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Courtship and Tank Spawning Behavior of Temperate Basses (Genus *Morone*)

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Abstract.—Special arenas were used to observe and describe courtship and spawning behavior of captive striped bass *Morone saxatilis,* white bass *Morone chrysops,* and white perch *Morone americana.* To induce final gonadal maturation and spawning, fish were either implanted with gonadotropin-releasing hormone analog, injected with human chorionic gonadotropin, or both. Behaviors were videotaped and systematically quantified. Broodfish displayed courtship behavior for at least 5 h before spawning, characterized by one female and from one to five males releasing gametes at the water surface. Spawning lasted about 10 s for striped bass, 5 s for white bass, and less than 1 s for white perch. The best predictor of imminent spawning was a significant increase in male attending behavior, defined as extremely close and continuous following of the female, sometimes contacting her abdominal or vent area with the snout. Around the time of spawning, male striped bass attended females less intensely than did white bass or white perch. Just before and during spawning, male white perch and white bass displayed a stereotypical circling behavior whereas male striped bass did not. In volitional hybridization trials, white perch and white bass hybridized with one another, but striped bass and white bass did not. Electro-olfactogram recordings from juveniles of all three *Morone* species did not reveal sensitivity to any known teleost steroid or prostaglandin pheromone.

Striped bass *Morone saxatilis* and its hybrids with white bass *M. chrysops* (striped bass × white bass and reciprocal cross) support valuable sport and commercial fisheries and a rapidly expanding aquaculture industry in the United States (Whitehurst and Stevens 1990). Striped bass have been produced in hatcheries to restock fisheries, and methods for artificially inducing their final maturation and spawning in tanks are well developed (Smith and Whitehurst 1990; Woods and Sullivan 1993). However, very little is known about normal prespawning and spawning behavior of this and other *Morone* species. More detailed knowledge of these behaviors would contribute to basic understanding of *Morone* reproductive biology and could lead to improved methods for captive spawning, including volitional spawning across species to produce hybrids for aquaculture (Woods et al. 1995).

Prior accounts of *Morone* reproductive behavior are largely anecdotal and incomplete. Most published descriptions of natural striped bass spawning behavior suggest that from 5 to 50 fish are involved in a single spawning event (Worth 1903; Merriman 1941; Woodhull 1947; Raney et al. 1952; Lewis and Bonner 1966). Spawning is said to include one female and many males, usually involving a great disturbance of the water that can be seen from considerable distances. Apparently some physical contact occurs between the fish before spawning; they have been reported to swim erratically and occasionally roll over on their sides while splashing at the surface. Reports of striped bass spawning in hatchery tanks indicate that males aggressively ''court'' the females before spawning, which may include ''observation'' of her vent area with their snouts, ''butting'' her sides, or even occasional biting of her pectoral fins (Bishop 1975; Smith and Whitehurst 1990).

Descriptions of white bass spawning behavior indicate that a single female will spawn with 6–12 males in a ''confused scramble'' and that males often ''bump the abdomen'' of the female before spawning at the water surface (Riggs 1955; Webb and Moss 1967). Descriptions of prespawning behavior are lacking, but observations of actual spawning indicate that it lasts less than 5 s.

Much less is known about spawning behavior of white perch *M. americana.* Prior reports indicate

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that, as spawning approaches, larger individuals are followed by smaller fish. The females are said to approach the water surface where they release eggs, joined by males releasing milt. A single female may spawn two or three times (Mansueti 1961).

Much remains unknown regarding the details of *Morone* courtship and spawning. The primary objective of this study was to describe reproductive behavior of species in this genus in detail, focusing on the sequence of specific behaviors leading up to and including spawning. We also sought to make comparisons of reproductive behavior among striped bass, white bass, and white perch.

Included in these comparisons were volitional hybridization trials to verify whether or not differences in courtship or spawning behavior are associated with failure to hybridize in captivity. Most aquaculture production of *Morone* species is based on hybrids between striped bass and white bass because the hybrid is perceived to be a superior culture stock. Because these two species do not successfully spawn together in captivity (Woods et al. 1995), hybrids must be produced by an arduous process of inducing and detecting ovulation within a narrow window of time so that fertile ova can be collected for in vitro fertilization (Rees and Harrell 1990). Identification of differences in the spectrum or frequency of behaviors associated with failure to hybridize could be the first step toward developing methods for inducing desired behaviors to enable volitional spawning and simplify production of hybrids.

Methods

Experimental animals.—In experiments conducted at the North Carolina State University Pamlico Aquaculture Field Laboratory (PAFL), we used captive striped bass broodstock maintained in outdoor tanks (Hodson and Sullivan 1993; King et al. 1994b; Hodson et al. 2000). At the start of experiments, females weighed 7–12 kg and males 4–5 kg. All of the striped bass had previously matured (spermiated or completed oocyte growth) or spawned in captivity. White perch and white bass were from stocks maintained at PAFL for several generations (Jackson and Sullivan 1995; Hodson et al. 2000). At the time of behavioral testing, the white perch weighed 0.15–0.25 kg and white bass weighed 0.30–0.40 kg. All experiments were conducted in April and May of 1994 and 1995.

Spawning induction.—The maturational status of striped bass females was assessed by ovarian biopsy under anesthesia (King et al. 1994b). In 1994, females had initiated final maturation (King et al. 1994a; 1994b) when they were first biopsied (oocyte stage <15 h; Rees and Harrell 1990). They were induced to complete maturation and spawn with an injection of human chorionic gonadotropin (HCG; 330 IU/Kg body weight). In 1995, females were less mature when initially biopsied. If their maximum oocyte diameter equaled or exceeded 850 μm, females were selected for spawning and implanted with 150 μg of a synthetic analog of human gonadotropin-releasing hormone, des-Gly^{10}, [D-Ala^{6}]-LH-RH Ethylamide (GnRHa), which was pelleted in a matrix of cholesterol and cellulose (Woods and Sullivan 1993). Females were placed in a spawning arena and captured by seine 24 h later for ovarian biopsy. If their oocytes showed significant coalescence of oil droplets (oocyte stage 12–14 h; Rees and Harrell 1990), they were given an HCG injection to induce maturation and spawning (Hodson and Sullivan 1993). All females spawned within 17–48 h after receiving an HCG injection. Male striped bass were spermiating naturally at the start of experiments, but they were given an injection of HCG (165 IU/kg body weight) before each spawning trial to sustain spermiation and reproductive activity.

We assessed the maturational status and induced spawning of female white bass and white perch as described above, except that females were biopsied using small glass microhematocrit tubes and selected for spawning if their maximum oocyte diameter equaled or exceeded 550 μm. The white perch and white bass were induced to spawn with HCG only.

Spawning arenas.—Striped bass spawning trials were conducted in two identical circular outdoor spawning arenas (7 m diameter, 1.5 m deep). These were modified, above-ground fiberglass swimming pools. Each arena had an adjacent observation tower constructed of commercial steel scaffolding with a viewing platform located 5.5 m above the ground. From these platforms we observed and videotaped fish behavior. To make night observations possible, we mounted 10 red 75-W floodlights spaced at equal distances along the top edge of each pool. The fact that fish could be easily touched by hand when reaching into the tank while the red lights were on at night indicated that they could not readily see outside the tank when illuminated. In daylight, observers could never approach or touch the striped bass, which would rapidly swim away from any approach. A system of blue lines painted on the white tank bottom formed a 1.75-m^2 grid used to estimate relative distances between fish. Well water flowed into the pool at a rate of 18 L/min. Water temperature ranged from 18.0°C to 19.5°C, and dissolved oxygen ranged from 6.5 to 8.0 mg/L. Outflow from spawning arenas passed through a standard striped bass egg collector (Smith and Whitehurst 1990) before draining into a nearby ditch. When not observed directly, the approximate time of spawning was estimated from the water temperature and stage of embryo development at recovery, and fecundity and fertility estimates were made for each spawn using established striped bass hatchery procedures (Rees and Harrell 1990).

White perch and white bass tank spawning and hybridization trials were conducted in two, circular tanks (1.8 m diameter, 1.2 m deep) located inside the hatchery at PAFL. Each tank was equipped with observation blind, video camera (described below), two red floodlights, and an egg collector. As for striped bass, water temperature was 18.0–19.5°C and dissolved oxygen was 6.5–8.0 mg/L. Small pieces of PVC pipe scattered on the tank bottom provided spawning substrate. Once eggs were detected, the broodfish were removed and the eggs were allowed to hatch in the tank.

Spawning trials.—Spawning trials were conducted to visually observe and record courtship and spawning behavior for all three species. The three 1994 striped bass spawning trials involved the same group of eight males and eight females in a single arena. Before trial I, all of the males and two of the females were injected with HCG as described above. Fish behavior was observed or recorded starting 18 h after administration of HCG and ending 24 h after the last (usually second) female spawned. All fish were removed from the arena 48 h later, and the two females previously injected with HCG were removed from the study. All of the males and two of the remaining six females were then injected with HCG and returned to the arena for trial II. This same procedure was repeated for trial III.

In April of 1995, five more striped bass spawning trials were conducted as before, except that only two striped bass females per trial were placed in the spawning arena and males were not reused in successive trials. In each trial, one female was implanted with GnRHa and later injected with HCG as described above, whereas the second (control) female was not implanted or injected with hormones.

Striped bass were not marked with external tags before being placed into spawning arenas. Weighing 3–8 kg less, males could be easily distinguished from females, although we could not identify individual males. Females were selected that were slightly different in size (1–2 kg) from one another so that control fish could easily be distinguished from hormone-treated spawners.

In August of 1994, striped bass behavior in the spawning arenas was observed outside the natural spawning season. To document behavior not associated with spawning, two trials were conducted as described above for 1994, except that the fish were not implanted or injected with hormones.

We conducted seven tank-spawning trials with white perch and with white bass. Each trial involved one female and four to seven males. There were no control females in the arena. Females weighed 0.1 kg more than males, so they could easily be recognized by observers. The fish were placed into the spawning tanks after receiving HCG injections and remained there for up to 72 h

Trials of volitional hybridization between the three *Morone* species were conducted. In four separate trials, four female white bass and seven small spermiating striped bass males (weight about 1 kg) were placed in a tank and allowed to interact for 3 d after HCG injection. White bass and white perch were placed together tanks for three separate hybridization trials. Two trials involved one or two white perch females and four or six white bass males, and one trial involved a white bass female and seven white perch males.

Behavioral observations.—In the 1994 spawning trials, preliminary observations of behavior were recorded using an audio tape recorder, handwritten notes, and a VHS video camera (Panasonic model ag-187u) equipped with a standard zoom lens (Panasonic model Power Zoom Lens -X12). These observations and recordings were used to construct an ethogram of specific female and male prespawning and spawning behaviors.

In 1995 spawning trials I and II, an observer recorded the frequency of typical prespawning behaviors (noted during 1994 experiments) that occurred during the interval between implantation of the female with GnRHa and the time when she was injected with HCG. This interval varied between 24 and 96 h, depending on how quickly the GnRHa implants stimulated oocyte maturation. Each day, the observer would record behavior for several 1-h periods during both the day and night. These recordings began 24 h after GnRHa implantation. These initial observations were conducted to provide a baseline of reproductive activity against which later behavior could be compared.

Each 1995 trial was videotaped as before, except that the camera was refitted with a wide-angle lens (Panasonic model Super Wide Pro 5050–0.5x) and polarizing filter (Tasco) and the field of view was adjusted to simultaneously record fish activity over the entire spawning arena. Videotaping began 18 h after administration of HCG to the female and continued for 6 h after a spawn, weather permitting.

Three white bass spawning trials and two white perch spawning trials were filmed as described above for the 1995 spawning trials and used for behavioral quantification. The remainder of the spawning trials were used to describe reproductive behavior of these species.

Two of the four white bass × striped bass hybridization trials were videotaped and analyzed as described above for the 1995 spawning trials. The other two trials were neither videotaped nor observed directly. The three hybridization trials conducted using white perch and white bass were not videotaped or observed due to time constraints and camera malfunction.

Quantification of specific behaviors.—Videotapes were viewed to identify segments containing apparent courtship and spawning behaviors. The time interval from 5 h before a spawn to 6 h after a spawn (11 h total) was selected for more detailed analysis, quantification, and statistical evaluation of reproductive behavior associated with spawning. We quantified levels and changes of specific behaviors using a Tandy M100 laptop computer and the Keyboard behavioral program (J. Baylis, University of Wisconsin). For striped bass, we selected five, 20-min segments of videotape per spawning trial for viewing and quantification of reproductive behaviors. We chose 20 min for the length of each viewing segment because it was long enough to verify the frequency of specific behaviors and enable statistical comparisons between time periods. The 20-min segments fell randomly within the following periods: 5–4 h before spawning (−5), 2–1 h before spawning (−2), 10 min before to 10 min after spawning (sp), 1–2 h after spawning (+2), and 5–6 h after spawning (+6). Each segment was viewed in its entirety at normal (real time) speed four times, once each focusing on the following fish or groups of fish: (1) spawning female, (2) nonspawning (control) female, (3) males located less than 2 m from the spawning female, and (4) males located less than 2 m from the control female.

White bass, white perch, and hybridization spawning trials were evaluated similarly, except that there was no control female. Viewing and evaluation of videotaped white bass and white perch hybridization trials was done as described above, except that data from 10-min segments were used. Three white bass and two white perch trials were used to evaluate behavioral changes associated with courtship and spawning. Hybridization trials between striped bass and white bass were viewed, but not quantified because duration of specific courtship behaviors was very low.

The unit of observation used for statistical analyses of the data for each type of behavior was cumulative seconds of this behavior observed per 10-min or 20-min viewing segment (seconds/segment). The mean (±SE) data for specific behaviors were converted to percent time for graphical presentation.

Electro-olfactogram recording.—Because some aspects of attending behavior suggested that males might be detecting substances released from the female urogenital tract, electro-olfactogram (EOG) readings were taken from *Morone* fingerlings of each of the three species exposed to potential pheromones. The set of prostaglandins (tested at 10 nM) and steroids (tested at 1 and 10 nM) used as stimuli for EOG recordings included all known and potential pheromones shown in earlier studies (reviewed by Stacey and Cardwell 1997) to be detected by the olfactory systems of other fishes (Salek 2000). The fingerlings were produced in April and May 1995 at the PAFL and shipped by air to the University of Edmonton, Alberta, for testing in December of 1995 by one of us (N.E.S.). Employing apparatus, procedures, and fish holding systems described by Cardwell et al. (1995), the EOG recording was used to assess olfactory responsiveness to eight prostaglandins (Cayman Chemical, Ann Arbor MI) and a large number (N = 111) of steroids and steroid conjugates (Sigma, St. Louis MO; Steraloids, Newport, Rhode Island; see Results). All fish (two white perch, two white bass, and one striped bass) were subjected to EOG recording procedures as subyearling (phase I) fingerlings (total length 50–149 mm).

Statistical analyses.—Using JMP statistical software (SAS Institute, Cary, North Carolina) for the 1995 striped bass spawning trials, we compared control female and spawning female behaviors, as well as male behaviors relative to these females. Behavioral data were analyzed by repeated-measures analysis of variance (ANOVA) with female as the between-subjects effect and time as the within-subject effect. The effects in the ANOVA model included spawning trial, female, time, time by female, and time by trial. We performed univariate repeated-measures analyses following verification of *H*-type covariance structure using the JMP sphericity test (Huynh and Feldt 1970; Latour and Miniard 1983). One-tailed *t*-tests were used for female effects because it was expected that spawning females would show a higher degree of prespawning and spawning behavior than control females. Striped bass trial V was excluded from analyses. Data from trial III did not include information for periods + 2 and + 6. Therefore, behavioral data from these two periods could not be analyzed using the repeated-measures ANOVA model because of the missing data and insufficient degrees of freedom. When the ANOVA indicated a significant time effect for a specific behavior, a posteriori contrasts (JMP least squares method) were performed for the remaining periods (−5, −2, and sp) to assess whether there were significant increases or decreases in specific behaviors leading up to spawning.

The white bass trials were analyzed for changes over time by one-way ANOVA. Statistical analyses of white perch data were not performed because the sample size was too low ($N = 2$). We did not perform statistical analyses on hybridization experiments because of the low level of interaction between males and females.

Results

Striped Bass

Adult male and female striped bass observed outside the spawning season (August) did not interact in any consistent or quantifiable way (data not shown). During the 1995 spawning trials, females observed from the time of HCG injection until 24 h before spawning tended to hover motionlessly or swim alone, whereas striped bass males tended to school together. Striped bass females would occasionally join these schools for short periods, but few clear interactions between females and males were apparent.

Commencing a few hours before spawning, striped bass displayed a suite of characteristic and quantifiable reproductive behaviors (described in Table 1, shown in Figure 1). Some common reproductive behaviors, such as leading, following, and in pack commenced as early as 15 h before spawning. Other frequent reproductive behaviors, such as attending, commenced as early as 5 h before spawning. The remaining behaviors (chase, shimmy, face-to-face, and side-to-side) occurred at a very low frequency and only within 1 h of spawning. A summary of the 1994 and 1995

TABLE 1.—Ethogram of striped bass spawning behaviors.

Behavior	Description
Female behaviors	
Lead pack	Female swimming ahead of two or more males, males remaining behind the female (Figures 1E, 1F).
Follow pack	Female swimming less than 1 m behind two or more males and changing direction to follow males as they change direction (Figure 1B).
In pack	Female swimming with males both in front of and behind her. Sometimes males swim alongside the female (Figure 1F).
Swim alone	Female swimming or stationary, not following or leading pack; no other fish within 1 m.
Motionless	Swimming female stops forward motion and hovers.
Headstand	Female motionless, head down and tail up, in a headstand posture. This often occurs at spawning and sometimes afterwards. The female is generally accompanied by males (Figure 1C).
Shimmy	Female stops forward motion and undulates body left and right, often in a headstand posture. This is part of the spawning act and generally occurs while males are alongside her making body contact (Figure 1C).
Spawn	Female begins by hovering motionless, often goes into a headstand posture, shimmies violently from side to side with all fins erected, but remains stationary by ''sculling'' backward with her pectoral fins. Her tail is oriented upward and sometimes out of the water, creating a mixing disturbance at the surface. The female releases one long continuous cloud of eggs for less than 10 s. This can be repeated as many as three times in an hour, but the first occurrence involves the major egg release. During this time, many males contact her and may turn her in a circle by physically pushing on her body (Figure 1D).
Flash	Female rapidly rolls over on one side and then quickly returns to a normal swimming posture.
Male behaviors	
Follow	Male swims less than 1 m behind a female, changing direction as she does (Figure 1F).
Attend	Extremely close following behavior with the male's snout repeatedly contacting the female's abdominal area close to the urogenital pore. Female makes no attempt to escape (Figures 1A and 1E).
Chase	Male approaches the female from behind and attends her. The female then swims away rapidly with the male in pursuit.
Flash	Male rapidly rolls over on one side and then quickly returns to a normal swimming posture.
Face-to-face	Male approaches a slowly moving female, head-on or from the side, places head against the female, and often begins to shimmy (Figure 1B).
Side-to-side	Male approaches slowly moving female, head-on or from behind, comes alongside her, places his body against her and often begins to shimmy (Figure 1A).

TABLE 1.—Continued.

Behavior	Description
Spawning	Males approach female from the side or behind, contact the female side-to-side or face-to-face, shimmy, and release milt. Their dorsal fins are erect and they sometimes flash as they release milt. Males often will turn the female in a circle by pushing against her as she releases eggs (Figure 1D).

striped bass spawning trials with regard to the stage of female maturity at the start of the trial, the time of spawning, and the fecundity and fertility of spawns is shown in Table 2.

In striped bass spawning trials, individual spawning acts lasted 5–10 s. The first spawning was always the longest and produced the largest gamete cloud. For all striped bass females, this first spawning was followed by two or three smaller spawns, approximately 10–20 min apart. Gamete clouds in these subsequent (minor) egg releases were much smaller than for the first spawning and sometimes difficult to discern. Male striped bass attended females (Figure 1E) more frequently as their time of spawning approached (Figure 2D). Males apparently courted females for many hours before spawning; they first followed the females and then became close enough to make contact. Contact usually involved one or more of a suite of specific behaviors (Table 1; Figures 1A–E). When striped bass females were approached by courting males well before spawning, they would swim away quickly. In contrast, during the actual release of eggs (spawning) the striped bass female would not swim away from males but would instead hover, allowing males to gather around her (Figure 1D).

Spawning striped bass females led the pack of striped bass males more frequently than control females ($F_{1,3}$ = 9.99, P = 0.025), and there was also a nonsignificant trend towards an increase in their leading behavior as the time of spawning approached ($F_{2,6}$ = 3.79 P = 0.08, Figure 2A). Control females followed the male pack more frequently than female spawners ($F_{1,3}$ = 8.79 P = 0.025; Figure 2B). We sometimes observed female spawners alone well before spawning, during period −5, but they were usually accompanied by males near spawning time ($F_{2,6}$ = 31.9, P = 0.0006). Females were often motionless just before or after spawning, although they were always active during spawning ($F_{2,6}$ = 15.1, P = 0.02). The repeated measures ANOVA model did not reveal any significant female, time, or female-by-time effects for the female-in-pack behavior. Headstanding, flashing, shimmying and spawning (Figure 1C–D) were shown by all female spawners but never shown by control females. As noted, these behaviors occurred at very low frequencies and only near or at the time of spawning. In fact, a spawning striped bass female often would do a headstand, begin shimmying, release eggs, and flash within less than 10 s.

Following behavior of striped bass males was generally directed at spawning versus control striped bass females ($F_{1,3}$ = 2.16, P = 0.11) and increased in frequency as spawning time approached ($F_{2,6}$ = 4.34, P = 0.06), but these changes were not statistically significant (Figure 2C). Striped bass males always attended spawning females more frequently than control females ($F_{1,3}$ = 20.44, P = 0.01) and began to preferentially attend spawning females more than 4 h before actual spawning. This attending behavior (Figure 1E) increased in frequency near the time of spawning ($F_{2,6}$ = 4.07, P = 0.035) and continued to increase during the period just after spawning (Figure 2D). The repeated-measures ANOVA model did not reveal any significant effects for male chasing behavior.

Shimmying, spawning, face-to-face, and side-to-side behaviors by striped bass males were directed only at female spawners. These behaviors occurred at very low frequencies and could not be tested for significant changes over time because they occurred only near the time of spawning. In fact, a single male often would initiate spawning by contacting the female spawner side-to-side or face-to-face, sometimes as she was in a headstand posture. Males would then begin shimmying, release their milt, and flash. Males would continue initiating side-to-side or face-to-face interactions whenever spawning females would hover after the first large egg release. These acts were not observed earlier than 1 h before spawning or more than 2 h after spawning, but striped bass males would continue following striped bass female spawners for at least 5 h after spawning.

Eggs were spawned in 1995 striped bass trial V, but they were largely infertile (Table 2). In this trial, striped bass males and females exhibited much lower frequencies of courtship and spawning behaviors (<3 times per behavior in each observation segment) than in trials generating more fertile spawns; however, levels of these behaviors were still higher than observed for striped bass during nonspawning periods (August 1994 or >24 h before spawning in 1995 trials).

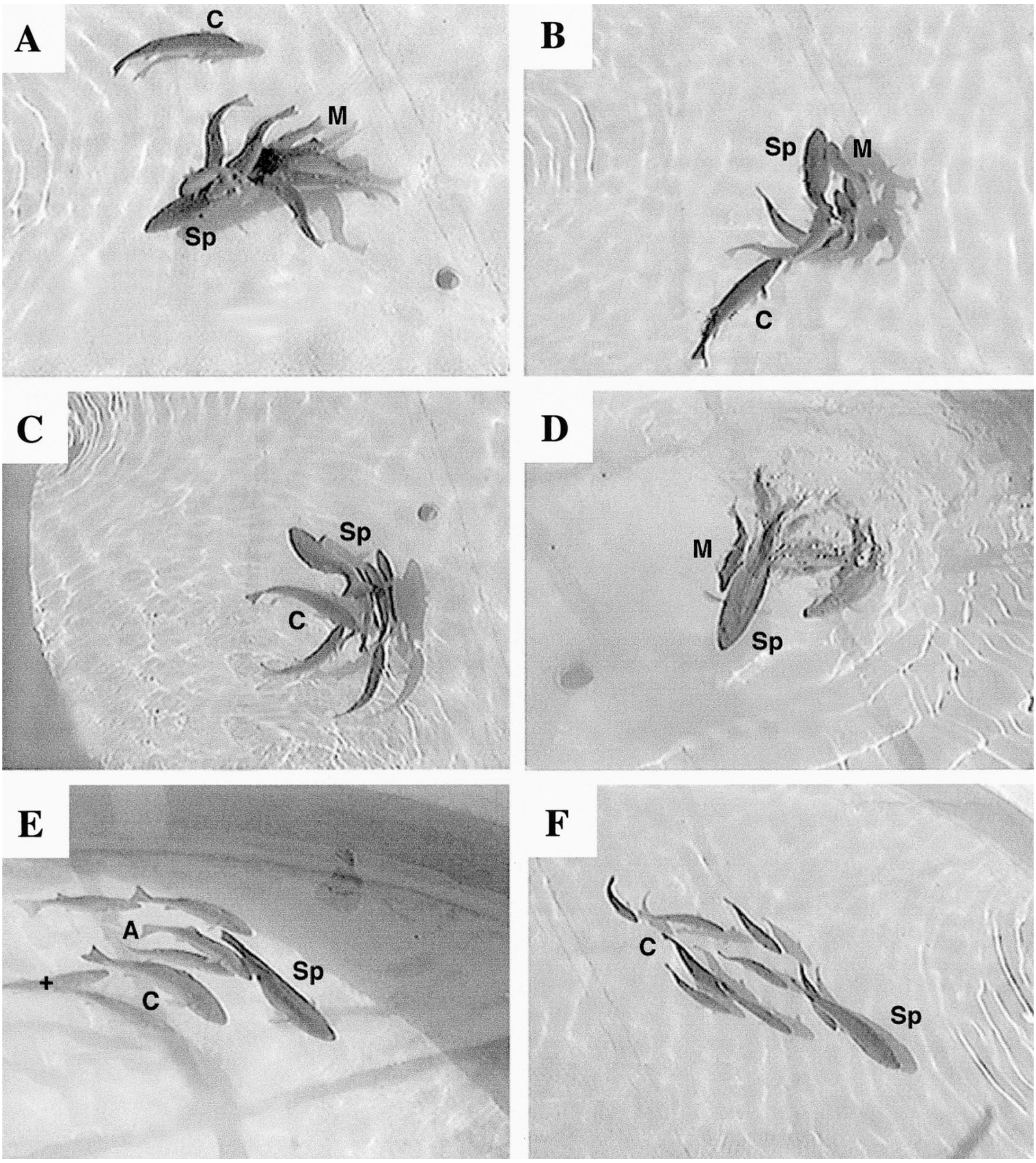

FIGURE 1.—Some courtship and spawning behaviors of striped bass (see Table 1 for definitions). **(A)** Smaller males (M) courting the larger spawning female (Sp). Male activities include attending and side-to-side behaviors. Note that the control female (C) is nearby but not involved in courtship. **(B)** Male exhibiting face-to-face behavior with reference to the spawning female. Note that the other males appear to be investigating the spawning female's urogenital region (attending behavior) and that the control female follows behind the group. **(C)** Spawning female in a headstand posture engaged in the shimmy behavior as males come alongside. Note that the control female is moving through the group in a different direction. **(D)** Males and female spawning. Note that the spawning female remains stationary by sculling backwards with her pectoral fins. Also note the absence of the control female. **(E)** Male (A) attending the spawning female including placement of his snout against her abdomen near the urogenital pore. Another male (+) approaches the control female immediately before attending her. **(F)** The spawning female leads the pack, the smaller males following her. The control female is in the pack.

TABLE 2.—Summary of striped bass spawning trials and reproductive data.

Trial	HCG[a] injection date (time)	Maximum oocyte diameter (μm)	Oocyte stage (h)	First-spawn date (time)	Fecundity[b] (number)	Fertility[b] (%)
1994 spawning trials						
1	Apr 6 (1730)	850	13	Apr 8 (0900)	577,986	64
			14	(1500)[c]	288,750	NA
2	Apr 11 (1400)	875	10	Apr 13 (0957)	216,000	61
			12		0	0
3	Apr 14 (2030)	850	10	Apr 16 (0247)	255,600	91
			15	(0328)[c]	NA	NA
1995 spawning trials						
1	Apr 1 (1443)	875	12	Apr 2 (1404)	395,696	33
2	Apr 6 (1450)	850	12	Apr 7 (1436)	629,303	67
3	Apr 7 (1645)	950	13	Apr 8 (1645)	481,231	45
4	Apr 19 (1600)	908	10	Apr 20 (0900)	36,400	18
5	Apr 20 (1748)	875	12	Apr 21 (1817)	24,500	<1

[a] Human chorionic gonadotropin.
[b] NA indicates that the female spawned but fecundity and/or fertility data were not collected. A value of zero for fecundity and fertility indicates the fish did not spawn.
[c] Females were not observed spawning; time of spawning estimated from the stage of embryo development.

White Bass and White Perch

White bass and perch males exhibited spawning behaviors similar to striped bass, including following, attending, and chasing, but when placed in the same arenas in which striped bass spawning was evaluated, they schooled together less and, instead, tended to hover in a small area. Consequently, observations of white bass and white perch behavior were done in smaller arenas, and results of these experiments (Table 3) are not completely comparable to those reported for striped bass. Female white bass and white perch were attended by males up to 70–80% of the time (Figures 3D, 4D). In addition, male white bass and white perch engaged in ''circling,'' (i.e., swimming back and forth in front of and sometimes around the female, immediately before spawning). Striped bass males never exhibited this behavior.

White bass showed an increasing frequency of female leading ($F = 6.94$ $P = 0.02$) and male attending ($F = 6.94$ $P = 0.02$) before spawning, behaviors similar to striped bass but longer in duration (Figures 3A, 3D). White perch males attended at frequencies similar to male white bass, both frequencies being much higher than for striped bass males (Figure 4D).

After a significant increase in attending, white bass males continually approached the female from the sides and below, placing their snouts and bodies against her. The entire group swam slower. Typically, at least one male circled in front of or around the female. The male(s) made bodily contact with the female, and the group swam in circles approaching the water surface. As they were circling, one or more males came alongside the female and turned on their sides so their ventral surface was closest to the female. The group began to shimmy, and their path of forward movement changed from circular to straight. Gametes were then released. The group separated when spawning ceased. All three white bass females that were filmed spawned in this manner three to four times within a 15–40-min period. Body contact, circling, and gamete release lasted 6–24 s, whereas actual gamete release lasted 3–6 s.

At spawning, female white perch became motionless, allowing males to gather beneath them. In a sudden rush, males and the female turned in a circle while releasing gametes. These rushes were quick, lasting less than 1 s. The first female white perch spawned six times in a 15-min period. The second female white perch spawned three times in a 12-min period. These spawns occurred at all water depths, not only at the surface. These spawning acts lasted less than 1 s. Male circling was observed in both trials, occurring once or twice in the hour before and during spawning. Although PVC pipes were provided as spawning substrate, both white bass and white perch were observed spawning in all parts of the these tanks and spawned eggs were found adhered to all tank surfaces.

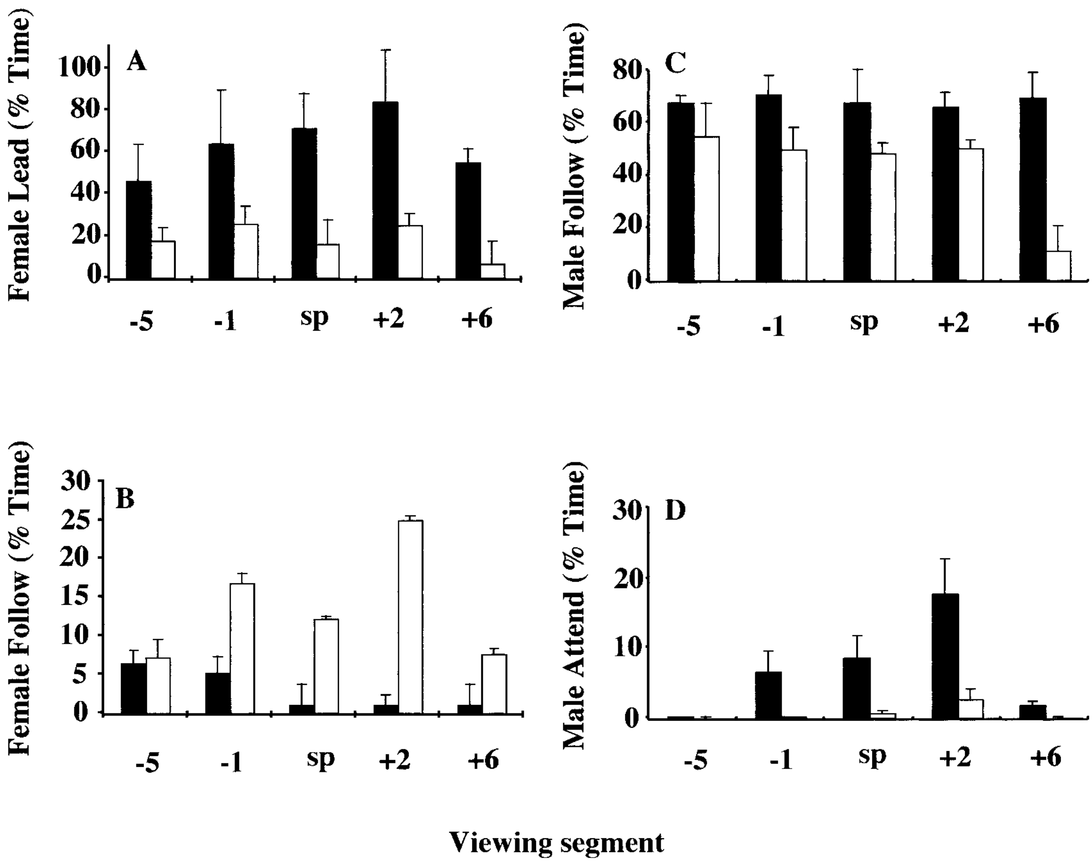

FIGURE 2.—Changing duration of some reproductive behaviors of female and male striped bass around the time of spawning in 1995 trials 1–5. Vertical bars indicate the mean duration of a behavior during each of the five 20-min viewing segments (−5, −2, sp, +2, and +6) calculated as percent time ([seconds/1,200] × 100). In panels **A** and **B,** black bars (+SE) indicate behaviors exhibited by spawning females and open bars indicate behaviors exhibited by control females. In panels **C** and **D,** black bars (+SE) indicate male behaviors directed toward spawning females and open bars indicate behaviors directed toward control females. All bars represent four trials.

Hybridization

All of the trials involving white perch and white bass produced viable fry; hybridization trials involving striped bass males and white bass females did not (Table 4). In fact, all of the female white bass ovulated but did not spawn. They withheld their ovulated eggs within their ovaries. Analysis of videotapes of these trials revealed that male striped bass did not follow, attend, or chase the white bass females.

Electro-Olfactogram Recording

Of the five fingerlings tested (two white perch, two white bass, and one striped bass), all consistently exhibited large amplitude (3–7 mV) responses to 10 mM L-alanine, indicating the olfactory receptor neurons were functional. However, none of the 111 unconjugated, glucuronidated, or sulfated steroids (1 and 10 nM) used as stimuli (Table 5) produced EOG responses in test fish greater than those induced by control water (0–0.2 mV) containing ethanol equivalent to that in the steroid test solutions. Likewise, none of the eight prostaglandin test solutions induced EOG responses greater than those elicited by solvent controls.

Discussion

The results of this study provide detailed descriptions and quantification of specific courtship behaviors culminating in spawning of striped bass, white perch, and white bass and reveal some clear interspecific differences that could underlie the block to volitional hybridization of white bass with striped bass. Our results verified that white bass

TABLE 3.—Summary of white bass and white perch spawning trials and data. The symbol NA indicates that the female spawned but injection time, oocyte diameter, spawning time, or fecundity data were not recorded or collected.

Trial	HCG[a] injection date (time)	Maximum oocyte diameter (μm)	First spawn date (time)	Fecundity	
				Fry	Fry/kg
White perch 1995 spawning trials					
1	Apr 10 (1343)	725	Apr 14 (NA)	502	2,952
2	Apr 10 (1354)	700	Apr 11 (NA)	1,741	9,163
3	Apr 16 (2056)	NA	Apr 18 (1200)	1,698	8,797
4	Apr 16 (2108)	NA	Apr 18 (1700)	803	4,461
5	Apr 16 (NA)	NA	Apr 18 (1250)	1,698	6,174
6	Apr 30 (NA)	675	May 1 (0338)	NA	NA
7	May 1 (NA)	700	May 3 (1745)	6,000	42,857
White bass 1995 spawning trials					
1	Apr 5 (0200)	NA	Apr 6 (1817)	NA	NA
2	Apr 10 (1343)	675	Apr 11 (NA)	53,500	178,333
3	Apr 10 (1354)	575	Apr 11 (NA)	14,064	33,485
4	Apr 16 (2054)	NA	Apr 18 (0300)	64,327	156,895
5	Apr 16 (2105)	NA	Apr 18 (0300)	76,849	258,750
6	May 1 (1720)	730	May 3 (1745)	40,878	148,647
7	May 2 (0355)	625	May 3 (1500)	NA	NA

[a] Human chorionic gonadotropin.

and white perch will spawn together in tanks and are more similar to each other than to striped bass with regard to certain reproductive behaviors. This study identified male attending (i.e., extremely close and continuous following of the female while sometimes contacting her abdominal or vent area with the snout) as a quantifiable behavior that accurately predicts imminent spawning. Attending differed between striped bass and the other two species in relative frequency.

This study is the first to report on experiments in which the complete sequence of courtship and spawning behaviors has been observed and quantified for any *Morone* species. Assessments of fecundity and the use of control females provided rational means to judge to what degree courtship and spawning behavior of these captive animals could be considered normal. While there may be differences between the behaviors described here and those of fish reproducing in nature, the detailed observations and quantification of behavior accomplished in these experiments would be impossible to achieve in the turbulent and often murky rivers where *Morone* species naturally spawn.

These observations show that the actual act of spawning, when milt and eggs are simultaneously released, is very brief, although courtship and spawning behavior may continue for up to 1 h after initial release of gametes. In fact, all three species showed a similar pattern, spawning at least two times but as many as six times. The largest release of sperm and eggs occurs during the first brief spawn. Prior observations did not document that a single striped bass female releases eggs several times instead of all at once. Contrary to published descriptions indicating that *Morone* species appear to mill around aimlessly before spawning (Worth 1903; Merriman 1941; Woodhull 1947; Raney et al. 1952; Lewis and Bonner 1966), we observed that they exhibit a predictable sequence of quantifiable behaviors leading up to and including spawning.

We observed distinct differences among the three *Morone* species in the levels and type of reproductive behaviors exhibited just before spawning. Specifically, the maximum level of prespawning attending shown by white bass and white perch males (up to 70–80% of time) was about four-fold higher than that shown by male striped bass (<20% of time; Figures 3D, 4D versus 2D). A second difference was that white bass and white perch remained nearly stationary or moved about over very short distances (<10 body lengths), whereas striped bass moved over large distances. Finally, a striking difference between species was that white perch and white bass males engaged in circling behavior, but not striped bass males. These observations also reveal that just before and during spawning, white bass and white perch females receive more tactile stimulation from males than do female striped bass. It also appears that white bass and white perch males do not have to constantly follow females for courtship (attending) as do striped bass males. Visual dis-

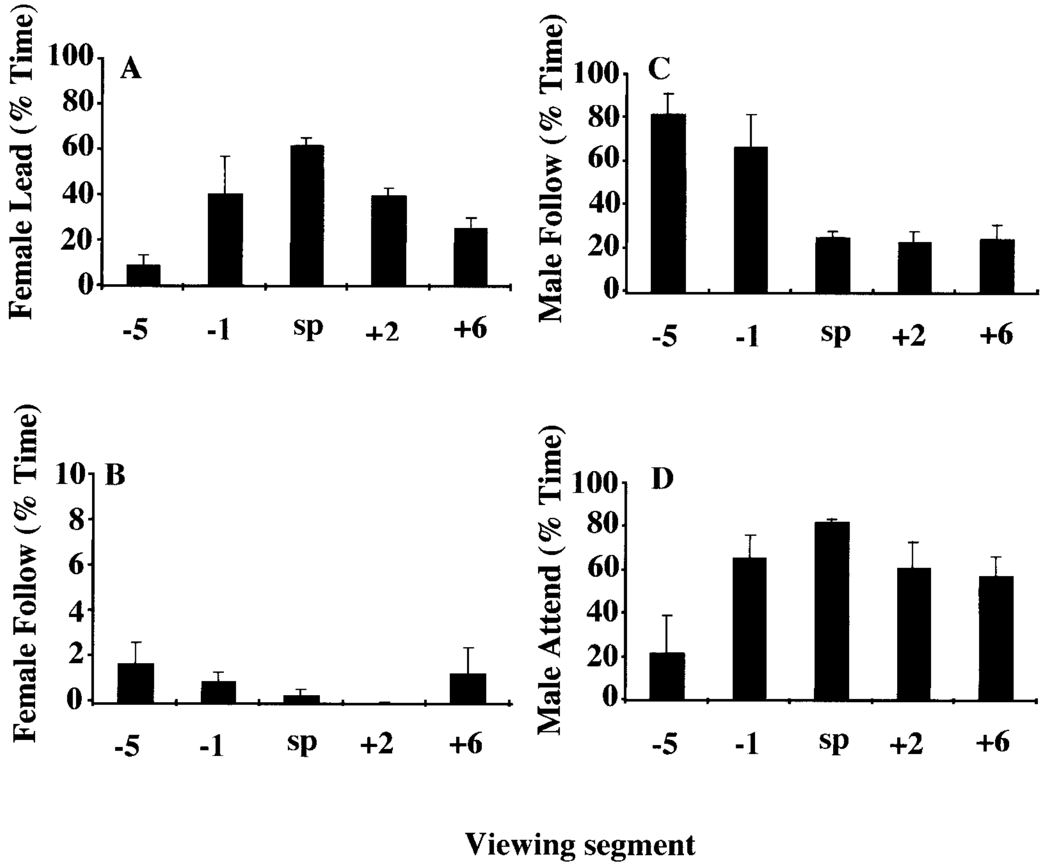

FIGURE 3.—Changing duration of some reproductive behaviors of female and male white bass around the time of spawning: **(A)** female lead, **(B)** female follow, **(C)** male follow, and **(D)** male attend. Vertical bars (+SE) indicate the mean duration of a behavior during each of the five 10-min viewing segments (−5, −2, sp, +2, and + 6) calculated as percent time ([seconds/600] × 100). All bars represent three trials.

plays, such as circling, may need to be observed by the female before she spawns. Some of these behavioral differences could underlie the block to volitional spawning between striped bass and white bass or white perch, preventing hybridization in nature. Most hybrids between striped bass and white bass found in nature can clearly be attributed to release of captive animals (feral hybrids) or volitional backcrosses of such hybrids to either parental line (Avise and Van Den Avyle 1984; Harrell and Dean 1988). Volitional hybridization of these two species in nature has never been documented, as discussed below, and it has been reported only once for captive animals (Woods et al. 1995).

One of the behaviors observed in this study (attending) suggested male responsiveness to chemical cues released from the female urogenital tract. Many fish use pheromones of gonadal origin to induce and synchronize spawning (Sorensen and Stacey 1991; Stacey and Sorensen 1991). However, our EOG recordings provided no evidence to support the idea that any known teleost pheromone can be detected by any of the three *Morone* species. A large array of unconjugated or conjugated steroids and prostaglandins were tested as EOG stimuli, including all those reported to function as sex pheromones in other fish species (see Stacey and Cardwell 1997 for review). We do not believe that this lack of olfactory responsiveness resulted from our use of juvenile *Morone.* In the few species in which EOG recordings of juveniles and adults were compared, juveniles exhibited detectable responses sometimes equivalent to those of adults (Cardwell et al. 1995; Irvine and Sorensen, 1993).

Lack of EOG responsiveness to prostaglandins in *Morone* species is consistent with results of studies of other perciform species (Stacey and

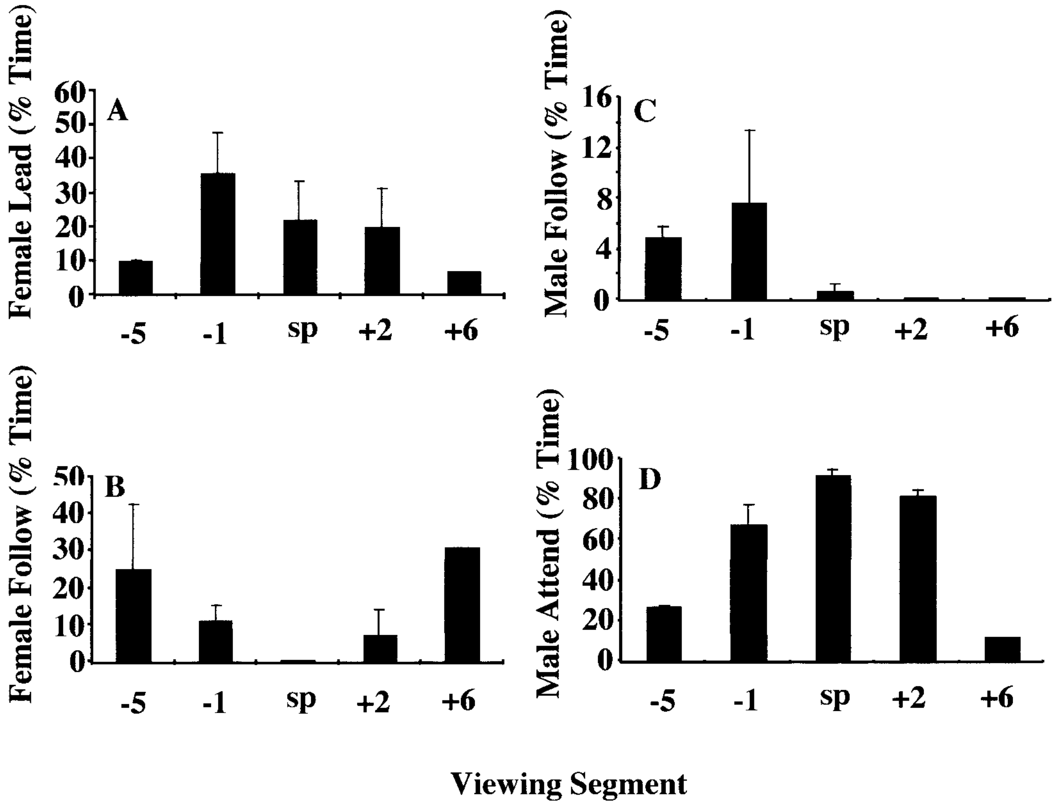

FIGURE 4.—Changing durations of some reproductive behaviors of female and male white perch around the time of spawning: **(A)** female lead, **(B)** female follow, **(C)** male follow, and **(D)** male attend. Vertical bars (+SE) indicate the mean duration of a behavior during each of the five 10-min viewing segments (−5, −2, sp, +2, and + 6) calculated as percent time ([seconds/600] × 100). For segments −5, −2, sp, and +2, N = 2 trials; N = 1 trial for segment +6.

Cardwell 1995; Robison et al. 1998; Murphy and Stacey 1999). Although the olfactory systems of some perciform fish are extremely and specifically sensitive to certain steroidal compounds (Robison et al. 1998; Murphy and Stacey, 1999), others are similar to *Morone* species in exhibiting no EOG response to the steroids tested in this study (Stacey and Cardwell 1997). Despite our failure to find evidence that pheromones are involved in *Morone* spawning behavior, it is quite possible that these species release novel forms of steroids or prostaglandins or other substances not used as test stimuli in our EOG recording studies.

The studies reported here are also relevant to

TABLE 4.—Summary of hybridization spawning trials with white bass, white perch, and striped bass. The symbol NA indicates that the injection time was not recorded. A value of zero for fecundity indicates that the fish did not spawn.

Trial	HCG[a] injection date (time)	Female (number and species)	Male (number and species)	Fecundity (fry)
1	Apr 10 (NA)	2 white perch	6 white bass	29,771
2	Apr 21 (2111)	4 white bass	7 striped bass	0
3	Apr 21 (2124)	4 white bass	7 striped bass	0
4	Apr 21 (2131)	4 white bass	7 striped bass	0
5	Apr 21 (0622)	4 white bass	7 striped bass	0
6	Apr 27 (1400)	1 white bass	7 white perch	5,220
7	May 2 (NA)	1 white perch	4 white bass	1,255

[a] Human chorionic gonadotropin.

TABLE 5.—Categories and numbers of conjugated and unconjugated steroids and specific prostaglandins[a] used as stimuli for electro-olfactogram recordings.

Steroid category	Unconju-gated	Glucuroni-dated	Sulfated
21-carbon steroids (progestin- and corticosteroid-like)			
4-pregnen	23	3	7
5-pregnen	5	1	3
5a-pregnan	13		
5β-pregnan	20	1	
9-carbon steroids (androgen-like)			
4-androsten	9	1	1
5-androsten	2	1	1
5a-androstan	3		1
5β-androstan	3	3	
18-carbon steroids (estrogen-like)			
1,3,5(10)-estratrien-3α	4	2	4

[a] Prostaglandins used were as follows:

prostaglandin E1	15-keto prostaglandin E2
prostaglandin E2	15-keto prostaglandin F2α
prostaglandin F1α	13,14,dihydro-15-keto prostaglandin F2α
prostaglandin F2α	
prostaglandin F3α	

practical aspects of fish reproduction—for example, production of hybrid striped bass for aquaculture (Harrell and Webster 1997). Growth of the hybrid striped bass culture industry would benefit from techniques for inducing volitional hybridization of striped bass with white bass in hatchery tanks. Currently, all hybrid striped bass for commercial culture are produced via in vitro fertilization, which necessitates repeated biopsy of the female over hours to days, followed by repeated verification of their status of ovulation by manual abdominal palpitation with or without anesthesia (Rees and Harrell 1990). These procedures are inherently stressful and sometimes damaging to the fish. Furthermore, predicting the exact time of ovulation is required to obtain fertile eggs from striped bass. There is, at best, a short window around ovulation time that large numbers of highly fertile eggs can be obtained, and predicting when it will occur for individual females is often unsuccessful and more art than science (Sullivan et al. 1997).

Prior hybridization attempts using striped bass females paired for tank spawning with white bass males routinely resulted in the females spawning or aborting overripe eggs (Woods et al. 1995). It was surmised that the presence of males of a different species either did not provide appropriate synchronizing cues for spawning ovulated eggs or actually inhibited spawning. In our study, while attempting volitional hybridization to generate the reciprocal hybrid cross (white bass female × striped bass males), we found that male striped bass would barely court female white bass at all. However, white perch and white bass readily spawned together as both original (white perch female × white bass male) and reciprocal crosses. This is not surprising because naturally occurring hybrids of these latter two species are known to exist (Todd 1986) and because courtship and spawning behaviors of these species are quite similar, although very different from those of striped bass.

It appears that achieving volitional hybridization of striped bass with white bass will require, as a foundation, more detailed knowledge of how courtship and spawning behaviors are regulated. As a first step along this path, studies of the role of androgens and the neurotransmitter, arginine vasotocin (AVT), in regulating male attending behavior of white perch have been conducted (Salek 2000).

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