HYBRIDIZATION POTENTIAL AND SPAWNING BEHAVIOR OF RIO GRANDE SILVERY MINNOW (*HYBOGNATHUS AMARUS*) AND PLAINS MINNOW (*HYBOGNATHUS PLACITUS*)

FINAL REPORT

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U.S. FISH AND WILDLIFE SERVICE
ECOLOGICAL SERVICES FIELD OFFICE
ALBUQUERQUE, NEW MEXICO 87113
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Rio Grande silvery minnow
Photo by C. Caldwell and C. Sykes

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INTRODUCTION

The presence of a variety of drainage systems in New Mexico (Great Plains, Chihuahuan Desert, Mexican Plateau, Sonoran Desert, Great Basin) has resulted in one of the greatest diversity of native fish faunal assemblages in the interior southwestern states (Sublette et al. 1990). Historically, sixteen families comprising 69 native species of fish have lived in New Mexico waters. At the time of the publication of Fishes of New Mexico (Sublette et al. 1990), eight species had been extirpated with 27 species having their historic ranges restricted. Native floral and faunal communities in the southwestern United States have been severely impacted by activities of pre-Columbian and post-Columbian man. Practices such as irrigation, municipal water use, damming, logging, mining, livestock grazing, and the introduction of nonnative terrestrial and aquatic species have resulted in the decline and extirpation of native fishes. One species receiving considerable attention is the federally endangered and state-protected Rio Grande silvery minnow (*Hybognathus amarus*). Bestgen and Platania (1991) found *H. amarus* inhabiting 5% of its former range in the Rio Grande basin of New Mexico, Texas, and Mexico. In accordance with the Rio Grande Silvery Minnow Recovery Plan to re-establish, stabilize, and enhance populations within its historic range of the Rio Grande Basin, information is needed to determine the biological response of the species to altered habitat including the potential for hybridizing with other species.

Although historical records indicate the Rio Grande silvery minnow was abundant throughout parts of its range in New Mexico, Texas, and Mexico, alterations of its habitat including flow regulation with trans-channel barriers and water diversion from the river for agricultural purposes have contributed to the extirpation of the species throughout sections of the Rio Grande in New Mexico and Texas (Bestgen and Platania 1991). All surface water in the Rio Grande from Colorado to Texas is appropriated by compacts, treaties and purchased water rights and may actually exceed the annual mean flow of the river (Levings et al. 1998). The effect agricultural and urban land and water use has on surface water within the river is reflected by how the river water is used as it moves south. Over 90% of the water in the Rio Grande has been

allotted for irrigation which results in the water being diverted into irrigation ditches, applied to fields, and subsequently returned to the Rio Grande in reduced quantity and quality containing greater sediment and salt concentrations. In addition, urban and heavily industrialized areas contribute storm-water runoff, wastewater, metals, hydrocarbons, home-use pesticides; and, agricultural areas contribute chemicals from fertilizers, pesticides, and animal waste high in oxygen-depleting nutrients (Levings et al. 1998).

The disappearance of the Rio Grande silvery minnow from the middle portions of the Pecos River, New Mexico, (from Sumner to Avalon reservoirs) was presumed to coincide with the introduction and spread of the plains minnow (*H. placitus*) (Bestgen and Platania 1991). Until the accidental introduction of the plains minnow prior to the early 1960's, the Rio Grande silvery minnow was one of the most abundant *Hybognathus* species in the central portion of the Pecos River. Two specimens, collected in 1964, were characterized as *H. amarus* x *H. placitus* having intermediate morphological characteristics (Bestgen and Platania 1991). Although the Pecos River may provide suitable habitat for the re-introduction of the Rio Grande silvery minnow, additional work should be conducted to determine to what degree its disappearance was due to either hybridization and competition with other *Hybognathus* species, or habitat alterations. The objective of this study was to determine the potential for hybridization between the Rio Grande silvery minnow and plains minnow and investigate behavioral spawning interactions between adult congeners.

This work will contribute to the enhancement and stabilization of existing populations of Rio Grande silvery minnow and help managers assess the feasibility of re-introducing the fish throughout portions of its historic range. As information is developed on the environmental requirements and genetic integrity of populations, it will be necessary to transplant captive-reared fish into areas that were historically occupied. Specifically, this research is working toward addressing a narrative recovery task listed in the Rio Grande Silvery Minnow Recovery Plan (U.S. Fish and Wildlife Service 1999): [2.4.1] Determine the level and rate of hybridization between Rio Grande silvery minnow and plains minnow with the ultimate goal of reestablishing the Rio Grande silvery minnow at appropriate locations within its historic range.

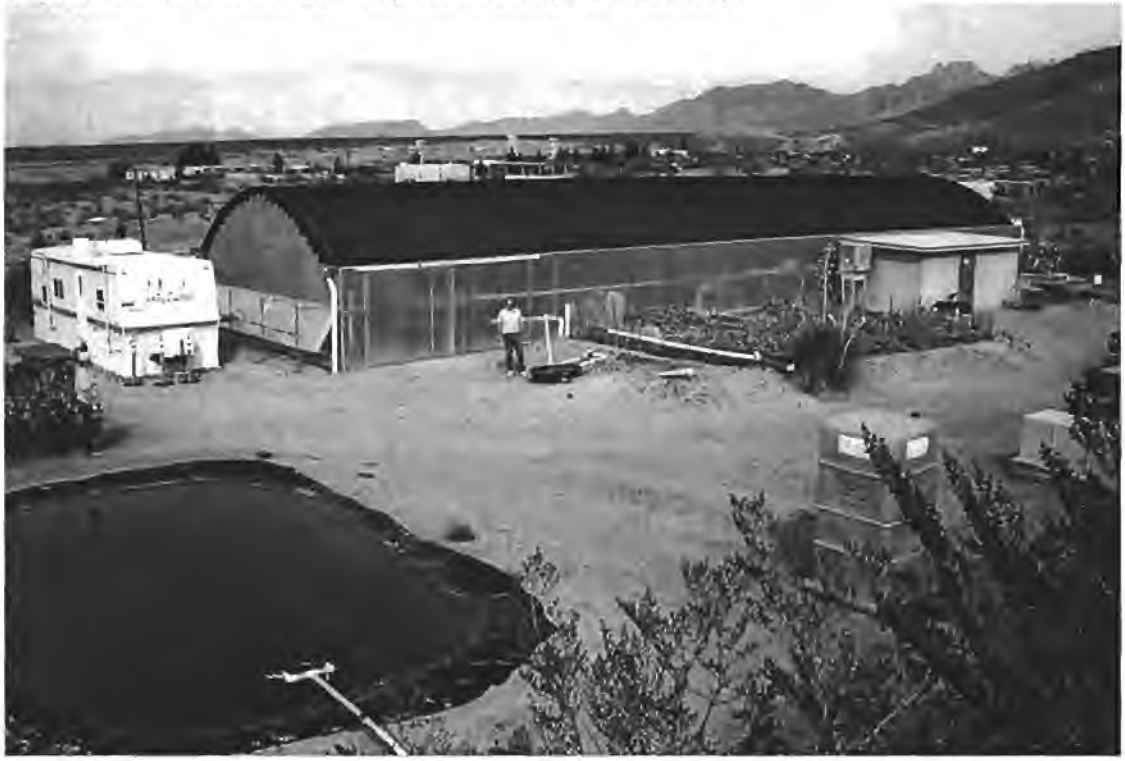
METHODS

Broodstock Maintenance

On 22 March 2002, 136 adult *H. placitus* were collected from the Pecos River (48 km north of Roswell, New Mexico, Chaves County). These fish were transported to the NMSU Fisheries Research Laboratory and placed into a 1514 L (400-gallon) circular tank with fresh water flow-through (8 L/min) and aeration and airlifts to maintain dissolved oxygen levels (6.5 - 7.4 mg/L). Air is provided by a compressor and filtered after it enters the Laboratory prior to entering the fish tanks. The Laboratory is serviced by a deep well (120 meters in depth) resulting in optimal water quality parameters for fish growth and maintenance (pH 7.7; electrical conductivity, 565 micromhos/cm; total dissolved solids, 342 mg/L; chloride, 44.9 mg/L; alkalinity, 145 mg/L as $CaCO_3$; hardness, 200 mg/L as $CaCO_3$). Adult fish were fed 4 to 6 times per day a ration (2.5% body weight) of a flake diet containing 3 parts spirulina, 1 part brine shrimp, and 1 part plankton/krill/spirulina.

Adult *H. amarus* were collected the fall of 2000 from the Rio Grande near San Marcial, New Mexico (Soccorro County) and held at the U.S. Fish and Wildlife Service fish holding facility in Albuquerque until the fall of 2001 when they were transported to the NMSU Native Fish Culture Facility (4.8 km from campus), Las Cruces, New Mexico (Dona Ana County) (Photo 1). While at the facility, *H. amarus* were maintained using the same flake diet as *H. placitus* described above. The entire culture system is enclosed in a double-wall arched greenhouse structure (9.2-m x 29.3-m; 270m^2). Recirculated water is treated by either an artificial wetland filter or bead and sand filters designed to remove hazardous nitrogenous wastes prior to reuse. Water quality is improved (greater than 96%) by more complex treatment of ultra-violet sterilizers to irradiate and control potential pathogens. A centrifugal air blower provides oxygen input via air stones and circulation via air lift pumps to the tanks.

Photo 1. Native Fish Culture Facility at New Mexico State University and in cooperation with U.S. Fish and Wildlife Service, Las Cruces, New Mexico.



Experimental System

The hybridization study was conducted in a 3293-liter (870-gallon) recirculating system in the Fisheries Research Facility at NMSU (Photo 2). Well water is fed into a 946-liter (250-gallon) sump tank. A 1/8 h.p. pump moves the water through a 113-lpm (30-gpm) BBF2P bubble bead filter (Aquatic Eco-Systems, Inc.) using a filter media of low-density polyethylene beads with a 56.6 cm^3 (2 ft^3) bead capacity. A Rainbow Lifegard UV97 sterilizer is attached behind the bead filter with 3 UV bulbs totaling 120 watts and an exposure of 30,000 :ws. From the sterilizer, water continues into a 378-liter (100-gallon) head tank which is aerated with a 122-cm (4-ft) bio-weave type diffuser hose (supports 7.26-kg [16-lbs] of fish) (Photo 3). Water is then gravity fed into fifty-two 38-liter (10-gallon) glass aquaria on stainless steel racks (Photo 4). Each aquarium has a globe valve for individual water adjustments and an airline with a 10-cm (4-inch) diffuser (supports 0.772-kg [1.7-lbs] of fish). Aquaria drain from bulkhead fittings centered in the front glass panel and gravity feeds the water through a 7.62-cm (3-inch) slotted PVC pipe back into the sump tank. Nitrite (mg/L) and ammonia (mg/L) were monitored using a

HACH spectrophotometer (DR/2010) and HACH methods 8507 and 8155, respectively. Temperature was maintained as ambient and ranged from 19.5 to 21.0°C.

Photo 2. Recirculating experimental fish culture system at NMSU Fisheries Research Laboratory.

Photo 3. Bead filter, UV sterilizers, head and sump tanks.

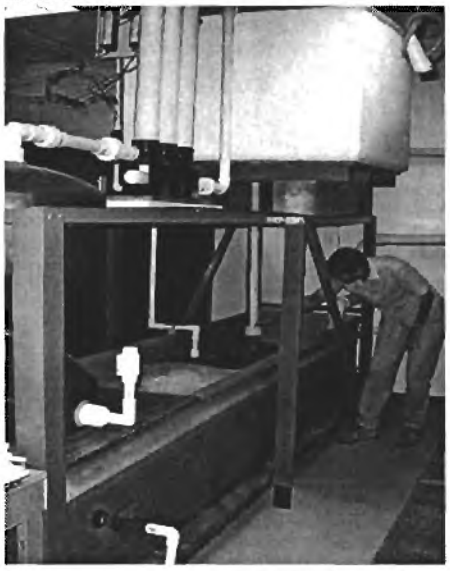


Photo 4. Thirty-eight liter glass aquaria.

Hybridization Experimental Design

Fish larvae were propagated from each of two groups (female parent is indicated first; *amarus x amarus, placitus x placitus*) and the two reciprocal crosses (*amarus x placitus, placitus x amarus*). Thus, the experimental design included four crosses in five replications of the female x male cross. The five replications of each cross were randomized throughout the experimental system described above.

The hybridization study began 23 May 2002 at 1430 with the injection of a refined carp pituitary extract (CPE) (Sigma Chemical Company; Catalog Number P3034). Each fish was anaesthetized (50 mg/L Tricaine Methanesulfonate) and injected with 1.0 mg of CPE using a 1-cc syringe and a 24-gauge needle. Prior to each injection, female fish of both species were weighed to the nearest milligram. The left pectoral fin of each *H. amarus* was clipped for identification of the species. The weight of female *H. amarus* ranged from 3.9 to 8.6 g resulting in a dosage of CPE ranging from 256 to 116 ug/g. In contrast, female *H. placitus* were twice as large with weights ranging from 6.8 to 15.1 g. Thus, the dosage of CPE was lower ranging from 147 to 66 ug/g, respectively. Two male fish were placed with one female fish per aquaria. Within 24 hours of spawning, adult fish were moved from aquaria and placed in holding aquaria directly above spawning aquaria for observation through the end of the study. Mortality was recorded and fish were preserved in 10% formalin for necropsy. All *H. amarus* were archived for transmittal to the University of New Mexico for archival purposes.

A qualitative fish health assessment was performed on all adult fish at the end of the study. Briefly stated, all fish were given a cursory examination during necropsy using an ordered observation of external and internal tissues and organs to assess the relative health and condition of the adult fish at the end of the study. Although this autopsy-based method has been standardized as a quantitative index to describe changes in the health of fish populations (Goede and Barton 1990), we applied the methodology for descriptive purposes only.

Larvae Maintenance and Development

Within 48 h post-hatch, freshwater rotifers (*Brachionus sp.)* and *Artemia* nauplii were presented to the larvae to overlap resorption of the yolk sac with time to first exogenous feeding. Rotifers were provided to larvae twice each day until the end of the study. The rotifers were initially harvested from an outdoor pond, and subsequently cultured in laboratory conditions (0:24 dark:light; 27°C; enhanced with a microalgae formulation of *Crypthecodinium*). *Artemia* cysts were obtained commercially and harvested in laboratory conditions (0:24 dark:light; 27°C; 20 ppt salinity). *Artemia* nauplii were provided to the larvae four times each day until the end of the study. Tanks were cleaned of feces and uneaten food beginning 11 d post-spawn to reduce chances of siphoning larvae from tank.

We chose to initially provide freshwater *Branchionus sp.* due to their small size when compared to *Artemia* nauplii. Mouth size has been shown to be a limiting factor in successful feeding of larval fish (see review by Hoff and Snell, 1987). It is not the overall size of the prey, but the width of the item that is important in prey selection and larvae survival. *Branchionus plicatilis* ranges in width from 114 to 199 μm while some commercial *Artemia* nauplii are 250 μm in width (Hoff and Snell 1987). As the larvae increased in size, the size of prey ingested also would need to increase. Thus, we anticipated survival of larvae would be improved if provided prey that overlapped in size as the larvae learn to search, capture, and ingest prey items.

Spawning Behavioral Observations

Spawning behavior of adult fish in two crosses (*placitus x amarus* and *amarus x placitus*) were videotaped beginning 14 h post-injection from 0330 to 1330 hours (May 25, 2002). One replicate of the *placitus x placitus* cross was briefly recorded to gain a general understanding of typical spawning behavior of both *H. amarus* and *H. placitus* reported by Platania and Altenbach (1998). The fish were filmed using an 8-mm video camera recorder in ambient light. Six undred minutes of videotape was reviewed to evaluate spawning behavior between the two species.

RESULTS

Hybridization Study

There was no immediate mortality in the adults as a result of either anaesthesia or injection. Spawning began at approximately 0200 May 24 (12 h post-injection). By 0800 (18 h post-injection), adults in eight of 20 aquaria representing all four crosses had spawned (2 *amarus x amarus*; 2 *placitus x placitus*; 1 *amarus x placitus*; 3 *placitus x amarus*). Successful spawning was indicated by the presence of eggs, however, the number and quality of eggs varied within and among the four crosses. Two additional spawns occurred at 1245 (*amarus x amarus*; 22 h post-injection) and 1430 May 25 (*amarus x placitus*; 48 h post-injection).

The *placitus x placitus* cross was the first to spawn resulting in several thousand eggs (not enumerated) that hatched within 48 h post-spawn (see Table). Larvae from this spawn survived through the end of the study (d 24 post-hatch). An additional spawn in a second aquarium containing *placitus x placitus* resulted in very few eggs. The perivitelline space within the eggs filled with water resulting in the eggs becoming semibuoyant. These eggs, however, clouded within 24 h and did not hatch. Upon microscopic examination, the eggs were unfertilized. Thus, two out of five aquaria or 40% of the *placitus x placitus* cross spawned with one of the two replicate aquaria resulting in hatch and surviving larvae through the end of the study (see Table). Microscopy of a small sample of eggs and larvae at hatch indicated some variation in developmental stages. No external developmental abnormalities were observed.

Three of five aquaria or 60% of the *amarus* x *amarus* cross resulted in spawns of several hundred eggs within each aquaria (not enumerated) (see Table). Microscopy of a sample of eggs and larvae collected at hatch indicated normal external development with larvae surviving until the end of the study (24 d post-hatch). One of the three aquaria contained several hundred eggs (not enumerated) in which the perivitelline space within the eggs filled with water resulting in the eggs becoming semibuoyant. These, however, did not hatch. Upon microscopic examination, the eggs were unfertilized.

Two of the five aquaria or 40% of the *amarus x placitus* cross resulted in spawns of several hundred eggs within each aquaria (not enumerated) (see Table). A moderate hatch of several hundred larvae (not enumerated) was observed in one of the two aquaria. These larvae exhibited intermittent directional swimming toward the surface within 24 h of hatch. Within 48 h of hatch, however, all larvae had died. A sample of larvae collected and preserved did not reveal external developmental abnormalities.

Three of the five aquaria or 60% percent of the *placitus x amarus* cross resulted in spawning within each aquaria of very few eggs (not enumerated) (see Table). A poor hatch of less than 20 larvae was observed in two aquaria and no hatch was observed in the third aquarium. In one aquarium, the larvae exhibited intermittent directional swimming toward the surface. Within 96 h of hatch, however, no larvae remained. Within a second aquarium, larvae did not survive to 24 h post-hatch. Microscopy of a sample of larvae collected within 24 h of hatch revealed one possible spinal deformity. Microscopic examination of eggs collected from the third aquaria having no hatch of larvae indicated the eggs were not fertilized.

Table. Summary of results of four crosses in which *H. amarus* and *H. placitus* were crossed (female x male) to evaluate spawning, hatch, and larvae survival until the end of the study at 24 d post-hatch. Percent spawn is calculated from five replicates for each cross. The percent hatch is calculated from the number of replicates that spawned. Larvae survival represents the number of replicates containing larvae living at the end of the study.

Treatment (female x male)	**Spawn**	**Hatch**	**Larvae Survival (24 d post-hatch)**
amarus x amarus	60% (n=3)	67.7%	100%
placitus x placitus	40% (n=2)	50%	100%
amarus x placitus	40% (n=2)	50%	0%
placitus x amarus	60% (n=3)	67.7%	0%

Behavioral Observations of Spawning Adults

Spawning was initially observed in one *placitus x placitus* aquaria. Spawning had begun prior to 0300 as indicated by several thousand eggs in the aquaria at the time videotaping began. Video captured one spawning episode of the *H. placitus* male and *H. placitus* female (see Video Segment 1). This episode was similar to that described by Platania and Altenbach (1998) with the male aligning laterally alongside the female and wrapping the lower portion of his body around the trunk of the female appearing to squeeze her midsection. The density of eggs within the tank at the time of the spawning episode made it difficult to view the release and fertilization of eggs. Viewing the classical spawning behavior of the "wrap and release" as described by Platania and Altenbach (1998) provided an excellent baseline for observing the spawning behavior of the *Hybognathus* crosses.

Two crosses (*amarus x placitus* and *placitus x amarus*) were chosen adjacent to one another to provide simultaneous observations throughout the video taping. A series of failed spawning attempts were observed in the *amarus x placitus* tank within 13 h post-injection (see aquarium on left in Video Segment 2). These overtures were recognized as spawning attempts when the male attempted to align himself alongside the female. These episodes were aggressive and sometimes violent as the male *H. placitus* pursued the female *H. amarus* throughout the tank. No successful spawning was observed. The female *H. amarus* died within 5 days post-injection. Necropsy revealed large translucent eggs loose within her abdominal cavity indicating viable eggs at the time of her death. Necropsy of the male remaining at the end of the study revealed that he was in poor condition with an external fungal infection. Except for the brief pre-spawning episodes, the male *H. placitus* swam continuously exhibiting agitated and sometimes frantic behavior while the female *H. amarus* remained relatively calm and sedentary at the bottom of the tank.

Within 13 h post-injection, fish within the *placitus x amarus* treatment began spawning behavior (see aquarium on right in video Segment 3). Eggs were noted within seconds of the first spawning encounter with the second *H. amarus* swimming through and eating the eggs. Subsequent spawning episodes were noted by an initial and aggressive (sometimes violent) pursuit by the male *H. amarus* of the female *H. placitus* that would end in a rapid "wrap and release" that

were often difficult to observe (see aquarium on right in video Segment 4). These spawning episodes of the *H. placitus* and *H. amarus* were in sharp contrast to the less aggressive "wrap and release" described above in the *placitus x placitus* spawning episode seen in Video Segment 1. By 24 hours post-injection, the *placitus x amarus* treatment resulted in only several hundred eggs (not enumerated). The hatch resulted in very few larvae that did not survive past 24 h post-hatch and the female *H. placitus* died within 5 days post-injection. Necropsy revealed ovaries containing cloudy white eggs being resorbed indicating that she was perhaps beyond the optimum reproductive window for viable gametes. Necropsy of fish remaining in the tank at the end of the study revealed one male *H. amarus* with well developed testes and one female *H. amarus* containing eggs. The female *H. amarus* was small (70 mm) and thus mistaken for a male. Videography did not reveal spawning behavior between the two *H. amarus* adults. Thus, the eggs most likely belonged to the female *H. placitus*.

Post-Spawning Observations

Within 72 h post-injection, we began to observe mortality in adult fish that varied between the two species and genders. By the end of the study (24 d post-spawn), 17 of 60 adult fish (28.3%) had died. Of these fish, 14 (82%) were female and distributed equally between *H. amarus* (7) and *H. placitus* (7). In contrast, one male *H. amarus* and two male *H. placitus* died prior to the end of the study resulting in 17% mortality for male fish. Prior to death, female fish exhibited extremely swollen and distended abdomens. The majority of these fish had not spawned or spawned with very few eggs that were not fertilized. Three female fish were euthanized due to extensive fungal growth.

At the end of the study, remaining adults were euthanized in a lethal dose of anaesthetic (approximately 200 mg/L) and necropsied to confirm gender and general health status. Necropsies were performed on fish that had died throughout the study. The necropsies revealed seven of the 20 aquaria had an incorrect ratio of two female to one male fish. Aquaria containing two female fish were the result of a fish having undeveloped ovaries and thus being mistaken for a male. None of the aquaria had either all male or all female fish.

Necropsies performed on fish remaining at the end of the study revealed the majority of the female fish of both species were feeding (indicated by the yellow-green tint of the gall bladder). In these fish, the general condition of the liver was considered normal and was in contrast to fish that had not resumed feeding indicated by the dark green tint of the gall bladder, hemorrhage and necrosis of the liver. Ovaries within individual female *H. amarus* and *H. placitus* were packed and contained eggs of various sizes and color from clear to cloudy and in later stages of resorption. Eggs also varied in size becoming smaller anteriorly. It is unknown why these fish did not spawn at the time of injection. The majority of male *H. amarus* had well developed testes and were feeding regularly with internal organs and tissues in generally good condition at the time of the necropsy. In contrast, a few male *H. placitus* had not been feeding. In these fish, the internal organs appeared necrotic. It is interesting to note, of the aquaria that resulted in a successful spawn and hatch, no mortality in the adults was observed.

Although necropsies performed on fish that died prior to the end of the study did not reveal the cause of death, they did provide some diagnostic information for future consideration. The majority of female fish that died prior to the end of the study had enlarged and discolored abdomens. Ovaries in the majority of the female *H. amarus* and *H. placitus* were extremely full of eggs from clear to cloudy and in later stages of resorption. In several fish, eggs were loose within the abdominal cavity prior to death. It is important to note the majority of these female fish either did not spawn, released very few eggs that were not fertilized, or resulted in a hatch in which no larvae survived to 48 h post-hatch.

DISCUSSION

Hybridization occurred between two closely related species of *Hybognathus* in an experimental setting. Spawning (production of eggs and larvae) was observed in both *Hybognathus* crosses (*amarus x placitus*, *placitus x amarus*). However, either hatch did not occur among hybrid crosses or the hybrid larvae died shortly after hatch. Is the loss of hybrid progeny the result of their lack of fitness (i.e., behavioral differences related to genetic incompatibility) or the result of poor timing and thus a reduction in gamete viability?

Our observations of reproductive behavior in both *Hybognathus* hybrid crosses indicate discordant or incongruous interaction between male and female fish. During spawning encounters, the male fish of both species exhibited extreme aggression that was at times violent in an attempt to spawn. Although our observations are based on a small sample size of each cross (one replicate per treatment), the spawning behavior was in contrast to the classic "wrap and release" behavior observed in both intraspecific crosses (Platania and Altenbach 1998). Nonetheless, hybrid progeny resulted from both *Hybognathus* crosses.

In an attempt to address the latter question of poor timing and thus reduced gamete viability, an *impromptu* study was initiated 29 May 2002 (day 6) and run concurrently to the original study. Twenty-two adult *H. amarus* and 22 *H. placitus* were injected with CPE to obtain progeny from (female parent indicated first) *amarus x amarus, placitus x placitus* and the two reciprocal crosses (*amarus x placitus, placitus x amarus*). The experimental design included two replications of the *amarus x placitus* and *placitus x amarus* cross and one replication each of the *amarus x amarus* and *placitus x placitus* cross. The purpose of this *impromptu* study was a close inspection and selection of female fish of both species appearing and thus presumed in optimal reproductive condition (i.e., not having grossly enlarged abdomens). Based on earlier observations, female fish began to exhibit enlarged abdomens in March 2002. As a result, we presumed egg resorption had begun to occur at the time of the study in mid-May resulting in the poor egg quality or the inability of the fish to release eggs. Of the four crosses, one *amarus x amarus* cross and one *amarus x placitus* cross resulted in a successful spawn. Of these, only the *amarus x amarus* treatment resulted in a hatch

with 18 larvae surviving through the end of the study. None of the eggs in the *amarus x placitus* cross hatched. None of the crosses having female *H. placitus* resulted in a spawn. Although anecdotal, these results indicate that egg viability may have been compromised by the timing of the study with the majority of female *H. placitus* as well as some *H. amarus* beyond the optimum window for gamete viability.

One additional consideration for poor reproductive performance or reduced gamete viability would be the timing and dosage of the CPE that was used to induce egg maturation, ovulation, and spawning. Egg maturation and ovulation in female fish is under the control of the hypothalamo-pituitary-gonadal axis which coordinates rapid responses to environmental cues (Redding and Patiño 1993). The teleost pituitary secretes gonadotropins to stimulate biosynthesis of gonadal steroid hormones which, in turn, mediate egg maturation and ovulation (Nagahama 1994). Injections of CPE essentially by-pass and/or augment the release of the gonadotropins which would, in a relatively short period of time, induce rapid growth and maturation of developing oocytes as a prerequisite for ovulation and eventual fertilization. The poor reproductive performance, aided by necropsies, indicated an excessive amount of CPE may have resulted in the majority of the developing oocytes to synchronously mature (personal communication: R. Patiño). This would result in the inability of eggs to pass through the oviduct for release. The adult female fish would appear as "egg-bound" with grossly distended abdomens that ultimately (from 4 to 8 days) resulted in death of the adult.

Both Lehtinen and Layzer (1988) and Taylor and Miller (1990) observed prolonged spawning seasons of *H. placitus* in the Cimarron River, Oklahoma. Both relied on gonadal weights and ovum diameters to identify the reproductive seasons which presumably began late April and ended in July or August with peak reproductive condition of the fish in June (well within the timeframe of our study reported here). Although gonadal indices of *H. placitus* correlated well with proximal environmental cues such as temperature and stream flow, the authors did not observe spawning nor did they collect eggs. Taylor and Miller (1990) relied on the presence of young-of-the-year to reflect timing of spawning in previous years. We received *H. placitus* late March of 2002 and observed by late April female fish developing distended abdomens indicating gonadal development. In one female *H. placitus*, the ovaries represented 40% of her body weight.

Necropsies performed on these fish mid-June revealed packed ovaries with some variation in egg size (eggs becoming smaller anteriorly) and a degree of egg deterioration and resorption occurring. Thus, we believe *H. placitus* was outside the window for optimum reproduction (i.e., producing viable eggs).

SUMMARY AND CONCLUSIONS

The purpose of the study was to determine the hybridization potential of two *Hybognathus* species in controlled conditions. Although we observed spawning behavior and subsequent hybridization between the two species, we believe the results to be somewhat anecdotal and thus inconclusive. Success in obtaining hybrid progeny was confounded with poor reproductive condition of female *H. placitus*. Thus, we believe it necessary to ensure that appropriate timing for reproduction is achieved to ensure optimal spawning success in both species. This would optimize the chances of obtaining viable hybrid progeny and thus determine the potential for hybridization to occur in the wild.

RECOMMENDATIONS FOR FUTURE STUDIES

An additional series of hybridization experiments would address several key concerns observed in this study. Briefly stated, additional tests would increase the potential for spawning success by selecting ripe and running male fish and carefully select against female fish with grossly distorted and extended abdomens (eggs presumed beyond optimum condition for fertilization). In addition, behavioral tests would be conducted in a series of replicates which would ensure maximum observational information. In other words, the tests would be designed to collect specific but descriptive behavioral information in replicate. This could be accomplished using a series of digital video cameras of broadcast quality to capture specific events. It is recommended that one male *H. amarus* and one male *H. placitus* be added to an aquarium containing either a female *H.*

amarus or *H. placitus*. This may provide behavioral information on the success of conspecific interaction and selection.

The Rio Grande silvery minnow has had a relatively unstable taxonomic history since it was first described by Girard in 1856. Species within the genus *Hybognathus* are morphologically similar and thus are difficult to separate using classical taxonomic methodology. Differences in the filtering apparati for particles or food were used to separate congeners within the genus (Hlohowskyj et al. 1989). Cook et al. (1992) identified several allozyme alleles that were specific to *H. amarus* separating it from its closest congener, the Mississippi silvery minnow (*H. nuchalis*). In a comprehensive study, Bestgen and Propst (1996) separated the Rio Grande silvery minnow from the plains minnow and the Mississippi minnow using extensive morphometric and meristic analyses. Although these analyses can be useful in identifying physical traits that are species specific, often these measurements are unable to distinguish hybrids with a great deal of certainty. Conservation biologists now have access to unique genetic tools that are relatively inexpensive and fast with respect to identifying genetic divergence and gene flow at the population level. Microsatellite DNA markers (repeating arrays of simple sequences that have high mutation rates) can be used for genetic monitoring to resolve taxonomic uncertainties and to identify genetically distinct populations and provide the necessary tool to assess and conserve genetic diversity of populations. They can be used to infer phylogenetic relationships, population structure, effective population size, impacts of captive-reared stocks on wild populations, hybridization among taxa, and to evaluate environmental stochasticity on rate of extinction. Thus, an important addition to this work would be to establish a genetic baseline using genetic markers from *H. amarus* from the Rio Grande throughout New Mexico, *H. placitus* from the Canadian and Pecos rivers, and *H. nuchalis* from the most proximate populations in Texas (such as the upper Red River and the lower Brazos River). Once hybrid larvae are obtained, diagnostic genetic markers can be used to assist in identifying hybrids of the two species in the wild. With hybrid progeny, morphological features (e.g., basioccipital process, filtering apparatus) would be identified using digital imagery on individuals from each group (see Bestgen and Propst 1996). This information would facilitate characterizing the morphology and meristics of *Hybognathus* interspecific hybrids obtained in the wild.

LITERATURE CITED

Bestgen, K.R. and S.P. Platania. 1991. Status and conservation of the Rio Grande silvery minnow, *Hybognathus amarus*. The Southwestern Naturalist 36:225-232.

Bestgen, K.R and D.L. Propst. 1996. Redescription, geographic variation, and taxonomic status of Rio Grande silvery minnow, *Hybognathus amarus* (Girard, 1856). Copeia 1996:41-55.

Cook, J.A. K.R. Bestgen, D.L. Propst, and T.L. Yates. 1992. Allozymic divergence and systematics of the Rio Grande silvery minnow, *Hybognathus amarus* (Teleostei: Cyprinidae). Copeia 1992:36-44.

Goede, R.W. and B.A. Barton. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. Pages 93-108 in S. M. Adams, editor. Biological indicators of stress in fish. American Fisheries Society, Symposium 8, Bethesda, MD.

Hlohowskyj, C.P., M.M. Coburn, and T. M. Cavender. 1989. Comparison of a pharyngeal filtering apparatus in seven species of the herbivorous cyprinid genus, *Hybognathus* (Pices: Cyprinidae). Copeia 1989:172-183.

Hoff, F.H. and T.W. Snell. 2001. Plankton culture manual. Florida Aqua Farms, Inc., Dade City, Florida. 162 p.

Hontela, A. and N.E. Stacey. 1990. Cyprinidae. In: Munro, A.D. Scott, A.P. and Lam, T.J., editors. Reproductive seasonality in teleosts: Environmental Influences. CRC Press, Inc., Boca Raton, Florida. Pp. 53-77.

Lehtinen, S.F. and J. B. Layzer. 1988. Reproductive cycle of the plains minnow, *Hybognathus placitus* (Cyprinidae), in the Cimarron River, Oklahoma. The Southwestern Naturalist 33:27-33.

Levings, G.W., D.F. Healy, S.F. Richey, and L.F. Carter. 1998. Water quality in the Rio Grande Valley. U.S. Geological Survey, Denver. Colorado.

Nagahama, Y. 1994. Endocrine regulation of gametogenesis in fish. International Journal of Developmental Biology 38:217-229.

Platania, S.P. and C.S. Altenbach. 1998. Reproductive strategies and egg types of seven Rio Grande basin cyprinids. Copeia 1998:559-569.

Redding, J.M. and R. Patiño. 1993. Reproductive physiology. In: Evans, D.L. editor. The Physiology of Fishes. CRC Press, Inc. Boca Raton, Florida. Pp. 503-534.

Sublette, J., M.D. Hatch, and M. Sublette. 1990. The Fishes of New Mexico. University of New Mexico Press, Albuquerque, New Mexico.

Taylor, C.M. and R. J. Miller. 1990. Reproductive ecology and population structure of the plains minnow, *Hybognathus placitus* (Pices: Cyprinidae), in Central Oklahoma. American Midland Naturalist 123:32-39.

U.S. Fish and Wildlife Service. 1999. Rio Grande silvery minnow recovery plan. Fisheries Resources Office, Albuquerque, NM. 141 p.