same location, which might not occur in the wild. While it is correct to characterize sturgeon as an open substrate spawner (Balon 1975), females in the artificial stream did not broadcast eggs widely over the substrate, but concentrated spawning in select areas. Because spawning occurs in moderate water currents, many eggs are transported by current to an unknown downstream location before they eventually sink to the bottom. The spawning of lake and shortnose sturgeon suggest the strategy of females is to place eggs in a discrete rough substrate, not to broadcast eggs over a wide rocky area. This strategy of females was suggested earlier by Richmond and Kynard (1995) and Kynard and Horgan (2002) and the information in the present study supports this idea.

The present study clearly shows the mating style of shortnose sturgeon is polygamous with females mating with multiple males and males mating with multiple females. This mating style maximizes the genetic diversity of offspring (Kirpichnikov 1981). Other sturgeon likely have the same or similar mating styles, i.e., lake sturgeon have a polygamous mating system (Bruch and Binkowski 2002). Lake sturgeon males compete for females, like shortnose sturgeon males, so male competition may be a common feature of the mating system in all sturgeon species. However, long-term mate selection as part of the mating system of shortnose sturgeon is not supported by the present study. In 2003, all females came from the downstream population segment and males came from the upstream segment, so they could not have established a mating relationship, yet widespread mating occurred between males and females.

Few eggs and embryos drifted during rearing in the slow velocity in the artificial stream, suggesting drift of these life stages in rivers is dependent on river flow during rearing. Water velocity in the artificial stream was low and stable for the entire 32 d rearing period, a condition unlikely to occur in natural spawning sites. The artificial stream data suggest that when river flows are stable during rearing of ELS, there will be almost no drift of eggs, and only a low drift of embryos. However, when river flow increases quickly and changes the velocity at the substrate, both eggs and embryos will be displaced, and the magnitude of displacement is likely in proportion to the magnitude of change by flow. The low number of drifting embryos we observed in 2003 (13/ d) is likely the level expected in a natural river system with stable flows during rearing. The zero embryos we captured moving downstream in 2002 was likely related to the low number of live embryos present.

Recent evidence suggests that sturgeons make acoustic calls during final sexual maturation (Tolstoganova et al. 1999, Johnston and Phillips 2003). Researchers interpreted the sounds they

recorded from fish in tanks as signals between males and females that are used in reproduction, not just incidental sounds from internal organs or body movements when rubbing tanks. However, the sounds yet recorded cannot be placed into a life-history context and were affected by fish density, water quality, and handling. Thus, they do not have the characteristics expected of an innate stylized call used for a reproductive signal. Signals used for communication during reproduction should be highly repeatable and little affected by environmental factors. Male lake sturgeon make sounds associated with spawning that can be heard by ear (Bruch and Binkowski 2002), but unfortunately, they were not studied. We detected no sounds during spawning of shortnose sturgeon using the hydrophone 2 m from the fish. (The primary author also monitored calls of pre-spawning shortnose sturgeon adults in a tank in 1993. While the male did much rubbing and prodding of the female's body during courtship, only the squeaks and sounds from their bodies rubbing on the tank bottom were heard--no acoustic calls were heard.) We also observed no movement of adults during spawning that appeared to physically or visually connect a female and male during egg release, so we do not know how a male knows the female has released eggs, i.e., when to release sperm. If present, the sound may be very subtle, and only detectable close to the fish.

The same pattern of downstream movement of ELS in the small laboratory channel used by Kynard and Horgan (2002) was also found in the artificial stream, with a few exceptions. In the small channel, embryos did not move downstream (Kynard and Horgan 2002); however, a few did move downstream in the artificial stream and in the river (Taubert and Dadswell 1980). The day and night downstream movement of a few embryos is not a dispersal migration (Kynard and Horgan 2002), but likely represents embryos seeking different habitat, or perhaps, dying. Mean velocity was 5 cm/s in the laboratory channel, about 25% of the velocity in the artificial stream.

Perhaps, at the higher velocity, embryos that change position and encounter increased velocity have difficulty re-establishing residency and tend to keep moving. It seems clear that in a natural spawning area with variable river flows, there will be a low number of drifting embryos present during the entire embryo rearing period.

The larval dispersal described by Kynard and Horgan (2002) was for 50 larvae that migrated during 5 d (90% during 3 d). These 50 fish were from eggs fertilized at the same time and hatched within 24 h of each other, so development and behavior were very synchronized. In contrast, larvae in the artificial stream were from eggs fertilized during 24 h (2002) or 48 h (2003) periods, so more variation in larval migration timing was expected. In spite of the longer periods of egg fertilization, migration of larvae was brief with 90% of the larvae in 2002 captured in 4 d and 90% in 2003 captured in 3 d. Thus, dispersion peak was the same whether eggs were spawned at one time (laboratory channel study) or during 1–2 d (present study). However, the duration of migration was slightly different between these two studies, with a 3 d longer dispersal period by larvae from eggs fertilized during 24–48 h. Observations on dispersal timing and CTU development of embryos and larvae in the artificial stream also support observations in the laboratory channel.

A major advantage of using an artificial stream rather than traditional fish culture techniques is that it allows a more natural selection of mates and exchange of gametes between the sexes. The artificial stream used a subset of males, and like in the river, males competed for mates. Presently, there is no indication from life history that mates are selected prior to spawning (Kieffer and Kynard present volume-a). Traditional fish culture does not permit mate selection and sperm from all males usually has an equal opportunity to fertilize the same number of eggs. Some males were dominant in the artificial stream, and some males were dominant during

spawning in the river (Kieffer and Kynard present volume-a). Males clearly compete for females during spawning, and males fertilize different numbers of eggs, and they did in the artificial stream. In life-history situations like with sturgeon, where reproductive fitness is determined by competitive ability of males, it is detrimental to the long-term genetic fitness of the population to use equal volumes of sperm to fertilize eggs (Kirpichnikov 1981). This allows each male to fertilize the same number of eggs and results in a decreased fitness of the entire cohort (Calaprice 1969). If repeated for many generations, the fitness of the entire population will be reduced.

A second advantage of the artificial stream is that it does not relax selection on eggs or sperm during fertilization or on egg stickyness. Eggs and sperm spawned naturally must function in fast moving water, which is very different than during artificial fertilization, where contact time of sperm with eggs is much longer than in swift current. When we fertilized shortnose sturgeon eggs in 2003 using eggs placed into a shallow pan from a wild female running eggs and added a sperm and water mix from males captured with her, we estimated the fertilization rate was very high, likely >90%. And after several days, we estimated 70% were still alive in the hatching jar. So, fertilization was high and the resulting survival for several days was high. However, our count of live and dead eggs in the artificial stream show that many do not hatch. We suspect many eggs were not fertilized in the first place. If true, then artificially fertilizing eggs enables a great number of eggs to be fertilized and survive that would not be fertilized and survive in swift currents of natural spawning. A low fertilization rate of eggs also occurs in Chinese sturgeon (Wei 2003). Additionally, egg stickyness likely contributes greatly to survival of eggs. Eggs that do not quickly become sticky after contact with water are carried downstream by the current and away from the spawning habitat where they likely have poor survival (Kynard and Horgan 2002). However, egg stickyness does not contribute to survival and fitness of cultured eggs

because all eggs are deadhesed by coated them with a substance to remove stickyness and make them suitable for rearing in a hatching jar (Conte et al. 1988, Goncharov 2001). This allows the survival of many unsticky eggs that would die in the wild.

A third advantage of the artificial stream is that selection for rearing in the natural environment is not relaxed during rearing of ELS. Eggs in the river and in the artificial stream are subject to many potential sources of mortality from sedimentation, poor long-term adherence ability, resistance to abrasion, resistance to bacteria, etc. Artificial culture of sturgeon allows embryos to swim-up out of the hatching jar and enter a smooth-bottom, cover-less rearing tank where selection for photonegative and concealment seeking, which would determine their survival as a wild embryo, is totally relaxed. This major relaxation of selection is avoided by spawning and rearing of ELS an artificial stream. Also, we made no effort to exclude small fish and predatory invertebrates smaller than the 3-cm mesh screen on the water supply pipe during rearing of ELS. The artificial stream did not have a natural fauna during spawning because of the necessity for draining and construction, but after several days, many invertebrates were present and some siltation was occurring. For Connecticut River shortnose sturgeon, the lack of predatory fishes in the artificial stream is not a relaxing of selection, because fish predation is very low on ELS at the natural spawning area (Kynard and Horgan 2002). This might be different for other populations, but predators could be added in these cases.

The results from 2003 show that a small artificial stream can produce thousands of larvae that are much closer to the phenotype-genotypes that result from spawning and rearing in the wild. The estimated rearing area for eggs and embryos in the artificial stream was only 18.9 m^2 when 8,309 larvae were produced in 2003. Thus, given the number of females and techniques used, the present results suggest that about 420 larvae/m^2 can be produced in our stream. It will take

additional years of study to determine the best combination of environmental factors and number of adults to maximize production of this type of rearing stream. The present results indicate our stream can produce 8,000–10,000 larvae. These larvae would be far superior for use in conservation stocking than artificially cultured larvae. We believe artificial streams have great potential to contribute to conservation stocking of sturgeons, even those with large bodies.

Acknowledgments

Funding for the study was provided by the S.O. Conte Anadromous Fish Research Center. Shortnose sturgeon were studied under Scientific Permit No. 1239 to Boyd Kynard from the National Marine Fisheries Service and Scientific Permit No. 429-AC from the Massachusetts Division of Fisheries and Wildlife.

References

Balon, E. P. 1975. Reproductive guilds of fishes: a proposal and definition. Journal Fisheries Research Board of Canada 32: 821-864.

Bams, R. A. 1983. Early growth and quality of chum salmon (Onchorhynchus keta) produced in keeper channels and gravel incubators. Canadian Journal of Fisheries and Aquatic Science 40: 499-505.

Bayunova, L, I. Barannikova, and T. Semenkova. 2002. Sturgeon stress reactions in aquaculture. Journal of Applied Ichthyology 18: 397-404.

Bruch, R. M. and F. P. Binkowski. 2002. Spawning behavior of lake sturgeon (Acipenser fulvescens). Journal Applied Ichthyology 18: 570-575.

Buckley, J. and B. Kynard. 1985. Habitat use and behavior of pre-spawning and spawning shortnose sturgeon, Acipenser brevirostrum, in the Connecticut river. pp. 111-117 in F. P. Binkowski and S. I. Doroshov, editors. North American sturgeons: biology and aquaculture

potential. Dr W. Junk, Dordrecht, The Netherlands.

Calaprice, J. R. 1969. Production and genetic factors in managed salmonid populations. pp. 377-388. in Symposium on salmon and trout in streams. University of British Columbia, Vancouver.

Conte, F. S., S. I. Doroshov, P. L. Lutes, and E. M. Strange. 1988. Hatchery manual for the white sturgeon, Acipenser transmontanus Richardson, with application to other North American Acipenseridae, Cooperative Extension Publication Number 3322, University of California, Davis.

Dadswell, M. J. 1979. Biology and population characteristics of shortnose sturgeon, Acipenser brevirostrum LeSueur 1818 (Osteichthyes: Acipenseridae), in the Saint John river estuary, New Brunswick, Canada. Canadian Journal of Zoology 57: 2186-2210.

Goncharov, B. F. 2001. In vitro approach to studying the mechanisms of oocyte maturation in sturgeons: a review of fundamental and applied aspects. Journal of Applied Ichthyology 18: 368-375.

Gross, M. R., J. Repka, C. T. Robertson, D. H. Secor, and W. V. Winkle. 2002. Sturgeon conservation: insights from elasticity analysis. American Fisheries Society Symposium 28: 13 -30.

Johansson, N. 1981. General problems in Atlantic salmon rearing in Sweden. Ecological Bulletin 34 (Stockholm): 75-83.

Johnston, C. E., and C. T. Phillips. 2003. Sound production in sturgeon Scaphirhynchus albus and S. platorhynchus (Acipenseridae). Environmental Biology of Fishes 68: 59-64.

Kieffer, M. C., and B. Kynard. 1996. Spawning of shortnose sturgeon in the Merrimack River, Massachusetts. Transactions of the American Fisheries Society 125:179-186.

Kieffer, M., and B. Kynard. Spawning of Connecticut River shortnose sturgeon. American Fisheries Society Monograph. Present volume - a.

Kirpichnikov, V. S. 1981. Genetic basis of fish selection. Springer-Verlag, Berlin.

Krebs, J. R., and N. B. Davies. 1978. Behavioral ecology: an evolutionary approach. Sinauer Associates, Inc., Sunderland, MA.

Kynard, B. 1997. Life history, latitudinal patterns, and status of the shortnose sturgeon, Acipenser brevirostrum. Environmental Biology of Fishes 48: 319-334.

Kynard, B., and M. Horgan. 2002. Attraction of pre-spawning male shortnose sturgeon to odor of pre-spawning females. Journal of Ichthyology 42: 205-209.

Kynard, B., and M. Horgan. 2002. Ontogenetic behavior and migration of Atlantic sturgeon, Acipenser oxyrinchus oxyrinchus, and shortnose sturgeon, A. brevirostrum, with notes on social behavior. Environmental Biology of Fishes 63: 137-150.

Kynard, B., and M. Kieffer. 2002. Use of a borescope to determine the sex and egg maturity stage of sturgeons and the effect of borescope use on reproductive structures. Journal Applied Ichthyology 18: 505-508.

Kynard, B., M. Kieffer, M. Burlingame, P. Vinogradov, and B. E. Kynard. Biological significance of spawning by shortnose sturgeon at Holyoke Dam. American Fisheries Society Monograph. Present volume - a.

Lucas, M. C., and E. Barras. 2001. Migration of freshwater fishes. Blackwell Sciences, Ltd., Oxford, England.

Parsley, M. J., P. J. Anders, A. I. Miller, L. G. Beckman, G. T. McCabe, Jr. 2002. Recovery of

white sturgeon populations through natural production: understanding the influence of abiotic and biotic factors on spawning and subsequent recruitment. American Fisheries Symposium 28: 55-66.

Rakes, P. L., J. R. Shute, and P. W. Shute. 1999. Reproductive behavior, captive breeding, and restoration ecology of endangered species. Environmental Biology Fishes 55: 31- 42.

Richmond, A., and B. Kynard. 1995. Ontogenetic behavior of shortnose sturgeon. Copeia 1995: 172-182.

Taubert, B. D. 1980. Biology of the shortnose sturgeon, Acipenser brevirostrum, in the Holyoke Pool, Connecticut River, Massachusetts. Doctoral Dissertation. University of Massachusetts, Amherst.

Taubert, B. D. and M. J. Dadswell. 1980. Description of some larval shortnose sturgeon (Acipenser brevirostrum) from the Holyoke Pool, Connecticut River, Massachusetts, U.S.A., and the Saint Johns River, New Brunswick, Canada. Canadian Journal of Zoologist 58:1125-1128.

Tolstoganova, L, K., O. S. Bukovskaya, A. Ronyai, and D. El. Kime. 1999. Acoustics and hormonal activities during final maturation and spawning in the Siberian sturgeon, (Acipenser baeri). Journal of Applied Ichthyology 15: 321.

Wei, Qiwei. 2003. Studies on Chinese sturgeon (Acipenser sinensis Gray): spawning ecology and adult and yearling abundance. Doctoral Dissertation, Institute of Hydrobiology, Wuhan, China.

Figure captions

Figure 1. Plan view of the rectangular artificial stream (1.5-m wide and 23.2 m outside circumference) showing the center wall, and (a) two water inlets, (b) two propellor systems (not shown is the screen covering to protect fish), (c) three drain pipes, (d) removable larval trap, (e) water drain, and (f) four louvers in each corner to keep water moving around the corners. Louver edges were covered with foam to protect fish. In 2002, none of the bottom was covered by screen, but in 2003, screen was placed over the bottom in the areas indicated. East and West transects used to sample eggs are designated as T1–T5. Spawning transect locations are indicated by E1–E5 (East side) and W1–W5 (West side). Direction of water flow is clockwise. Water depth during spawning was 58–65 cm.

Figure 2. Water velocities during 2002 tests showing velocities at 0.6 water depth and 5 cm above the bottom during adult spawning, egg and embryo rearing (incubation), and larval dispersal.

Figure 3. Water temperatures during spawning, egg and embryo rearing, and larval dispersal in 2002 and 2003.

Figure 4. Percent of body weight decrease during spawning of females and males in 2002 and 2003, i.e., pre-spawning weight - post-spawning weight.

Figure 5. Number of mating bouts and number of bouts/h during spawning of females and males during 2002 and 2003. Bars = number of bouts; filled circles = number of bouts/h.

Figure 6. Number of mating bouts by females and males during day 1 and day 2 of spawning in 2003.

Figure 7. Number of males that each female mated with during 2002 and 2003.

Figure 1

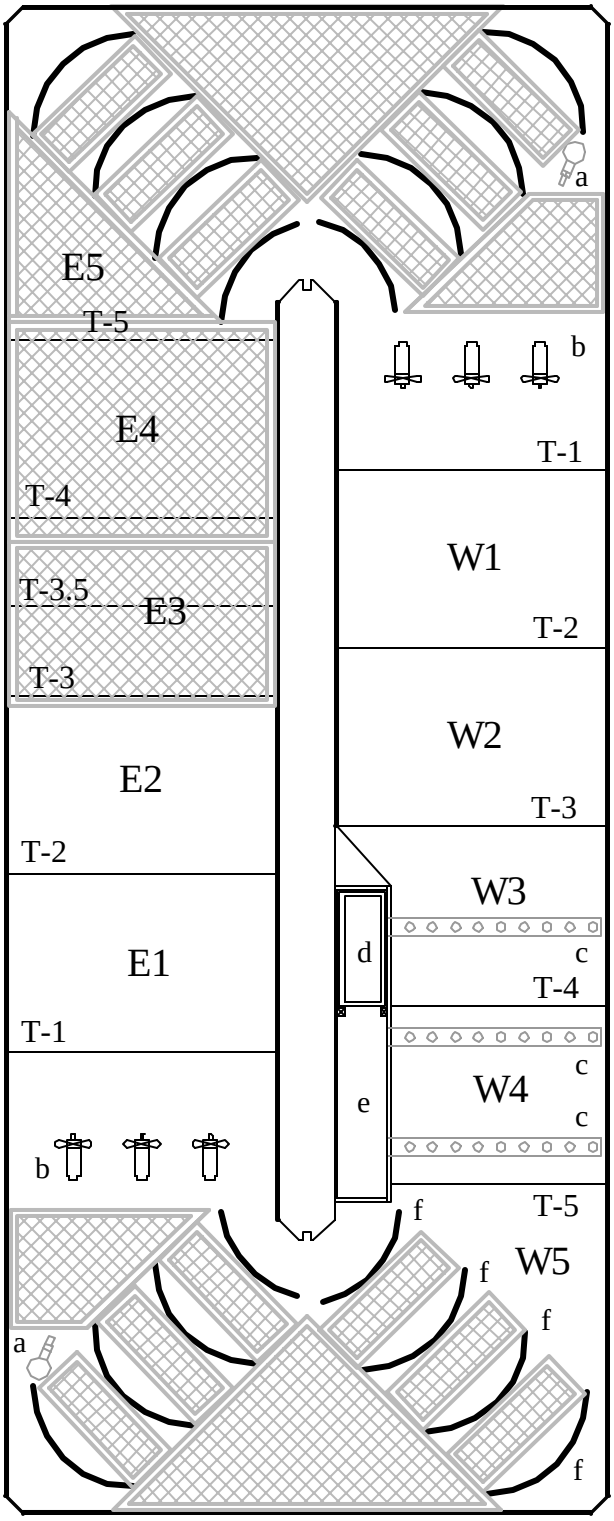


Figure 2

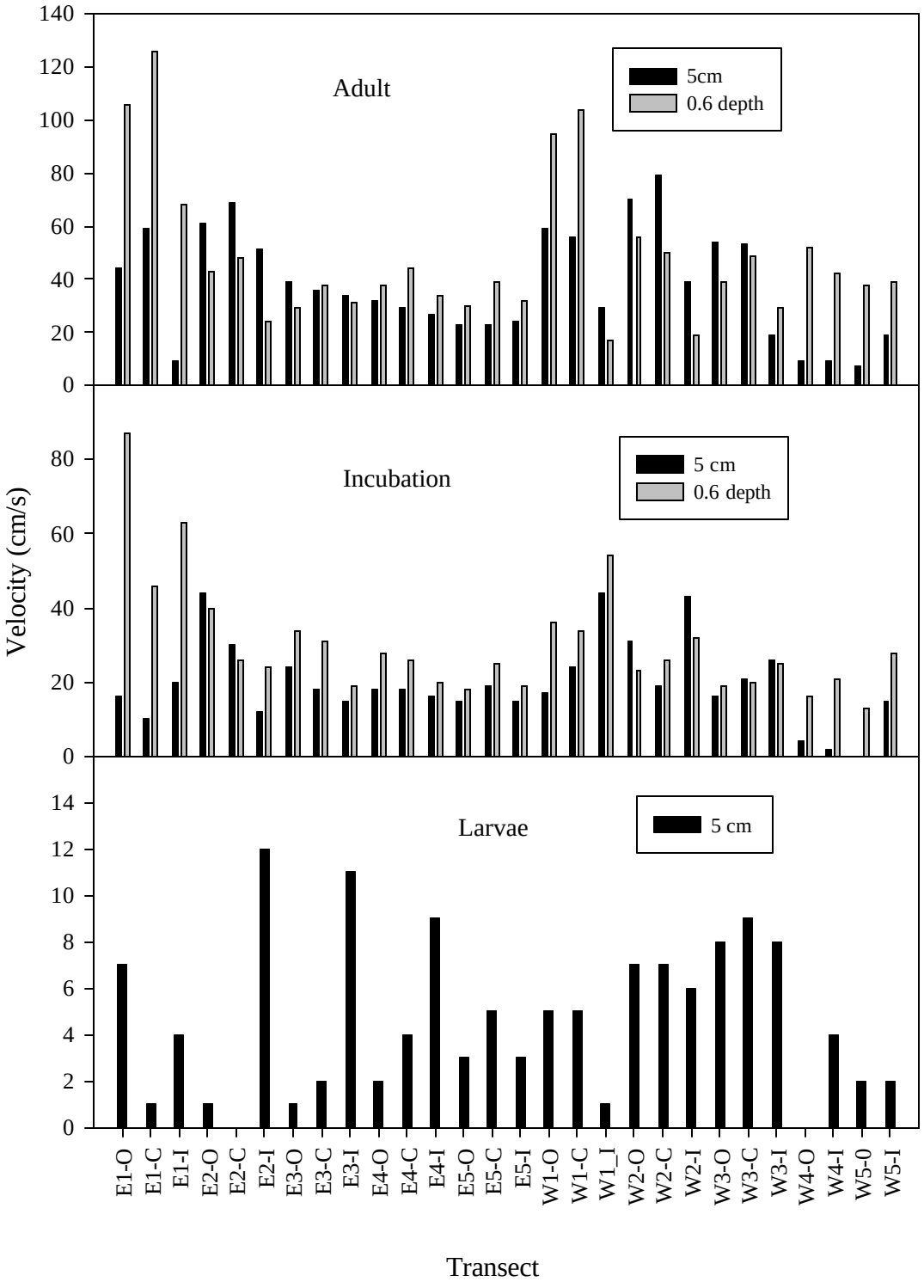

Adult
5cm
0.6 depth
Incubation
5 cm
0.6 depth
Larvae
5 cm
Velocity (cm/s)
Transect
E1-O
E1-C
E1-I
E2-O
E2-C
E2-I
E3-O
E3-C
E3-I
E4-O
E4-C
E4-I
E5-O
E5-C
E5-I
W1-O
W1-C
W1_I
W2-O
W2-C
W2-I
W3-O
W3-C
W3-I
W4-O
W4-I
W5-0
W5-I

Figure 3

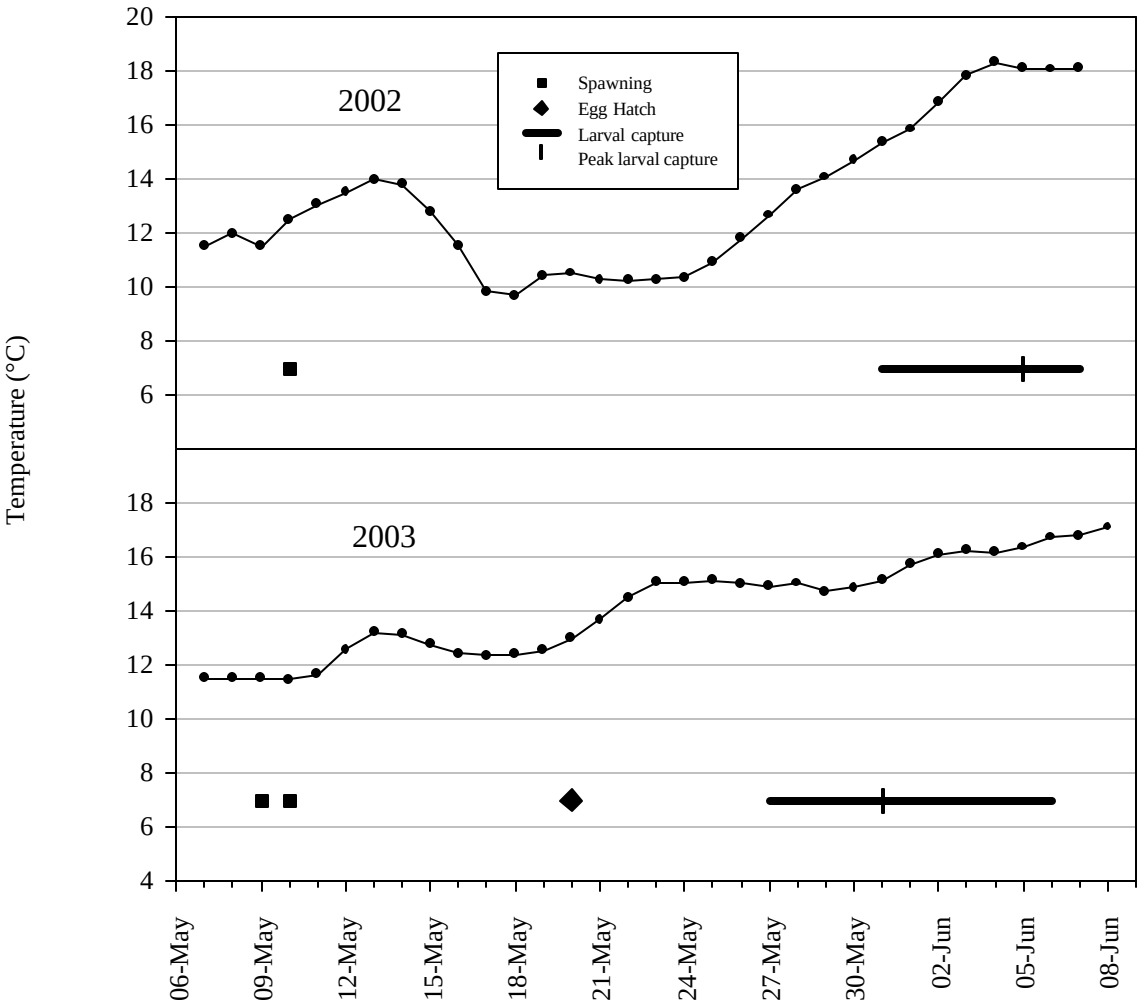

Temperature (°C)
2002
2003
Spawning
Egg Hatch
Larval capture
Peak larval capture
20
18
16
14
12
10
8
6
4
06-May
09-May
12-May
15-May
18-May
21-May
24-May
27-May
30-May
02-Jun
05-Jun
08-Jun

Figure 4

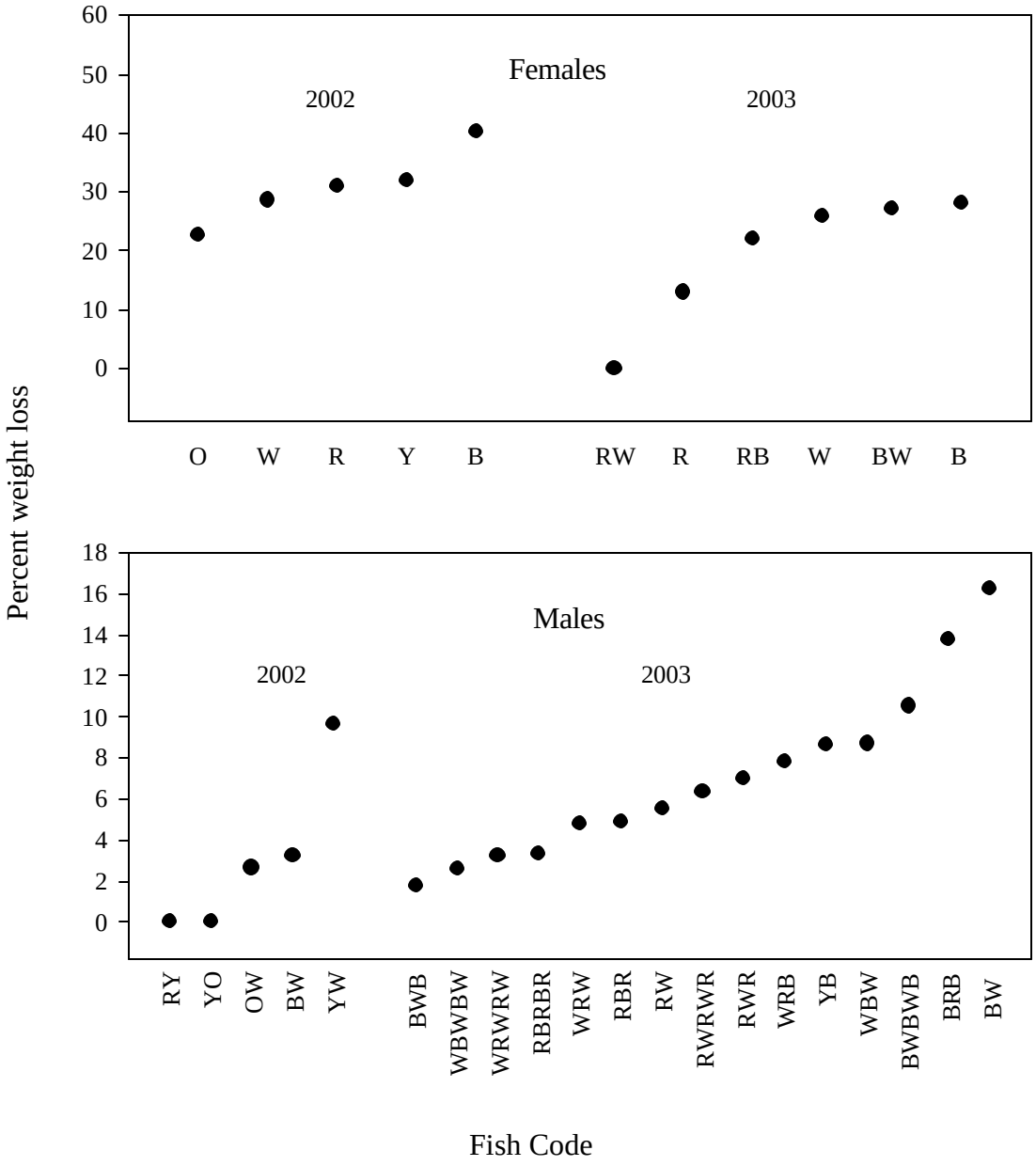

Females
2002
2003
60
50
40
30
20
10
0
O
W
R
Y
B
RW
R
RB
W
BW
B
Males
2002
2003
18
16
14
12
10
8
6
4
2
0
RY
YO
OW
BW
YW
BWB
WBWBW
WRWRW
RBRBR
WRW
RBR
RW
RWRWR
RWR
WRB
YB
WBW
BWBWB
BRB
BW
Percent weight loss
Fish Code

Figure 5

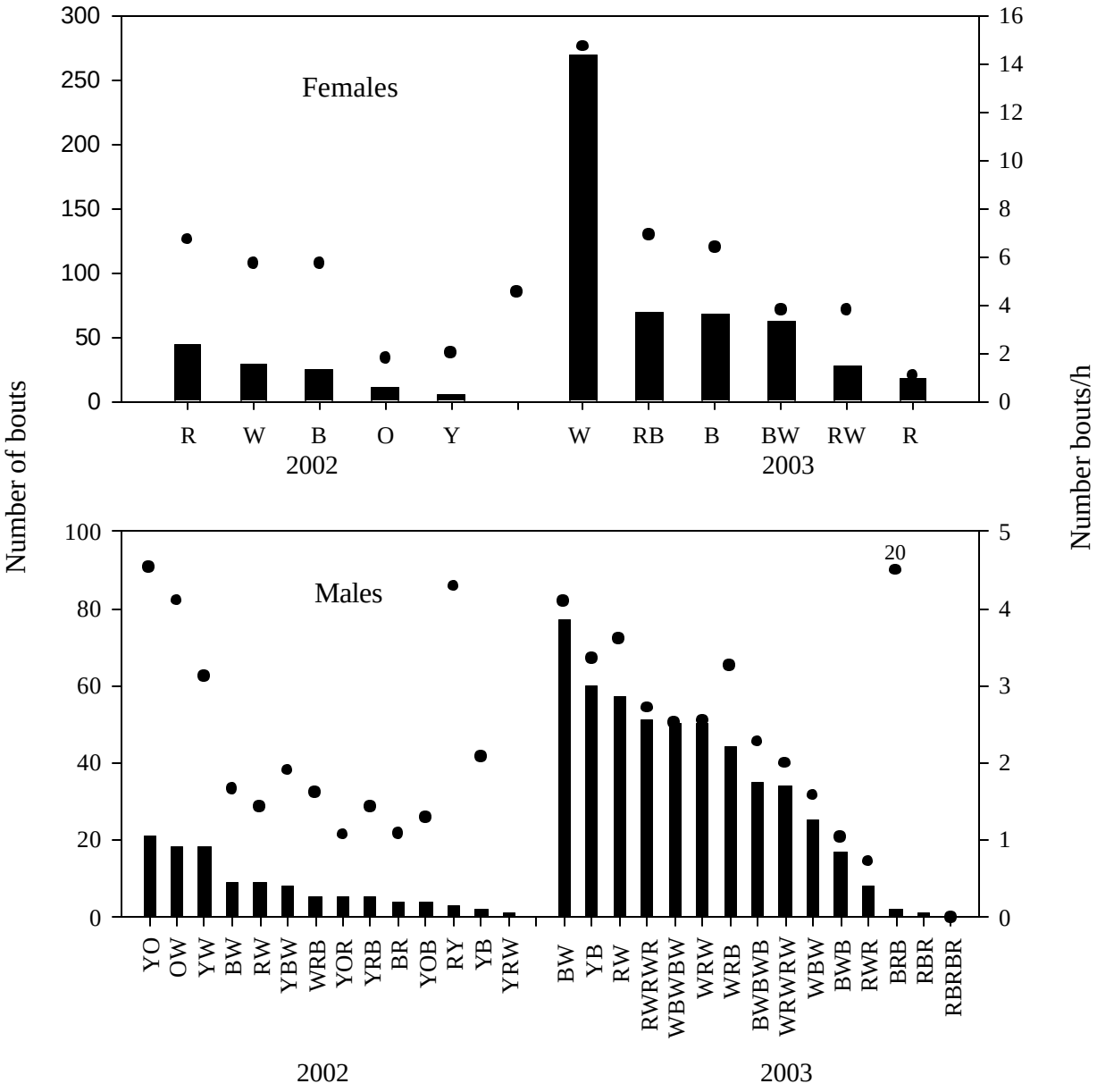

Females
Males
Number of bouts
Number bouts/h
300
250
200
150
100
50
0
16
14
12
10
8
6
4
2
0
R W B O Y W RB B BW RW R
2002
2003
100
80
60
40
20
0
5
4
3
2
1
0
20
YO OW YW BW RW YBW WRB YOR YRB BR YOB RY YB YRW
BW YB RW RWRWR WBWBW WRW WRB BWBWB WRWRW WBW BWB RWR BRB RBR RBRBR
2002
2003

Figure 6

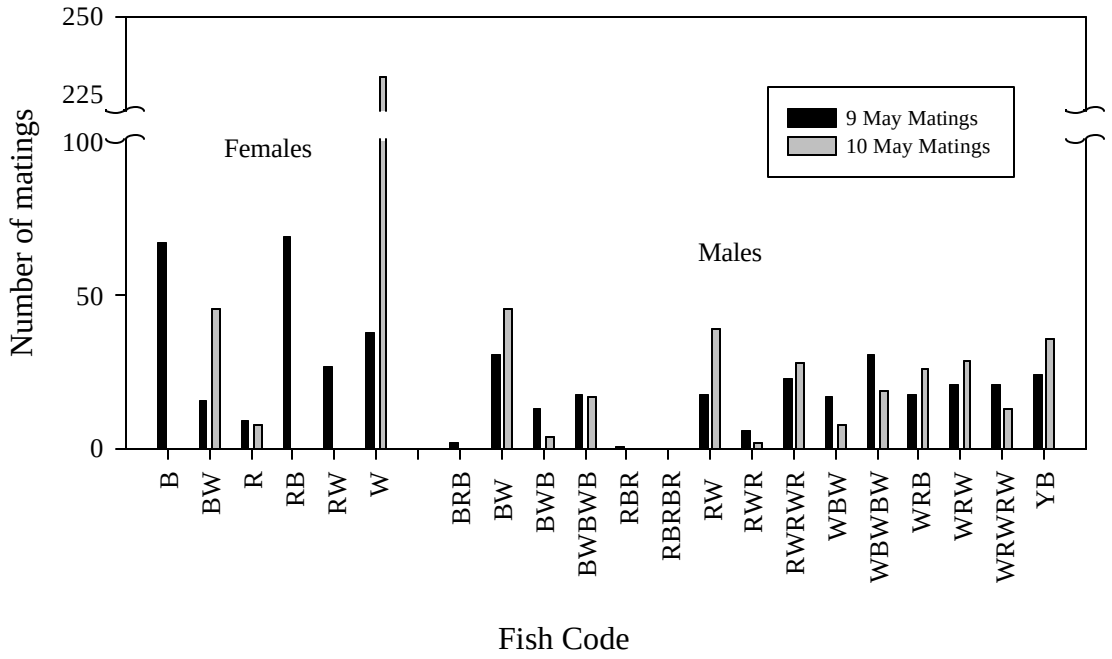

250
225
100
50
0
Number of matings
Females
Males
9 May Matings
10 May Matings
B
BW
R
RB
RW
W
BRB
BW
BWB
BWBWB
RBR
RBRBR
RW
RWR
RWRWR
WBW
WBWBW
WRB
WRW
WRWRW
YB
Fish Code

Figure 7

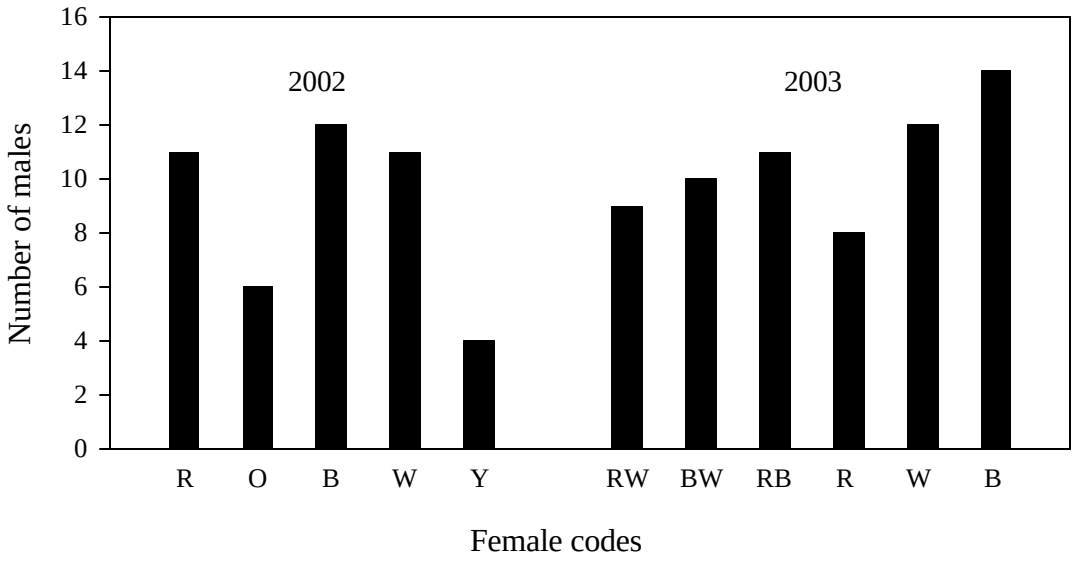

16
14
12
10
8
6
4
2
0
Number of males
2002
2003
R
O
B
W
Y
RW
BW
RB
R
W
B
Female codes

Figure 8

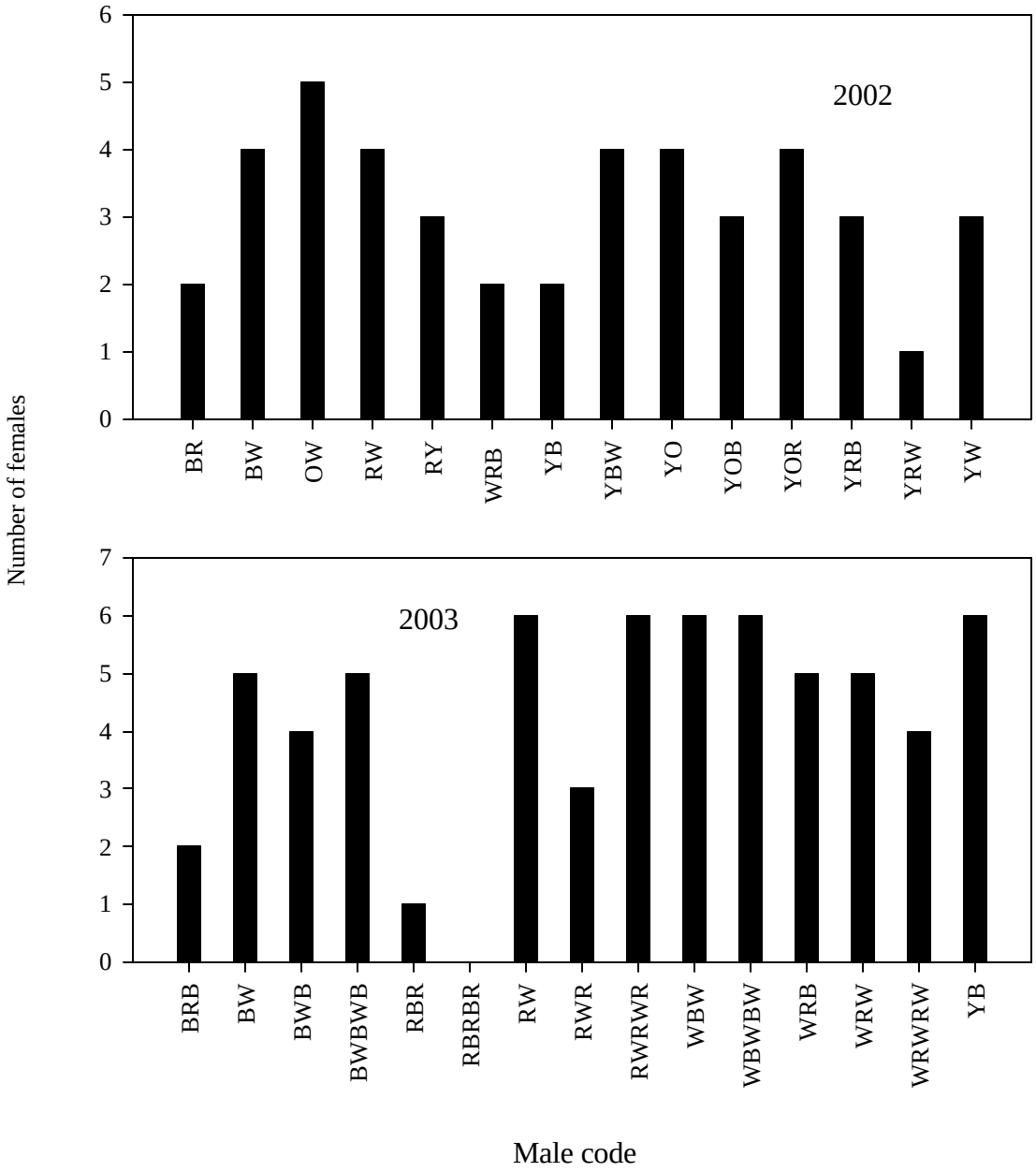

2002
2003
Number of females
Male code
BR
BW
OW
RW
RY
WRB
YB
YBW
YO
YOB
YOR
YRB
YRW
YW
BRB
BW
BWB
BWBWB
RBR
RBRBR
RW
RWR
RWRWR
WBW
WBWBW
WRB
WRW
WRWRW
YB

Figure 9

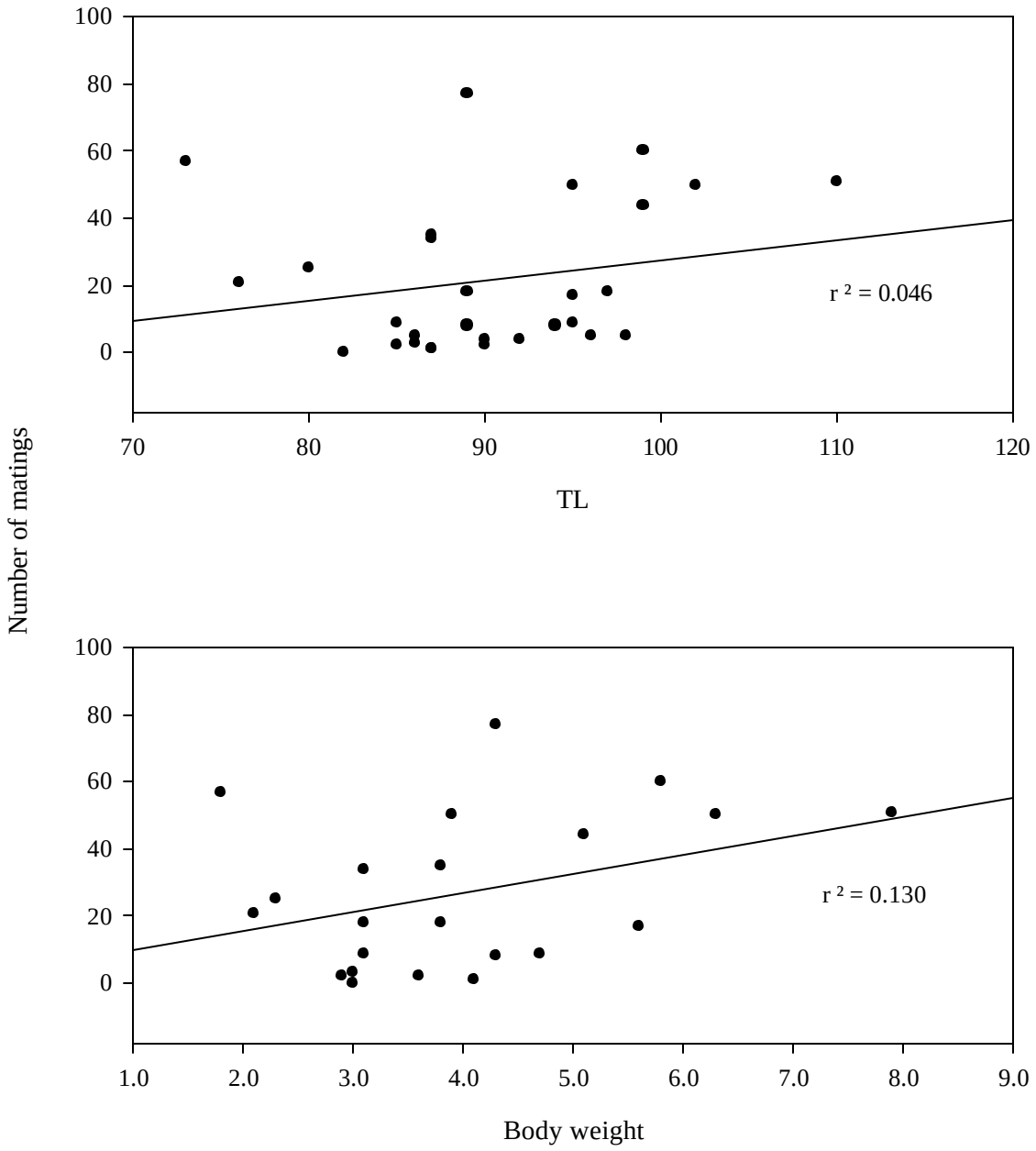

Number of matings
100
80
60
40
20
0
70
80
90
100
110
120
TL
r ² = 0.046
100
80
60
40
20
0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
9.0
Body weight
r ² = 0.130

Figure 10

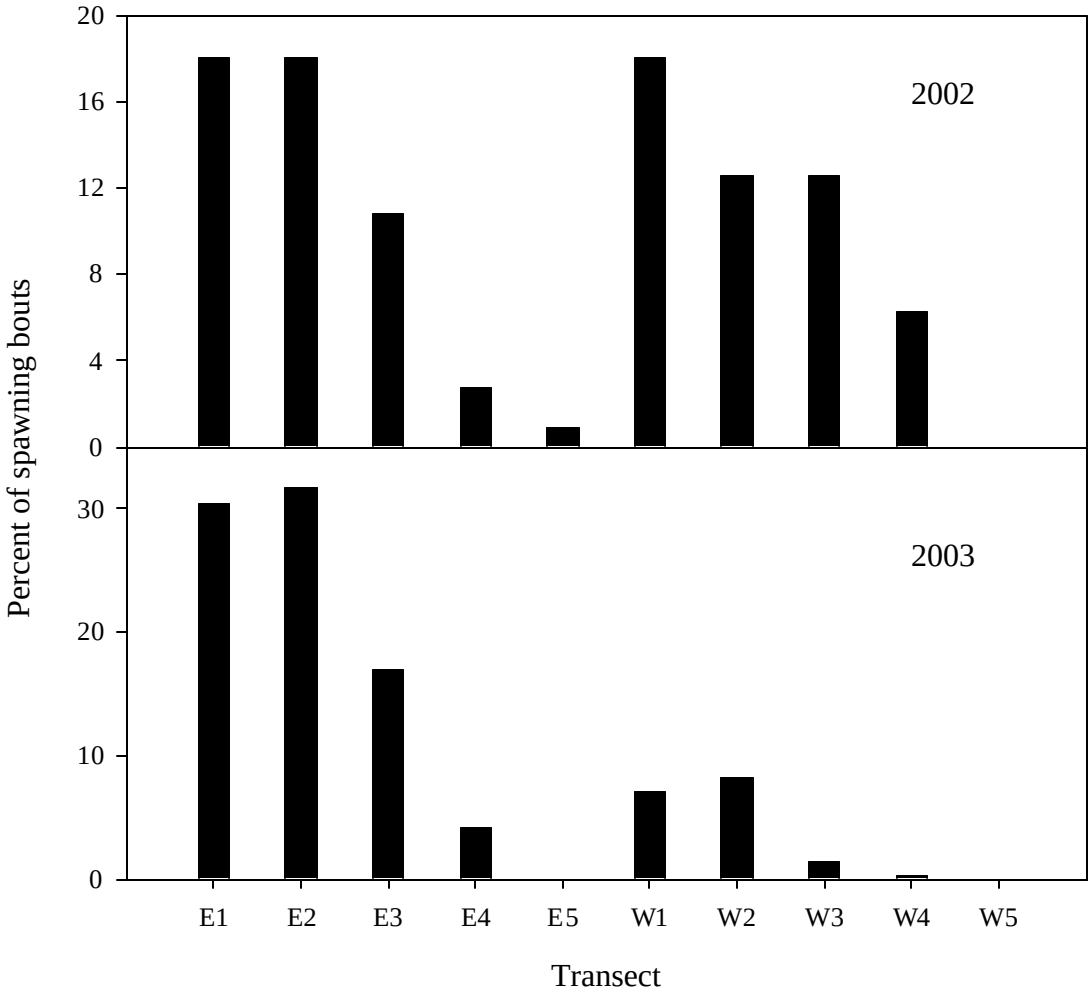

Percent of spawning bouts
20
16
12
8
4
0
2002
30
20
10
0
2003
E1
E2
E3
E4
E5
W1
W2
W3
W4
W5
Transect

Figure 11

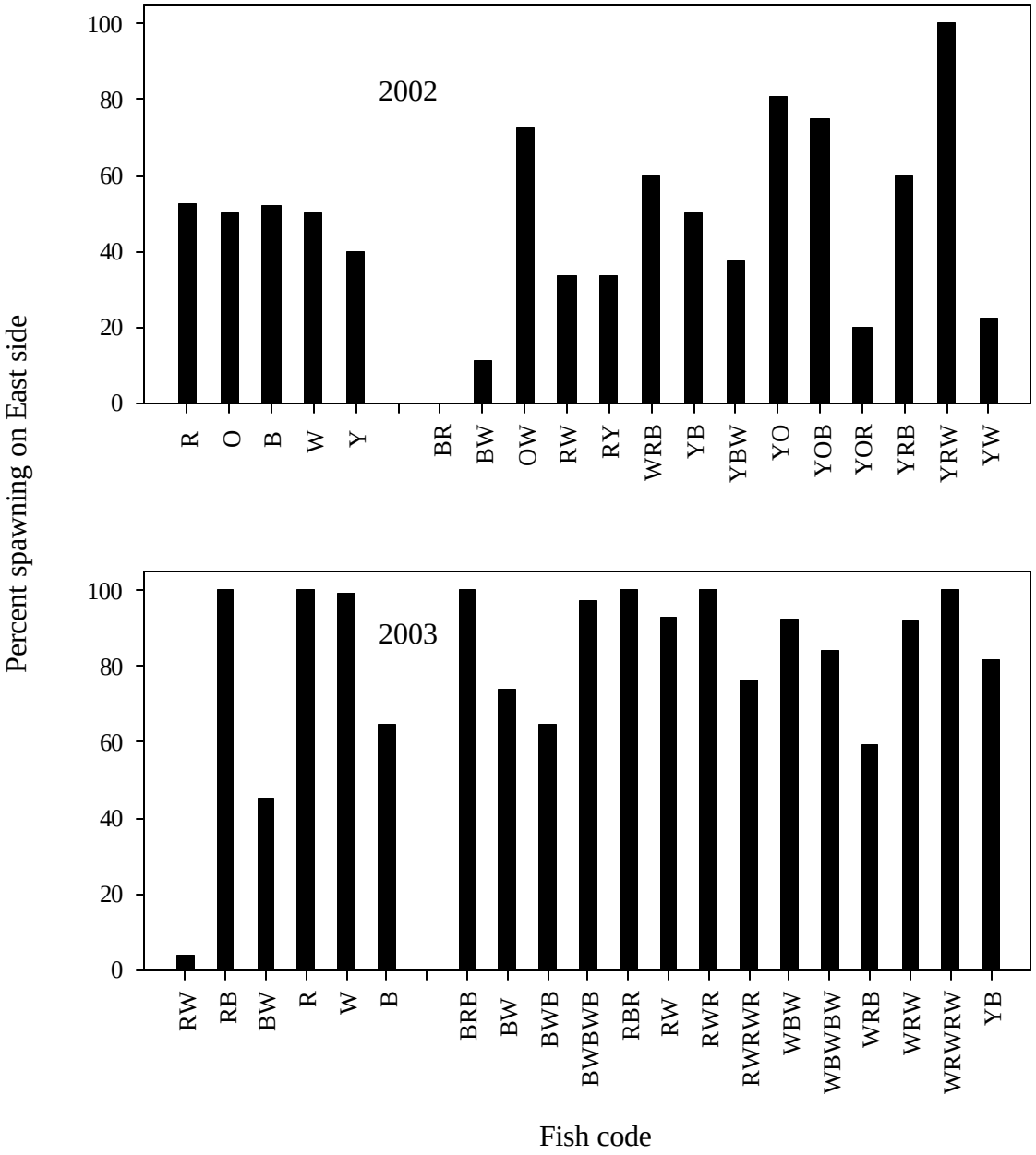

Percent spawning on East side
2002
100
80
60
40
20
0
R
O
B
W
Y
BR
BW
OW
RW
RY
WRB
YB
YBW
YO
YOB
YOR
YRB
YRW
YW
2003
100
80
60
40
20
0
RW
RB
BW
R
W
B
BRB
BW
BWB
BWBWB
RBR
RW
RWR
RWRWR
WBW
WBWBW
WRB
WRW
WRWRW
YB
Fish code

Figure 12

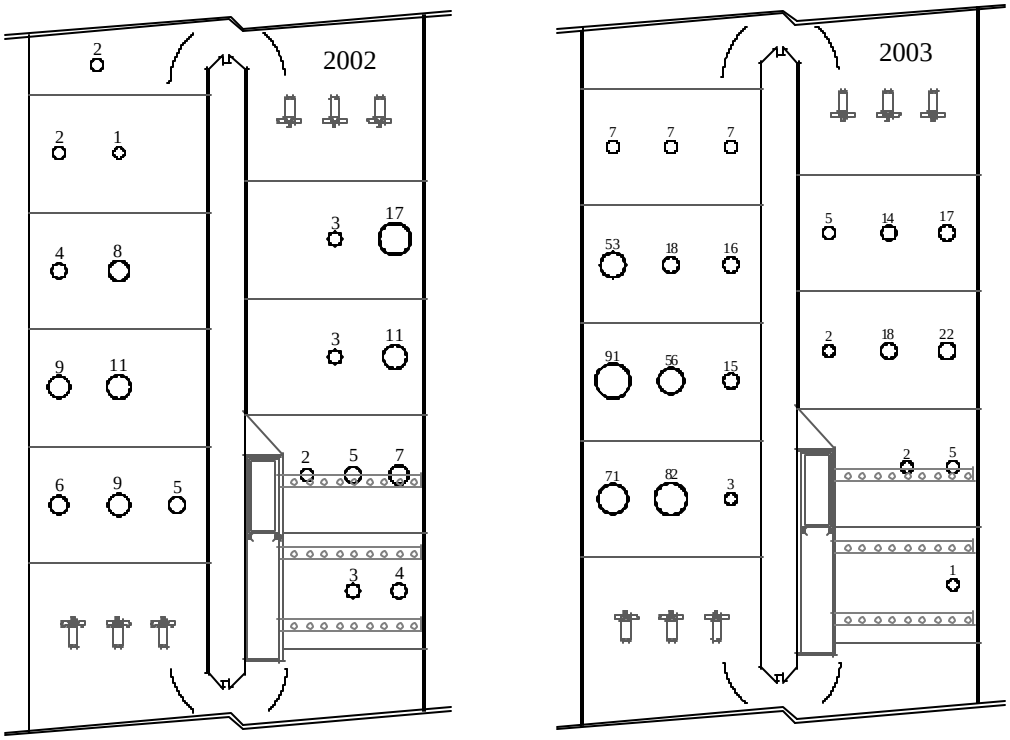

2002
2
2 1
3 17
4 8
3 11
9 11
2 5 7
6 9 5
3 4
2003
7 7 7
5 14 17
53 18 16
2 18 22
91 56 15
2 5
71 82 3
1

Figure 13

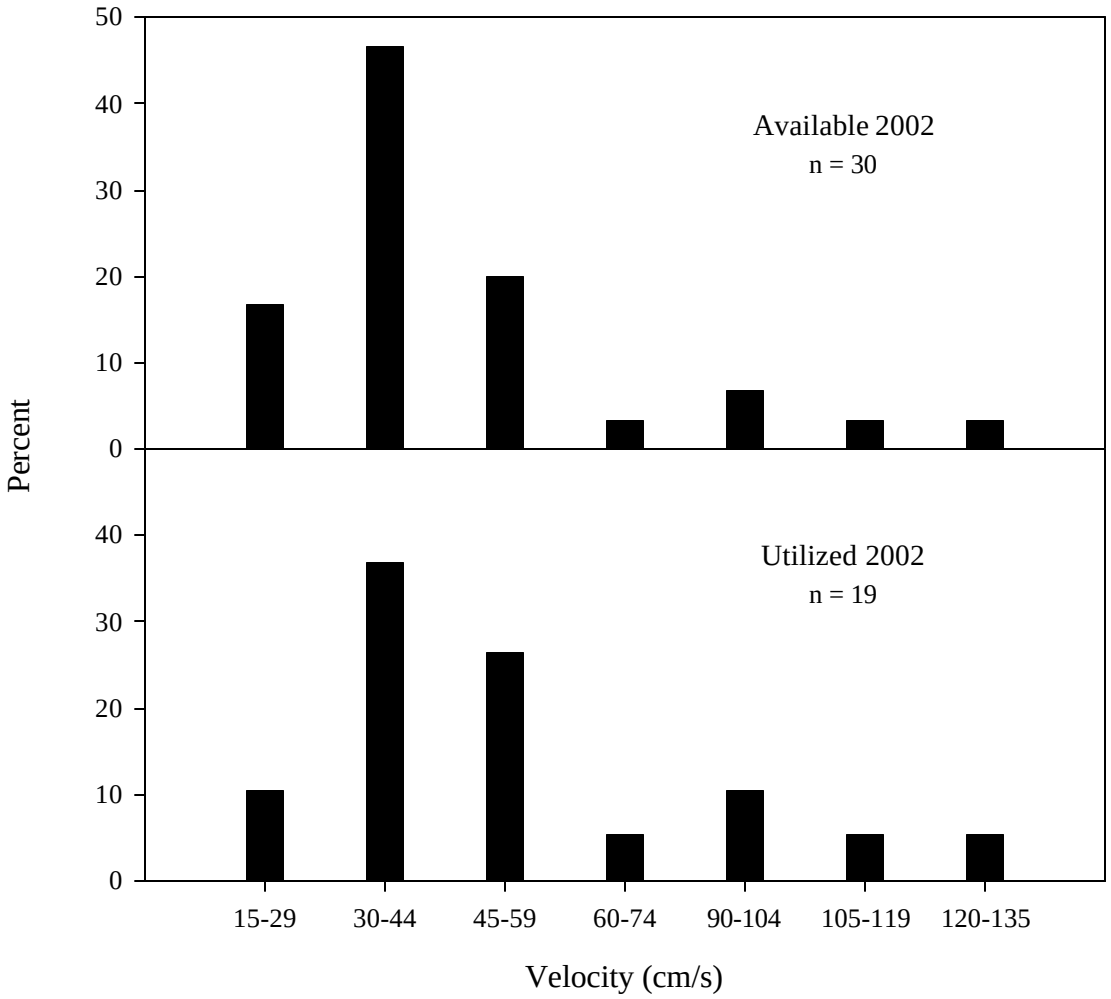
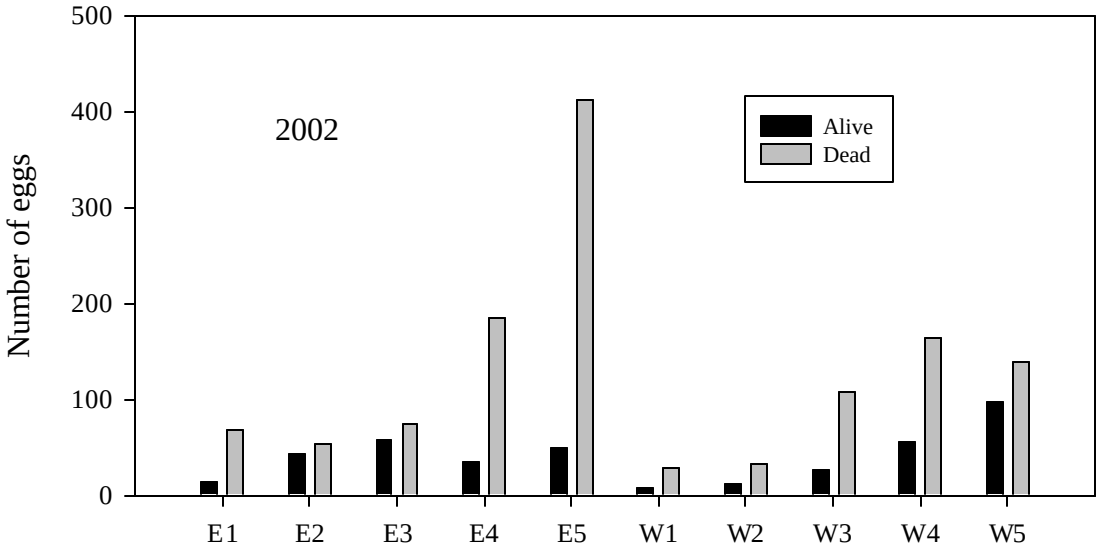

Available 2002
n = 30
Utilized 2002
n = 19
Percent
50
40
30
20
10
0
15-29
30-44
45-59
60-74
90-104
105-119
120-135
Velocity (cm/s)

Figure 14


2002
Alive
Dead
Number of eggs
500
400
300
200
100
0
E1
E2
E3
E4
E5
W1
W2
W3
W4
W5

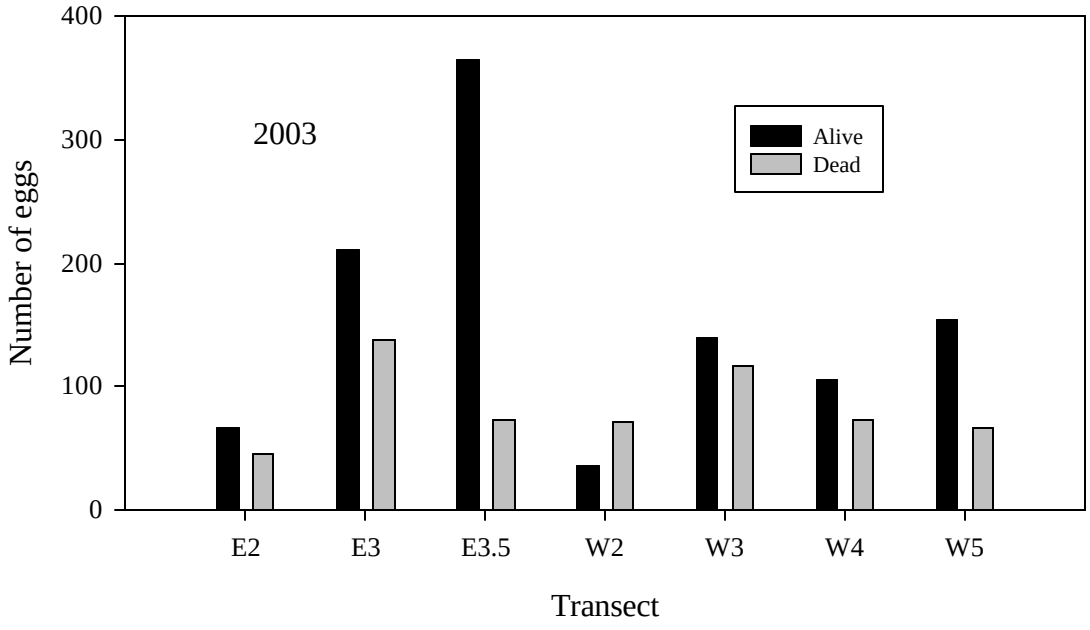

2003
Alive
Dead
Number of eggs
400
300
200
100
0
E2
E3
E3.5
W2
W3
W4
W5
Transect

Figure 15

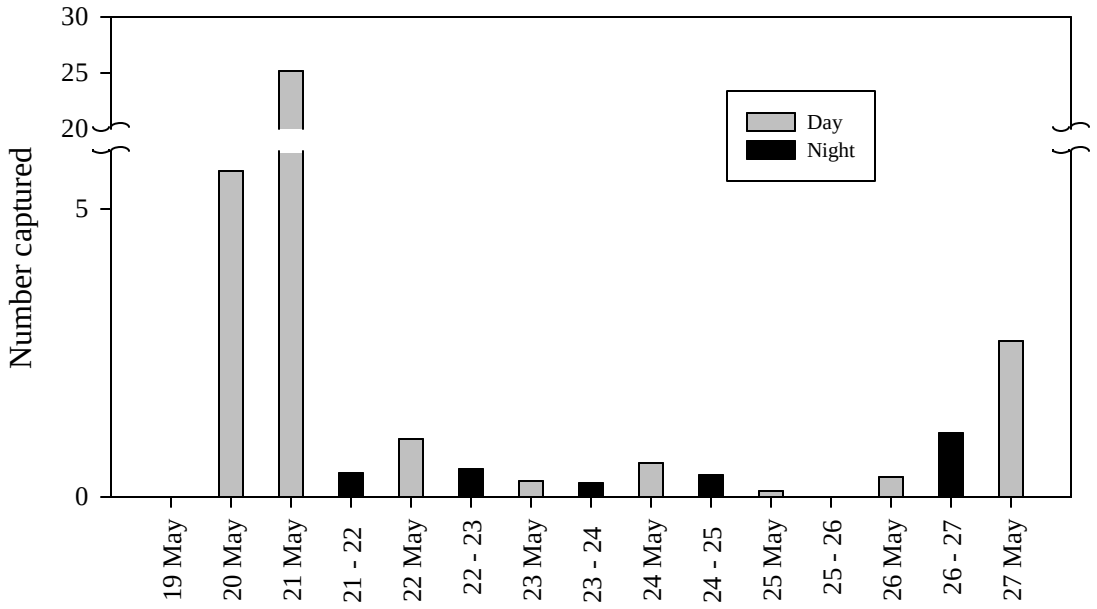

30
25
20
5
0
Number captured
Day
Night
19 May
20 May
21 May
21 - 22
22 May
22 - 23
23 May
23 - 24
24 May
24 - 25
25 May
25 - 26
26 May
26 - 27
27 May

Figure 16

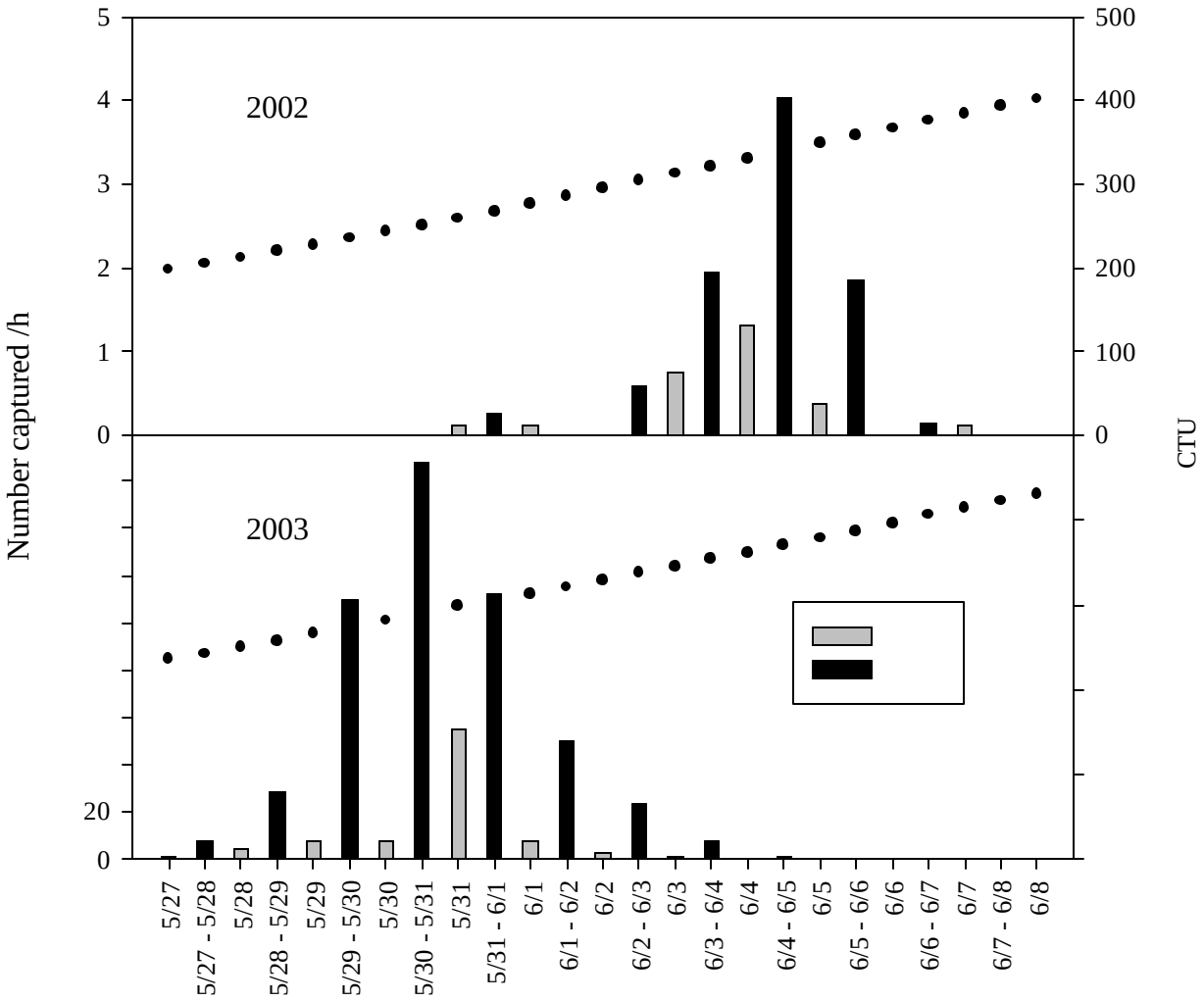

2002
2003
Number captured /h
CTU
5
4
3
2
1
0
500
400
300
200
100
0
20
0
5/27
5/27 - 5/28
5/28
5/28 - 5/29
5/29
5/29 - 5/30
5/30
5/30 - 5/31
5/31
5/31 - 6/1
6/1
6/1 - 6/2
6/2
6/2 - 6/3
6/3
6/3 - 6/4
6/4
6/4 - 6/5
6/5
6/5 - 6/6
6/6
6/6 - 6/7
6/7
6/7 - 6/8
6/8

Table 1. Summary table of adult shortnose sturgeon used in spawning tests during 2002 and 2003.

Test Year	Sex	Tag Color	Population segment [4]	Mean (range) of winter temperature [5]	TL (cm)	Weight (kg)		
						pre-spawn	post-spawn	Loss
2002	F [1]	B	Down	9.3 (6.5 11.0)	97.0	8.2	4.9	3.3
	F [1]	O	Down	9.3 (6.5 11.0)	114.0	10.2	7.9	2.3
	F [1]	R	Down	9.3 (6.5 11.0)	114.0	8.1	5.6	2.5
	F [2]	W	Up	9.6 (6.5 - 11.5)	87.0	3.5	2.5	1.0
	F [2]	Y	Up	9.6 (6.5 - 11.5)	83.0	2.5	1.7	0.8
	M	BR	Down	river [6]	90.0		2.8	
	M	BW	Down	river [6]	85.0	3.1	3.0	0.1
	M	OW	Down	river [6]	97.0	3.8	3.7	0.1
	M	RW	Down	river [6]	95.0	4.7	4.5	0.2
	M	RY	Down	river [6]	86.0	3.0	3.0	0.0
	M	WRB	Down	river [6]	86.0		2.4	
	M	YB	Up	river [6]	90.0	3.6	3.4	0.2
	M	YBW	Up	river [6]	94.0		3.4	
	M	YO	Down	river [6]	76.0	2.1	2.1	0.0
	M	YOB	Up	river [6]	92.0		2.8	
	M	YOR	Up	river [6]	98.0		4.1	
	M	YRB	Up	river [6]	96.0		3.5	
	M	YRW	Up	river [6]	87.0		2.6	
	M	YW	Up	river [6]	89.0	3.1	2.8	0.3
2003	F [2]	B	Down	9.2 (6.8 - 11.0)	91.0	3.9	2.8	1.1
	F [2]	BW	Down	9.2 (6.8 - 11.0)	110.0	9.6	7.0	2.6
	F [1]	R	Down	9.2 (6.8 - 11.0)	84.0	3.1	2.7	0.4
	F [3]	RB	Up	ambient [7]	102.0	5.9	4.6	1.3
	F [3]	RW	Up	ambient [7]	99.0	6.5	6.5	0.0
	F [1]	W	Down	9.2 (6.8 - 11.0)	117.0	9.7	7.2	2.5
	M	BRB	Up	river [8]	85.0	2.9	2.5	0.4
	M	BW	Up	river [8]	89.0	4.3	3.6	0.7
	M	BWB	Up	river [8]	95.0	5.6	5.5	0.1
	M	BWBWB	Up	river [8]	87.0	3.8	3.4	0.4
	M	RBR	Up	river [8]	87.0	4.1	3.9	0.2
	M	RBRBR	Up	river [8]	82.0	3.0	2.9	0.1

M	RW	Up	river [8]	73.0	1.8	1.7	0.1
M	RWR	Up	river [8]	89.0	4.3	4.0	0.3
M	RWRWR	Up	river [8]	110.0	7.9	7.4	0.5
M	WBW	Up	river [8]	80.0	2.3	2.1	0.2
M	WBWBW	Up	river [8]	95.0	3.9	3.8	0.1
M	WRB	Up	river [8]	99.0	5.1	4.7	0.4
M	WRW	Up	river [8]	102.0	6.3	6.0	0.3
M	WRWRW	Up	river [8]	87.0	3.1	3.0	0.1
M	YB	Up	river [8]	99.0	5.8	5.3	0.5

[1] Internal tag.

[2] Incision only (control).

[3] No tag or incision.

[4] The up-segment is upstream of Holyoke Dam, down-segment is downstream of Holyoke Dam (river km 140).

[5] All 2002 te ambient temperature and four were held in heated water, 30 Jan-15 Apr.

[6] Ambient temperature in winter 2001-2002: mean = 3.2 (range 0.8-12.3); winter 2002-2003: mean = 2.4 (range, 0.8-7.5).

[7] River temperature for period of heated water: mean = 1.8 (range, 0.7-7.9)

[8] River temperature for period of heated water: mean = 1.9 (range, 0.7-6.9).

Table 2. Number of multiple spawnings by males, 2002 and 2003.

Year	Male Code	Number of multiple matings on day 1 of spawning	Number of multiple matings on day 2 of spawning	Total number of multiple matings
2002	BW	1	-	1
	OW	4	-	4
	RW	1	-	1
	RY	1	-	1
	WRB	1	-	1
	YBW	1	-	1
	YO	4	-	4
	YOB	3	-	3
	YRB	1	-	1
2003	BW	0	4	4
	BWB	1	0	1
	BWBWB	1	5	6
	RW	1	9	10
	RWRWR	2	4	6
	WBWBW	1	3	4
	WRB	0	6	6
	WRW	1	4	5
	YB	1	0	1
	WRWRW	0	5	5
	YB	0	7	7

Table 3. Number of eggs spawned by females in the artifical stream, 2002 and 2003, as estimated . using two methods: total body weight of pre-spawning females [1] and weight loss of post-spawned females [2].

Year	Fish code	Pre-spawning weight (kg)	Number of spawned eggs [1]	Yearly egg Total [1]	Weight loss (kg)	Percent weight loss	Number of spawned eggs [2]	Yearly egg Total [2]
2002	B	8.2	94,300		3.3	40.2	150,150	
	O	10.2	117,300		2.3	22.5	104,650	
	R	8.1	93,150		2.5	30.9	113,750	
	W	3.5	40,250		1.0	28.6	45,500	
	Y	2.5	28,750		0.8	32.0	36,400	
				373,750				450,450
2003	B	3.9	44,850		1.1	28.2	50,050	
	BW	9.6	110,400		2.6	27.1	118,300	
	R	3.1	35,650		0.4	12.9	18,200	
	RB	5.9	67,850		1.3	22.0	59,150	
	RW	6.5	-		0	0.0	-	
	W	9.7	111,550		2.5	25.8	113,750	
				370,300				359,450

[1] Number of eggs based on female weight x 11,500 egg/kg body weight (Dadswell 1979).

[2] Number of eggs based on weight loss x 45.5 ± 7.6 egg/g (Dadswell 1979).

Table 4. Percent survival of eggs to larvae in the artifical stream, 2002-2003.

Year	Number of live and dead eggs in stream [1]	Number of live eggs in stream [1]	Number of larvae captured [2]	Percent larval survival from all eggs [3]	Percent larval survival from live eggs [4]
2002	30,270	7,070	156	0.52	2.21
2003	191,401	68,339	7,935	4.15	11.61

[1] Number estimated by extrapolating transect samples to entire rock-covered area of stream.

[2] Number captured in the box trap.

[3] Number of captured larvae divided by number of live and dead eggs x 100.

[4] Number of captured larvae divided by number of live eggs x 100.