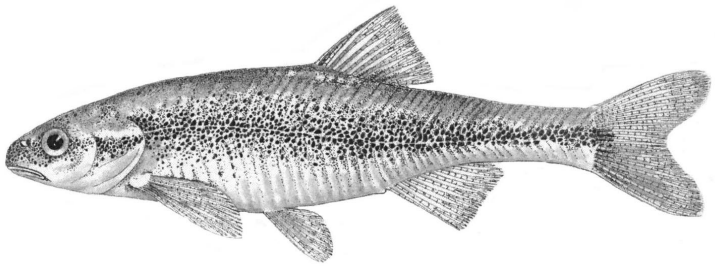
Captive Propagation and Rearing of Spikedace, Meda fulgida



Final Report

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23 August 2004

frontispiece: *Meda fulgida*, spikedace, illustration by
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EXECUTIVE SUMMARY

The primary objectives of this 2001-2003 study were to develop propagation techniques for spikedace, *Meda fulgida*, in the laboratory and provide recommendations for adaptation of those techniques to hatchery production. Adult spikedace collected during 2001-2003 from the Gila River, New Mexico, were transported to the laboratory where they were treated for diseases and parasites and subsequently used in attempts to induce spawning. Age 2 spikedace were the principal cohort used in this study due to the lack of spawning by age 1 individuals in initial trials and the difficultly of accurate gender determination in fish of that age and size.

Wild spikedace in this study proved extremely susceptible to outbreaks of *Ichthyophthirius multifiliis.* A health management protocol, designed to treat and control this parasite while minimizing fish mortality, was developed and implemented during the course of this investigation. In addition, a protocol for successful transport of specimens was also developed and tested. Both of these health management regimes will be necessary and extremely valuable in future artificial propagation efforts of spikedace.

At least 30 spikedace spawning events were documented during 2002-2003 with the reproductive effort occurring February through July. Attempts to induce spawning through the injection of selected hormones into reproductively active spikedace were not successful. Conversely, manipulation of environmental variables (primarily photoperiod and water temperature) in aquaria often resulted in spawning by this species. While the aforementioned environmental manipulation portion of the study was successful (i.e., spikedace spawned), those results were inconclusive as spikedace in control aquaria spawned as often and readily as those in test aquaria.

This study showed that spikedace are communal, broadcast spawners that produce small (2 mm diameter) demersal, adhesive eggs. Spawning occurred over several days and appeared to involve multiple spawning events by individual fishes. Female spikedace did not exhibit a preference for substrate type or structure at the time of egg release.

Fertilized eggs settled in the interstices of the substrate where, at 19°C, they hatched in approximately seven days. Upon hatching, spikedace were about 5 mm TL and had a relatively small yolk-sac. Development of the swim bladder and initiation of exogenous feeding occurred about two-three days post-hatching. Laboratory-spawned spikedace progeny ranged in length from about 30-45 mm SL about one year after hatching.

Results of this study suggest that spawning and rearing of spikedace could also be implemented on a larger scale such as is available in highly regulated and controlled endangered fish hatcheries. Knowledge gained from this study suggest that holding, spawning, and rearing of spikedace is a labor intensive effort especially compared to long-lived, large-bodied fish such as Colorado pikeminnow, *Ptychocheilus lucius*, or razorback sucker, *Xyrauchen texanus*. Conversely, the number of young produced from any individual or lot of individuals will be extremely low, compared to the aforementioned species, due to the relatively small size of spikedace. These items (high maintenance and low fecundity) are only two of many issues common to hatchery production of short-lived, small bodied fishes. At present, the primary issues to be addressed in attempts to produce spikedace in a hatchery setting (as identified in this study) are parasite and disease control, replicating natural photoperiod to stimulate spawning, and providing a means to separate adults from eggs to minimize egg cannibalism.

This study provided important fundamental information regarding aspects of artificial propagation of spikedace. It also resulted in the development of conditions and protocols necessary to hold and rear both adult and juvenile spikedace while minimizing mortality. Many additional avenues of spikedace reproductive biology still need to be investigated if the goal of artificial propagation of this species for repatriation to the wild is to be achieved. We believe the results of this study provide basic information necessary for development of the initial framework necessary to achieve this goal.

INTRODUCTION

Plagopterini, or spine-fins, are comprised of six species in three genera and are endemic to watersheds drained by the lower Colorado River in Utah, Nevada, Arizona, and New Mexico. Of the three Plagopterini genera, *Lepidomeda* is represented by four species (spinedace) while both *Meda* (spikedace) and *Plagopterus* (woundfin) are monotypic genera. Members of this tribe of fishes (Plagopterini) are characterized by spine-like dorsal and pelvic fin rays and bright silver body coloration.

Spikedace, *Meda fulgida* Girard, is a small, laterally compressed obligate stream cyprinid that reaches a maximum total length of 68-70 mm (U. S. Fish and Wildlife Service, 1990; Minckley, 1973). Scales on this species are markedly reduced and deeply embedded, the eyes relatively large and mouth terminal. Spikedace are silvery to brassy on lateral surfaces, white ventrally, brown or olive dorsally, and mottled by irregular black speckles on the dorsal and lateral surfaces. Breeding adult spikedace are tuberculate, and breeding males exhibit yellow coloration at fin bases and lateral surfaces.

Spikedace is endemic to the Gila River drainage in southwestern New Mexico and central and southeastern Arizona, and possibly northern Sonora (Koster, 1957; Minckley, 1973). While thought to have been abundant throughout its historical range, spikedace populations currently persist only in the upper Verde River, Eagle Creek, and Aravaipa Creek in Arizona (Marsh et al., 1991) and in low and declining numbers in the East Fork Gila River and West Fork Gila River in New Mexico (Propst, 1999). This taxon is a species of special concern in Arizona (Arizona Game and Fish Department, 1996), threatened (Category I) in New Mexico (Propst, 1999), and federally threatened throughout its range (U. S. Department of the Interior, 1986).

Habitats occupied by spikedace vary seasonally and by ontogenetic stage (Propst et al., 1986). Adult spikedace tend to be most frequently collected in shallow riffles and runs of moderate depth, whereas larval and juvenile individuals are more commonly associated with quiet, shallow waters or pool margins (Propst et al., 1986; Rinne, 1991). Adult spikedace feed primarily upon ephemeropteran nymphs, while juveniles prey largely upon dipterans (Schreiber and Minckley, 1981; Barber and Minckley, 1983; Propst et al., 1986).

Spikedace reproduce during mid-spring and early summer depending upon spring runoff (magnitude and duration), water temperature and velocity (Barber et al., 1970; Propst et al., 1986). During the reproductive season, gravid female spikedace move from pools to riffle areas occupied by males. Each female may be attended by several males and paired spawning is rare (Barber et al., 1970). Spawning occurs mid water column over sand and gravel bottoms, and adhesive demersal eggs remain to develop in the substrata (Barber et al., 1970; Rinne, 1999). Spikedace life expectancy has been reported up to age 4, but age 1 individuals were most common in the Cliff-Gila Valley reach of the Gila River sampled by Propst et al. (1986) while the breeding populations in Aravaipa Creek consisted primarily of two age classes (Barber et al., 1970). Lengths (39-59 mm) of Aravaipa Creek spikedace reported by Barber et al. (1970) suggest those were age 1 and age 2 individuals.

Reduction in spikedace range and abundance due to loss of habitat and deleterious interactions with nonnative aquatic fauna prompted investigation of alternative management strategies that might contribute to persistence of remaining spikedace populations. Development of a captive propagation and repatriation program was one such strategy. Rearing and propagation techniques are lacking for Plagopterini and did not exist prior to this work for spikedace. The general objectives of this work (Table 1) were to develop successful holding, spawning, and rearing techniques for spikedace in the laboratory and provide recommendations for adapting those techniques to hatchery propagation.

Table 1. Study objectives of Federal Grants 01-FG-32-0030 and 03-FG-32-0020 entitled: "Development of Propagation Techniques for Spikedace."

STUDY OBJECTIVES	DETAILS
1. GENERAL OBJECTIVES	
A	Perform field work and submit feasibility report detailing all aspects of the first 12 months of research, assess the probability of successful fulfillment of the overall goals of the project.
B	Provide detailed methods for successful propagation of spikedace in the laboratory and recommendations for adapting results to hatchery production
2. SPECIFIC OBJECTIVES	
A	Assemble and review published and gray literature dealing with husbandry and reproduction of Plagopterini
B	Visit streams occupied by reproducing populations of spikedace and attempt to observe courtship and spawning, determine habitat variables associated with spawning, and acquire live specimens
C	Through experimentation, develop and document conditions required to hold and maintain spikedace in artificial laboratory systems
D	Through experimentation, develop and document conditions required to spawn spikedace in artificial laboratory systems
E	Investigate the efficacy of hormone induction of spawning if "natural" reproduction cannot be elicited
F	Develop and document conditions necessary for incubation, hatching, and normal growth of young spikedace through juvenile life stage
G	Produce annual and final reports for review by Reclamation that document all phases of the study

LITERATURE REVIEW: REPRODUCTION AND HUSBANDRY OF PLAGOPTERINI

Plagopterini as defined by Miller and Hubbs (1960) is comprised of three genera and six species of western cyprinids belonging to the subfamily Leucisinae: *Lepidomeda albivallis* Miller and Hubbs, *L. altivelis* Miller and Hubbs, *L. mollispinis* Miller and Hubbs, *L. vittata* Cope, *Meda fulgida* Girard*,* and *Plagopterus argentissimus* Cope. Nearly all Plagopterini taxa are federally listed as threatened or endangered and the Pahranagat spinedace (*L. altivelis*) is extinct. There has been debate whether the tribe should include *Gila copei* (Jordan and Gilbert)*,* which would require the resurrection of the monotypic genus *Snyderichthys.* Dowling et al. (2002) reported results from parsimony analysis of cytochrome *b* sequences that suggest *G. copei* should be included in the Tribe Plagopterini. This supports the conclusions of DeMarais (1992) and Simons and Mayden (1997). As this relationship remains somewhat tenuous and is peripheral to the scope of this study, *Snyderichthys (G. copei)* is not included in this discussion.

Plagopterini are characterized by spinelike ossification of the first two dorsal and pelvic fin rays. This character is most extreme in *Meda* and *Plagopterus* and less evident in *Lepidomeda* (Miller and Hubbs, 1960). In addition, the number of pelvic rays is reduced (generally seven or less) as compared to that (eight) usually present in North American cyprinids (Miller and Hubbs, 1960). *Meda* and *Plagopterus* are considered the more specialized genera of the tribe and *Lepidomeda* the more ancestral genus.

Members of the genus *Lepidomeda* (spinedace) spawn in late spring and sporadically throughout the summer. *Lepidomeda vittata*, Little Colorado spinedace, spawn in May-June usually after spring runoff and before monsoon events (Robinson et al., 2003). Spawning by this species is apparently initiated in response to environmental cues such as water temperature, flow regime, and increased photoperiod. Nuptial coloration ranges from milky yellow in Little Colorado spinedace to orange or red in *L. mollispinis*, Virgin spinedace. Similar to the other Plagopterini, female spinedace move into riffle areas from downstream pools (Blinn et al., 1998).

Spikedace reproduce from mid-spring through early summer and depend upon environmental cues such as water temperature and velocity to initiate spawning (Anderson, 1978; Barber et al., 1970; U. S. Fish and Wildlife Service, 1990; Propst et al., 1986). Male spikedace develop a yellow breeding coloration early in the breeding season and both sexes are tuberculate (Barber et al., 1970). Male spikedace do not appear to be territorial, but patrol shallow riffle areas awaiting the arrival of gravid females from downstream pools, which they attend singly, or in groups (Barber et al., 1970; U. S. Fish and Wildlife Service, 1990; Propst, 1999). Female spikedace broadcast adhesive, demersal eggs which remain unattended in the substrate after fertilization (Barber et al., 1970; U. S. Fish and Wildlife Service, 1990).

Plagopterus argentissimus, woundfin, appears to depend upon water temperature increase, runoff decline, and increased photoperiod to initiate spawning (U. S. Fish and Wildlife Service, 1994). Spawning in this species occurs from April through August (Williams, 1995). Male woundfin develop a pinkish to red hue during the breeding season. Spawning behavior appears to be very similar to that of spikedace with female woundfin moving from pools to riffle reaches attended by patrolling males. Some spawning by woundfin may also occur in areas that offer cover when water velocities are high (U. S. Fish and Wildlife Service, 1994). As with spikedace, eggs of woundfin are adhesive and demersal.

Blinn et al. (1998), in their 1992-1995 study of reproduction and growth of captive Little Colorado spinedace held in an earthen pond/stream near Flagstaff, Arizona, provided

the most extensive account of Plagopterini reproductive ecology to date. Details from that publication follow. Spinedace used in their study originated from Rudd Creek, Arizona, and were assumed to be age 1 (based on lengths) when transplanted to the pond in 1992. The 1,408 m² surface area pond had a maximum depth of 3.6 m and was connected to a stream (gravitational flow) created by recirculation of pumped pond water. There was no mention of a water filtration system or supplemental feeding of study specimens.

Male Little Colorado spinedace became reproductively active (ripe) in late April, before female spinedace, at water temperatures (surface) about 16°C. By early May, surface water temperatures consistently remained >16°C while water temperature in the lower 2 m of the pond had risen to about 13°C. At that time, 75% of male and 98% of female Little Colorado spinedace sampled were reproductively capable. Blinn et al. (1998) noted that male spinedace were ripe at about 70 mm TL while female spinedace were typically not gravid until they had achieved a length of about 80 mm TL.

Reproduction by Little Colorado spinedace was observed in the artificial stream 16-20 May 1994 and in early June 1995. Spawning runs of spinedace into the artificial stream were observed only during daytime (night surveys were conducted) and often occurred when the stream mouth was in direct sunlight. Stream water temperatures during these events ranged between 19-22°C and spinedace were rarely observed in the stream prior or subsequent to spawning events.

Little Colorado spinedace spawning was preceded by a subset of adult spinedace (10-40 fish) separating from larger schools in the pond and ascending the artificial stream where a group (10-15) of male spinedace would engage a single female spinedace. Males in the aggregation would continually bump and nip the vent region of the female with prespawning events usually occurring over gravel substrata. Blinn et al. (1998) reported the presence of strong secondary sexual characters in spawning fish in these studies including high levels of tuberculation and bright coloration.

A Little Colorado spinedace spawning event involved a single male and female and included rapid posterior movements, twisting around each other, and dorsal-ventral inversions. On occasion, spawning male spinedace would insert their nose in the substrata while their caudal fin rose above the waters surface. Spawning bouts typically terminated after 10-20 minutes. Mature stripped Little Colorado spinedace eggs were adhesive, 1.0-1.3 mm diameter, and yellow to orange-red in color. Conversely, immature eggs of this species ranged from white to a pale yellow.

Egg deposition by Little Colorado spinedace appeared to occur in relatively specific substrata. Spawning did not occur in (or on) aquatic vegetation, artificial stream reaches with fine sediment or gravel >25 mm diameter, or any of the gravel trays researchers had placed in the littoral zone of the holding ponds. Reproduction occurred over substrata containing gravel 2-16 mm (mean 6.4 mm ± 0.37 mm). Mean water velocity in areas selected for spawning was 14.2 cm/sec with mean water depth 3.8 cm. Blinn et al. (1998), using population size structure of young-of-year Little Colorado spinedace produced in their study, estimated up to three discrete spawning periods during both the 1994 and 1995 May to mid-June reproductive seasons.

Larval Little Colorado spinedace were observed about five days post-spawning and were 6-7 mm TL. Larval and juvenile spinedace did not remain in the artificial stream but instead moved into the pond and clustered in the littoral zone near aquatic vegetation, especially floating mats of algae. Once spinedace had achieved a length of about 20 mm TL, they tended to move into deeper water habitats in the pond. Schooling behavior was noted in the earliest developmental stages and was maintained throughout their life. Most

Little Colorado spinedace appeared to spawn at age 2 and, according to Blinn et al. (1998), live at least four years.

Bryan et al. (2002) maintained captive Little Colorado spinedace for a study of behavioral responses to predators. Those fish were held in 90-l aquaria containing untreated well water and not fed 24-h prior to behavioral experiment trials. Experiments were conducted in 530-l artificial streams and substrate used was 10 mm diameter gravel arranged to provide a variety of depths while gray translucent plexiglass plates provided cover. Water was recirculated through a charcoal filter and chilled to 13-15°C. Timed control of overhead lighting allowed a photoperiod of 14-h light and 10-h dark. They did not report any additional information related to holding or rearing procedures of Little Colorado spinedace (Bryan et al., 2002).

Robinson et al. (2003) used the same system and environmental controls as Bryan et al. (2002) in their investigation of habitat use by rainbow trout and Little Colorado spinedace. Water temperatures for this latter experiment were 17-19°C and a photoperiod of 14-h light and 10-h dark was maintained using fluorescent and incandescent lighting. Little Colorado spinedace used by Robinson et al. (2003) were fed flake food twice daily. No additional details of holding or rearing methodology were provided.

Spikedace were kept in captivity for use in swimming performance and behavioral studies on native and nonnative fishes of Arizona conducted at the Environmental Research Laboratory in Tucson, Arizona (Ward et al., 2003). They were held in 0.5 m^3 recirculating tanks at (20°C ± 2°C) for at least 24-h prior to testing, and were tested within 72-h of capture in a recirculating swim tunnel in water maintained at 20°C ± 1°C. Ward et al. (2003) found that swimming abilities of native and nonnative cyprinids were very similar and therefore not adequate to explain the disproportionate displacement of nonnative fishes during flood events.

Extensive efforts to induce captive-controlled reproduction and to develop methods for rearing of woundfin were initiated in 1979 at Dexter National Fish Hatchery and Technology Center (formerly Dexter National Fish Hatchery), Dexter, New Mexico. Captive reproduction in woundfin was first recorded in 1982. Woundfin have been held and reared at this facility in ponds, raceways, and fiberglass and glass aquaria. Holding temperatures ranged from about 18°C to about 34°C (Williams, 1993). In 2003, woundfin were transferred to indoor facilities during winter (January to March) and returned to outdoor ponds to spawn in April. Those ponds were equipped with screened baskets containing cobble spawning substrata and woundfin fed a commercially available trout diet (Williams, 1993). No additional information regarding this study were available.

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MATERIALS AND METHODS

Collection of spikedace

The New Mexico site for spikedace collection was the Gila River reach that flows through the U. S. Forest Service "Bird Area" property. The locality descriptor used in the Division of Fishes, Museum of Southwestern Biology (MSB) database is:

> New Mexico, Grant County, Gila River, ca. 0.5 river miles
> downstream of its confluence with Moonhull Canyon and
> ca. 8.8 road miles south of Riverside on Forest Road 809 at
> the U. S. Forest Service "Bird Area," Gila National Forest;
> UTM Coordinates 724003 easting; 3634222 northing; zone 12.
> (The MSB Accession number dedicated to this project is: Acc 2001-IV:13)

Spikedace were collected with a 3.05 m x 1.83 m x 4.76 mm mesh (10 ft x 6 ft x 3/16 in mesh) seine, sorted, counted and placed into plastic 19-l buckets (containing about 10 l of river water) while further collections were made. When possible, water quality was assessed using Yellow Spring Instruments (YSI) water quality meters and commercial water test kits (Table 2). A backpack electrofishing unit was taken on the first three sampling trips but was not used as seining always provided a sufficient number of study specimens.

Six collections of wild spikedace were obtained from 2001-2003. Each collection of individuals was designated, for tracking purposes, as a "Population." In this report "Population" is capitalized to distinguish its use as a tracking aid versus use of the term in its literal sense. Numbers (Roman Numerals) were sequentially assigned to Populations (i.e., Population I was the first lot of spikedace collected for the study) with the exception of the lot of Aravaipa Creek spikedace which was designated Population VI. Aquaria Identification Codes also incorporated the Roman Numeral Population identifier (i.e., Aquaria II-1 contained Population II spikedace).

Transport Procedures

Individuals were inspected visually prior to transport. Fish determined to have external parasites *(i.e., Lernaea)* or to be unhealthy (including overly stressed) were returned to the river at the study site. Retained specimens were placed in 4 mil polyethylene bags (total volume ca. 19-l) containing oxygenated river water, sodium chloride, and a water conditioner. About one-third of the bag volume was river water while the remaining two-thirds was oxygen (ca. 98% O^2) delivered from a 2.27 m^3 capacity steel oxygen cylinder fitted with a regulator. Non-ionized sodium chloride was added to river water in transport bags at a rate of 4.8 g/l to achieve a solution with a 0.5% (5 ppt) concentration of sodium chloride. Between 20-30 spikedace were placed in each transport bag. Polyethylene bags were carried in ice chests and small amounts of cubed ice added to the ice chests until the temperature of the transport water was 16-18°C.

Addition of sodium chloride to water minimizes loss of salt in fish by reducing the concentration differences between fish blood and water. Lowering the osmotic differences between fish blood and water lowers energy demands on fish which reduces stress and improves survivorship (Carneiro and Urbinati, 2001; Finstad et al., 2003). The natural slime coat of fish is a defense against invasion by bacterial, parasitic, and fungal pathogens. Even minor breaks in the slime coat or epidermis of fish provides a pathway for electrolyte (necessary for osmoregulation) loss thereby increasing stress. Handling fish makes them more susceptible to attack by pathogens and can result in further stress and disease.

Table 2. Selected environmental variables recorded at the spikedace collection site during 2001-2003.

Date: / Population Identifier: / Environmental Variable	2 Apr 2001 I	19 Apr 2001 II	19 Oct 2001 III	30 May 2002 IV	25 Oct 2002 V	12 Jun 2003 VI
Water Temperature (°C)	13	16	13	28	15	23
Air Temperature (°C)	19	21	19	37	22	30
Discharge [2] (cfs: feet^3/sec)	314	204	95	26	61	21
Salinity (ppt)	NA	NA	NA	NA	0.2	0.0
Dissolved Oxygen (mg/L)	NA	NA	NA	NA	6.7	7.4
Conductivity (*m*S)	NA	NA	NA	NA	358.2	NA
Ammonia (mg/L)	NA	NA	NA	NA	0	<0.2
Nitrite (mg/L)	NA	NA	NA	NA	0	<1.0
Nitrate (mg/L)	NA	NA	NA	NA	0	0
pH	NA	NA	NA	NA	7.8	8.8
Number of Seine Hauls	42	27	22	35	26	33
CPSH [3] -shoreline	NA	NA	NA	0-3	0	1-82
CPSH [3] -shallow riffle	NA	NA	NA	0-3	0-1	0-34
CPSH [3] -pool	NA	NA	NA	0	0	0-29
CPSH [3] -midchannel, deep riffle	NA	NA	NA	7-12	1-10	0-11
Total Catch	120	100	198	109	47	375
Transport Mortalities	0	0	0	0	0	NA [2]

NA = not available or not applicable
[1] = no fish were retained
[2] = Mean daily discharge (U.S.G.S. Gila River at Redrocks Gauging Station - # 09431500)
[3] = Catch Per Seine Haul

In an attempt to ameliorate the effects of collection and handling, a water conditioner, commonly known in the field of aquaculture as stress coat (Aquarium Pharmaceutical, Inc.), was added to water in the transport bag at a concentration of 1 ml of conditioner per 15-l of water. This solution forms a synthetic slime coat on fish and serves to protect and promote healing of damaged tissue. In April 2001, a broad-spectrum antibiotic for bacterial infections was also included in transport water as a prophylactic measure. However, that practice was discontinued as medication and treatment of spikedace were more effectively administered in the laboratory.

Captured spikedace were typically examined hourly during the 4-h trip between the collection site and aquatic research facilities at the University of New Mexico (UNM) so that their overall condition could be assessed and water temperature checked and corrected as necessary. Upon arrival at the fish holding facilities, spikedace were acclimated so that the temperature of water in the transport bag could be equilibrated with that of the holding tank (generally 20-30 min). Immediately prior to release into holding tanks, about half of the study specimens were measured (SL) and mean SL of the sample estimated.

Holding

Spikedace were held in 76-l or 189-l glass aquaria that contained air diffusing under-gravel filtration systems, or in one of several 151-l glass holding aquaria plumbed into a recirculating system. Water in the latter system flowed through sand and ultraviolet filtering devices before being pumped back into occupied aquaria. Aquaria and system water were changed at least weekly (ca. 10-30% of total volume) unless reduced water clarity or inadequate water quality dictated increased frequency or volume of water replacement.

There were few differences between holding aquaria environments except during attempts to induce spawning. Aquaria water temperature was generally that of air temperature (19°C-23°C) and pH was typically between 7.8-8.0. Ammonia, nitrite, and nitrate concentrations in aquaria water were maintained at <0.20 mg/l, <0.5 mg/l and <10 mg/l respectively through weekly monitoring of water quality and frequent water changes. Photoperiod varied seasonally but was generally between 10-h and 14-h light.

Spikedace were fed three times per day except during attempts to induce spawning or increase growth of larval fish. Commercial fish foods (spirulina flake food, Banquet™ community flake, Sweetwater™ freshwater zooplankton, Hikari™ bloodworms, and tubifex worms) were primary components of the daily diet. Vitamin supplements (vitamin C, spirulina powder) were added monthly to their diet to augment nutritional requirements.

Attempts to Induce Spawning

Attempts to induce spawning in spikedace focused both on achieving reproduction with the aid of hormone injections and creating conditions in aquaria under which fish would spawn. Fish were taken from aquaria at random for use in the hormone portion of this study. All observations of spawning behavior or presence of eggs or larvae in holding aquaria were noted.

Environmental parameters were manipulated to stimulate gonad maturation and gender specific reproductive activity. The conditions manipulated were water temperature, photoperiod, feeding regime, substrate, and cover availability and density. The primary variables manipulated were temperature, light, and food. In 2002, variables in spawning aquaria were changed on 4 February and maintained until 26 May while in 2003 variables were changed 7 February and maintained until 30 September. In all cases, changes were immediate as opposed to gradual (changes were not phased into existence).

In 2002, water temperatures were increased above ambient (19-20°C) in three experimental aquaria (21°C, 23°C, and 24°C). In 2003, water temperatures were again increased in three experimental aquaria (to 26°C) and reduced to 16°C in a fourth tank. Aquarium water heaters (one per aquarium) were used to elevate water temperatures while two water chillers (per aquarium) were necessary to lower and maintain water temperatures.

Changes in photoperiod were accomplished with suspended (above aquaria) fluorescent lighting systems connected to programmable timers (Figure 1). Natural photoperiod (sunrise-sunset) in Albuquerque (35.084N Latitude) is about 10-h in January, increases about 1-h per month through May (14-h) and peaks at 14.5-h in June. Aquaria in which photoperiod was manipulated were maintained together, away from other aquaria, enclosed in black 4 mil plastic and subjected to 14-h of light. The difference between aquaria in which food was a variable versus control was in total amount and frequency of feeding. Fish in those aquaria in which food was a variable were fed more frequently (four-five times per day) compared to three times per day.

Over 100-l of substrata was gathered from the Gila River study site during April 2001, returned to the laboratory, washed under high water pressure, soaked in commercial bleach for one day, and sun-dried for one week. Dried substrata were sorted and classified by size, following the modified Wentworth scheme for substrate particulate size (Cummins, 1962), and stored in 19-l buckets for use in this study. While the modified Wentworth scheme designates substrata with a diameter between 64-256 mm as cobble, none of the cobble used in this study was >120 mm (diameter) and almost all cobble was 64-100 mm.

Spikedace were presented a variety of substrata, both within and between aquaria, over which to spawn. The area of the aquarium bottom (ca. 1,800 cm^2) allowed for inclusion of considerable amount of substrata. In aquaria that contained both cobble and gravel substrata, the rocks were generally randomly mixed. However, in some of the aforementioned aquaria, rocks were placed so that cobble (with some gravel present in interstitial spaces) covered about half of the bottom of the aquarium and gravel (no cobble present) the remainder. A variety of artificial (i.e., plastic) aquatic plants were presented as single stalks and clusters (covering up to 10% of the aquaria bottom). The potential risk involved with use of live plants in aquaria was not deemed acceptable given the problems encountered with parasitic infestations of spikedace.

Appearance of secondary sexual characteristics was deemed adequate indication of the achievement of the developmental state necessary for initiation of reproduction. Routine inspections of spikedace representing each Population and treatment were made to preclude unexpected spawning events by fish that did not appear gravid or did not express nuptial coloration. This was accomplished by gently applying pressure to the abdomens of randomly selected individuals to attempt to induce expression of gametes.

Hormone injections were administered to spikedace on several occasions during this project (2001-2003). In 2002, the procedure was conducted relatively late in the spikedace reproductive season to avoid interfering with non-hormone induced spawning activity of Population III. The 2002 attempt to induce spawning through the introduction of hormones included individuals from both Populations III and IV.

Administering of hormones to spikedace in 2003 occurred during February, May, June, July, and August. Hormone injections were administered at least once to adult spikedace from Populations IV, V, and VI while most individuals in Populations II and IV received injections on multiple occasions. Populations V and VI contained individuals that exhibited morphological evidence of reproductive maturity (distended abdomens).

Hormone-injected spikedace were anaesthetized in a water-bath containing 20 mg/l tricaine methanesulfonate (MS-222; Finquel™) and given 0.1 cc intraperitoneal injection,

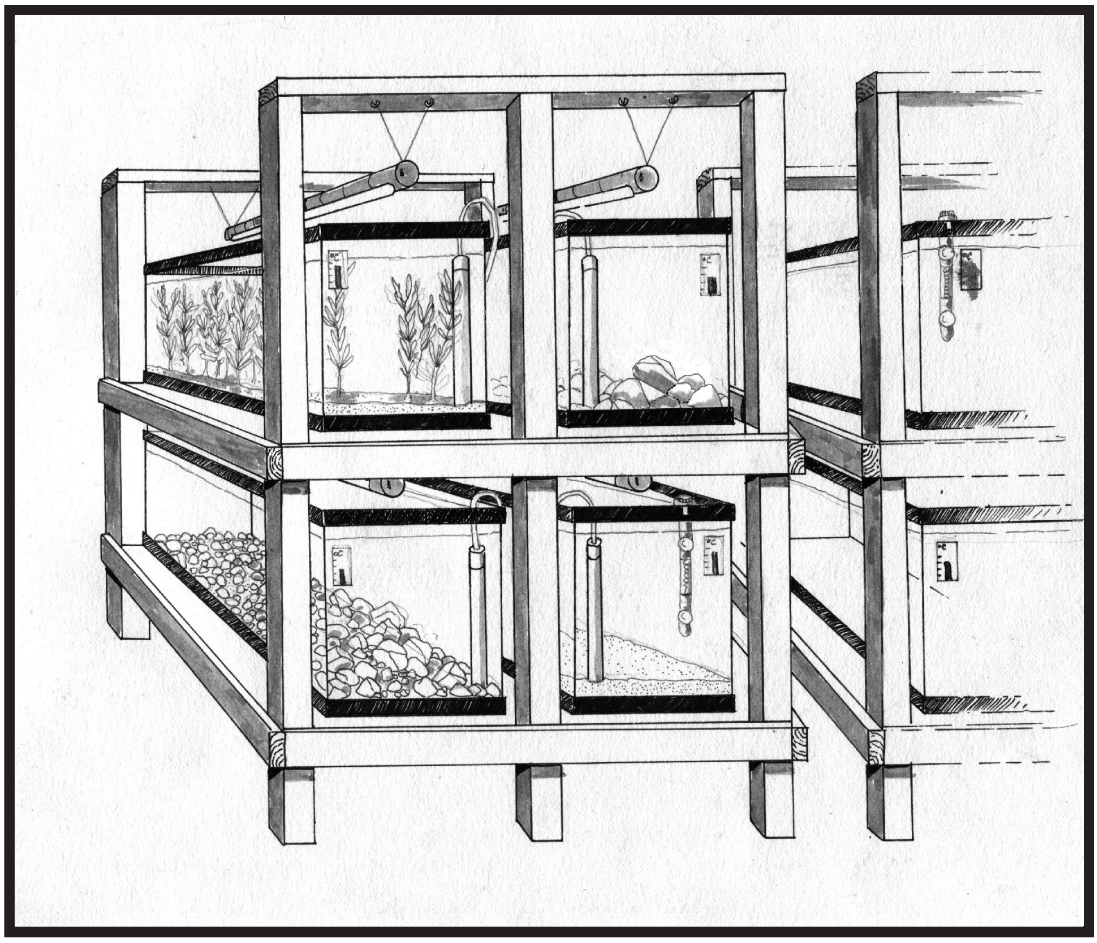

Figure 1. Illustration of spawning aquaria offering variety of substrata, photoperiods, and water temperatures.

immediately anterior to the insertion of the pelvic fin, of stock hormone solution. Stock solutions were produced via suspending or dissolving hormone into either sterilized water or physiologic saline. The following hormones were utilized in hormone induced spawning attempts: carp pituitary extract (CPE) at 125 mg/kg, 250 μg/kg and 500 mg/kg in water and saline solution, salmon pituitary extract (SPE) at 125 mg/kg and 250 mg/kg in water and saline solution, luteinizing-releasing hormone analogue (LH-RH) at 80-160 mg/kg in water, and human chorionic gonadotrophin at 1,000 IU/kg in saline solution. Injections were administered with a 28-gauge 12.7 mm (0.5 in) needle and 1 cc syringe (BD Micro-Fine™ IV Needle and Syringe; available at diabetic supply outlets). Injected spikedace were placed in spawning aquaria at gender ratios ranging from 1:1 to 5:1 (male to female).

When spikedace secondary sexual characteristics were not apparent, gender was determined based on pectoral fin morphology as defined in Barber et al. (1970). In that publication, Barber et al. (1970) determined that the pectoral fin of male spikedace was larger than that of female spikedace. The pectoral fin of male spikedace pectoral fin is wide and fan-shaped as compared to the slender pinnate shape of this fin in female spikedace.

Rearing of larval spikedace

Eggs and larvae of spikedace were recovered from aquaria by siphoning the substrata and straining the effluent though small mesh aquarium dip nets. Propagules were either preserved, transferred to an empty aquarium for later retention in a developmental series, transferred to an empty aquarium for rearing, or left to develop with parental spikedace. Developmental terminology (proto, meso, and metalarvae and juvenile) used in this report follow that of Snyder (1981; Appendix I).

In 2002, larvae were fed commercial fry food (BioKyowa™ fry feed A-250, particle size 250 microns) four or five times per day for the first two weeks of exogenous feeding (about 0.01 g/larval fish/day). They were subsequently fed a combination of BioKyowa™ A-250 mixed with crushed Banquet™ community flakes at a rate of 0.01-0.05 g/larval fish, four or five times per day. Most 2003 larvae were reared under the same conditions, except that their diet was supplemented with live *Artemia* about once per week.

A total of 227 larval spikedace (“J” aquaria series) from March 2002 spawning events were reared under identical conditions (i.e., water volume, chemistry, photoperiod) except that densities ranged from 16-63 individuals per aquarium. This experiment was to determine if there were any noteworthy differences in growth of larval spikedace reared at differing densities. After five months, 15 individuals from each aquarium were removed, at random, and measured (SL). The relatively small number of larval fish available precluded acquiring length measures from all specimens in this ancillary experiment. The effects of handling fish, especially small individuals, were deemed potentially detrimental to those fish which were necessary for other aspects (rearing to adult size) of this project.

A subsample of July 2003 larvae were selected for a cursory feeding trial and fed two different commercial fry foods (Azoo™ artificial rotifer and Cyclop-eeze™ bio-engineered freeze dried cyclops) to ascertain whether one food was more suitable for spikedace than the other. One hundred twenty eggs were distributed among four aquaria (A1: n=40, A2: n=20, A3: n=40, A4: n=20), allowed to hatch, and fed 0.06 g (total per day) of BioKyowa™ A-250. Starting on day-6, fish in aquaria A1 and A2 were fed artificial rotifer while those in the remaining two aquaria (A3 and A4) were presented freeze-dried cyclops. The ratio of fish to fish food mass remained the same between treatments except that the total mass of food per day doubled every week for one month (week 1: 0.12 g/day and week 4: 0.96 g/day) to accommodate fish growth. Larvae were enumerated, measured, and preserved in 5% buffered formalin at the end of this experiment.

RESULTS

Discharge in the Gila River was low during the period of study 2001-2003 (Figure 2) reflecting the severe drought that has enveloped the southwestern United States of America for the past several years. Spring discharge (defined here as 1 March through 31 May) was of short duration and magnitude in 2001 and 2003 and basically absent in 2002. Maximum mean daily spring discharge was 415 cfs in 2001, 327 cfs in 2003, and only 74 cfs in 2002. Mean discharge during the 92 day-period from 1 March through 31 May was 229 cfs in 2001, 141 cfs in 2003, and 52 cfs in 2002.

The six sampling efforts conducted during this project resulted in the collection of almost 1,000 spikedace and retention of about 625 individuals for aspects of this study. Although the 12 June 2003 sample yielded several hundred larval and juvenile spikedace, that sampling effort failed to produce adult spikedace thus none of those fish were retained. No mortalities occurred among spikedace during transport from the field site to the holding facilities.

Almost all individuals from Populations I and II were subjected to hormone injection in an attempt to induce spawning. Repeated attempts to induce spawning of (Populations I; 2 April 2001) spikedace through the use of hormone were unsuccessful. Population I individuals were sacrificed within a few days of the session of the final series of hormone injections so that accuracy of the gender determination effort could be assessed. Spikedace from the 19 April 2001 sample (Population II) were more reproductively active than those of the 2 April 2001 sample and were all age 2 or older. Population II spikedace were subjected to the same experimental protocol as Population I individuals (multiple hormone injections) including sacrifice of specimens to determine gender and reproductive condition.

The final 2001 trip for spikedace (19 October 2001; Population III) resulted in the collection of 198 age 2 spikedace. The purpose of this final 2001 sampling effort was to obtain specimens that could be held overwinter and spawned during spring 2002. In addition, holding of these non-reproductively active specimens would provide an opportunity (during a less critical life-stage) to acquire additional information on captive rearing practices for this species.

More than half of Population III spikedace were lost to parasitic infection. Mortality reached 54% (n=106) during November 2001, with the majority of individuals lost between 6-9 November 2001. This event resulted in a shift in emphasis of the study as it highlighted the immediate need for development of an intensive treatment and management protocol for captive spikedace (see: Treatment for Parasitic Infestation). Development of the treatment protocol allowed subsequent collection of additional specimens that could be held for long periods and provided the opportunity to refine aspects of culture and management. Individuals from the 19 October 2001 (Population III) and subsequent samples (30 May 2002 = Population IV, 25 October 2002 = Population V) comprised the pool of specimens for the remainder of the study and provided progeny used in numerous phases of this investigation (Table 3).

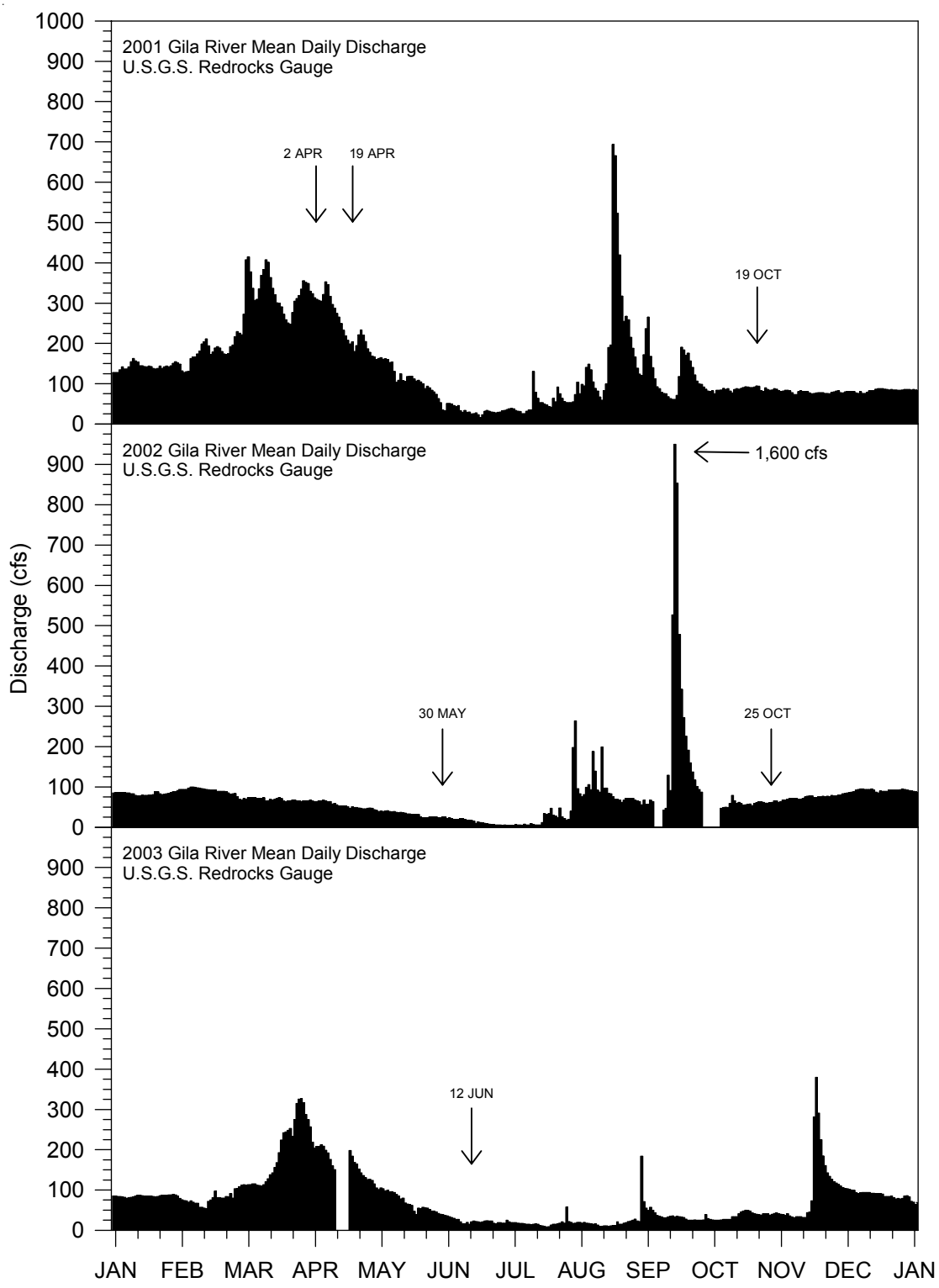

Figure 2. Hydrographs of discharge recorded during calendar years 2001-2003 at the U.S.G.S. Redrocks Gauging Station (# 09431500), Gila River, New Mexico. Arrows indicate sampling dates; blanks indicate gauging equipment malfunction.

Table 3. Growth and survivorship of laboratory adult spikedace populations.

Date Collected	Population III 19 Oct 2001	Population IV 30 May 2002	Population V 25 Oct 2002	Population VI Aravaipa Creek [1]
Mean SL - Arrival	62 mm	48 mm	50 mm	41 mm
Mean SL - Dec 2002	63 mm	64 mm	53 mm	NA
Mean SL - Jan 2004	64 mm	66 mm	60 mm	53 mm
Number of Individuals at Arrival	92	109	47	74
Number of Individuals - Dec 2002	87	107	38	NA
% Survivorship	95%	98%	81%	NA
Number of Individuals - Jan 2004	25	70	22	61
% Survivorship [2]	29%	65%	58%	82%

NA = not available or not applicable
[1] = specimens received 10 January 2003; date of collection unknown
[2] = calculated based on the number of individuals present in Dec 2002 (except Population VI)

Treatment for Parasitic Infestation

An important component of the spikedace health management protocol was the aggressive treatment regime designed to control the outbreak of *Ichthyophthirius multifiliis*. This protocol was first initiated with the 19 October 2001 collection of spikedace and subsequently modified as deemed necessary. A brief narrative of the life-history of *Ichthyophthirius multifiliis* follows to provide a better understanding of treatment rationale.

Ichthyophthirius multifiliis is a well-known single-celled ciliated protozoan with a relatively simple life-cycle that requires 8-14 days to complete at a water temperature of 20°C. The adult stage of this parasite (=trophont) is a white pustule about 0.5-1.0 mm in diameter and generally occurs on the eyes, gills, and epidermis of the host fish where it remains for about 7-10 days post-infestation. After the trophont detaches from the host, it sinks to the substrate where it attaches, encysts, and initiates asexual reproduction (=tomont). Cysts (tomont) rupture soon afterwards releasing large numbers of the infectious stage of the protozoan (=theront). Theronts generally live about 24-48-h but may survive up to 96-h. Upon contacting a fish host, members of this latter stage burrow into host tissue and grow into a pustule completing the life-cycle (Untergasser, 1989). While this parasite is naturally present in fish in the wild, it is a particularly serious parasite under conditions of confinement and high density.

Effective control of *Ichthyophthirius multifiliis* requires treating the water, not the fish, as encysted stages (trophont and tomont) are generally not affected by chemical treatments. The treatment protocol employed reduces the number of host-seeking parasites introduced into aquarium water by infected spikedace. Only free-swimming theronts are susceptible to chemical treatment (i.e., the treatment does not kill either the trophont or tomont cysts already infecting fish).

Epidermal trophonts present on spikedace collected in the Gila River likely detach soon after being placed in laboratory aquaria thereby exposing the study specimens to additional infestation (theronts) as soon as 24-h afterwards. The combined treatment of changing aquarium water and inoculation of the system with formalin is performed to reduce the number and density of host-seeking individuals (theronts) in aquaria. While the transfer of spikedace 24-h following the initial treatment further reduces contact with theronts, even a single trophont still present on a host will start the life cycle of this protozoan in the new aquarium and expose all fish to the host-seeking stage. Most of the theronts remaining in the original aquarium will have died within 48-h because of the presence of formalin and absence of hosts. This treatment protocol effectively and continuously reduces the numbers of infective theronts able to contact fish hosts. The addition of sodium chloride to aquarium water is an osmoregulatory aid to fishes meant to counteract the stress of treatment, handling, and trophont infestation.

The treatment protocol employed in this study required that wild spikedace be placed in and acclimated to holding aquarium water (5 ppt salt concentration) upon arrival from the field. Recently collected fish were provided food within two hours of arrival to initiate a more rapid transition to captive diet and support immunologic function during the stress of acclimation to captivity. Approximately 24-h after arrival (Day 1), a 25% water change was performed in the first holding aquarium, and formalin added to the system at a concentration of 25 ppm. Sodium chloride was added to aquaria water to maintain the concentration of 5 ppt. About 24-h after the first formalin treatment, fish were transferred to a second (recently unoccupied and cleaned) aquarium that is devoid of chemicals except for the 5 ppt concentration of sodium chloride (Day 2). About 24-h after the transfer to the second aquarium, a water change was performed (25%) followed by another formalin treatment (Day 3). Fish were returned to the first holding aquarium about 24-h later (Day 4)

where a 30% water change was done prior to the move (Table 4). This protocol was repeated for about 60 days at which time protocol efficacy was assessed. The treatment protocol was continued if symptoms persisted and reassessed weekly. Following successful implementation of this protocol, measures to manage fish health were limited to maintenance of holding aquaria environments and adherence to feeding schedules.

Several Population II spikedace (19 April 2001) were tentatively (incorrectly) diagnosed with a parasitic trematode (yellow grub) soon after (21 April) arrival at the laboratory. Praziquantel, the recommended treatment for yellow grub, was introduced into selected aquaria at 0.47 mg/1-l of water. Treated spikedace were observed (for signs of stress) for several hours immediately after the introduction of Praziquantel and reassessed the following day. Since the treatment did not appear to have a negative effect on spikedace at the aforementioned concentration, remaining infected specimens were subsequently exposed to this drug. An evaluation of the effectiveness of Praziquantel was not undertaken as, soon after treatment, all Population II spikedace were used in hormone induced spawning attempts and sacrificed soon after those trials.

During late October 2001, numerous Population III spikedace (19 October 2001) exhibited a flashing behavior often indicative of an external irritation. Necropsy of those individuals as well as a detailed examination of Population II specimens thought to succumb to a parasitic infection (yellow grub) revealed the presence of *Ichthyophthirius multifiliis* cysts in the fish tissue. Microscopic re-examination of all Population II (19 April 2001) specimens verified the presence of *Ichthyophthirius multifiliis* and absence of at yellow grub. Because of the aforementioned finding (absence of yellow grub), Praziquantel was subsequently eliminated from the disease and parasite treatment protocol.

Finally, Acriflavine, an antibiotic identified in the literature as effective at killing *Ichthyophthirius multifiliis,* was initially used (prior to development of the formalin protocol) in an attempt to control this parasite. While this compound is an effective treatment for this parasite, it must be presented at high concentrations. A single treatment (one aquarium) was attempted using Acriflavine. A previously empty treatment tank was sanitized using a relatively high concentration of formalin [0.18 ml/l] and elevated water temperature (33°C).

Spikedace exhibiting a heavy infestation of *Ichthyophthirius multifiliis* were transferred to the clean treatment tank which was devoid of substrate and structure. Acriflavine was initially applied at a moderate dose. Individuals in treated (Acriflavine) water often died before heavily infected spikedace maintained in untreated water. This suggested that higher concentrations of this chemical, necessary to control the parasite, would likely be fatal to spikedace and eliminated the possibility of use of Acriflavine in this study.

Gender Determination

Accurate determination of spikedace gender was necessary so that specific male:female ratios could be achieved in individual spawning aquaria. Based on post-mortem examination of specimens, gender of Population I spikedace (2 April 2001; the first sample) was correctly determined on 75% (n=90) of the study subjects. Examination of preserved Population I spikedace revealed the primary problem in assessing gender occurred with small fish (primarily age 1) that were presumed male. In most cases, these preserved individuals were reidentified as immature female spikedace.

Gonad examination of preserved specimens (19 April 2001; Population II) from the second spawning attempt revealed no mis-identifications of gender (n=100). This was due, in part, to the additional experience obtained by the field crew, but primarily because of the natural development of primary and secondary sexual characters in spikedace. These characters included distended abdomens (females) and bright-yellow hue (males). In

Table 4. Protocol for treatment of spikedace for *Ichthyophthirius multifiliis*. Treatment assumes maintaining a 0.5% (5 ppt) concentration of sodium chloride in aquarium holding water throughout the tenure of the procedure.

Day	Percent Water Change	Formalin [1] Concentration (ppm)	Aquarium Number	Cycle Number	Comments
0	NA	0	1	C1	Fish held 24 h in untreated water
1	25%	25	1	C1	Fish held 24 h in treated water
2	NA	0	2	C1	Fish held 24 h in untreated water
3	25%	25	2	C1	Fish held 24 h in treated water
4	30%	0	1	C2	Water changed prior to returning fish to aquaria - (Begin Cycle 2)

[1] = due to its relatively rapid degradation in well aerated systems (Moussavi et al. 2002), post-water change residual formalin is not considered when calculating the amount of formalin necessary to achieve a concentration of 25 ppm.

addition, sampling efforts concentrated on the collection of age 2 or older individuals as there was little question that those individuals would be capable of reproducing.

The most effective means of determining gender was to place spikedace in a bucket and observe their dorsum (view them from above). The pectoral fins of males were larger (both length and width), more robust, and more opaque than those of female spikedace. In lateral view, there appeared to be a difference in caudal peduncle depth with that of male spikedace being narrower than the caudal peduncle depth of female spikedace.

Spawning

Hormone Injection and Natural

There were 10 attempts to induce spikedace to spawn through injection of hormones and at least four discrete attempts to initiate spawning in spikedace through manipulation of environmental variables. Use of hormones to induce spawning did not appear to be successful. The phrase “did not appear” (versus “was not”) is used here regarding the success of hormone injection because, in 2003, eggs were discovered an aquarium containing spikedace (n=12) that had received two series of injections (CPE). The discovery was made several days after the fish were injected. This result is not conclusive as numerous uninjected spikedace from the same Population (IV) also spawned at about that same time. The concurrent spawning by injected and uninjected spikedace in combination with the failure of all previous and subsequent attempts to induce spawning strongly suggests that the aforementioned event was not related to the injections of CPE. While documentation of spawning through hormone injection did not occur, environmental conditions were provided that resulted in about 30 separate natural (i.e., not induced through injection of hormones) spikedace spawning events.

On 4 February 2002 experimental manipulation of selected environmental variables was initiated to determine the effect of the variables on spikedace reproduction. All individuals in this experiment were from Population III and were age 3 (age 2 when collected in October 2001). Water temperature, photoperiod, feeding regime, substrate type, and fish density were manipulated (Table 5). Environmental conditions in the control aquarium were 19°C water temperature, 11-h light: 13-h dark photoperiod and a three times per day feeding regime.

On 26 February 2002 at approximately 20:00 h, spawning behavior was noted in aquarium III-D. This behavior consisted of 1-3 male spikedace flanking and chasing a single female as she swam through the water column and along the sides of the aquarium. Males were observed using their snouts to make frequent contact with the female in the vent region. While expulsion of gametes was not witnessed, visual inspection of the substrate revealed the presence of eggs. Spawning behavior was witnessed intermittently from 26 February through 12 March 2002 in aquarium III-D. Similar behavior occurred in other aquaria on an irregular basis through March 2002 and had ceased by early April 2002.

During 2002, spikedace spawning occurred in four of the seven aquaria (including the control) established for manipulation of environmental variables. We were not able to determine which, if any, of the manipulated variables contributed most to spawning by spikedace. Most of the progeny from the 2002 spawn (February-March) were maintained in attempts to rear them to adult size so that they too could be used in spawning experiments.

In 2003, natural spawning occurred in seven of 12 aquaria and in all four Populations of spikedace being held. As in 2002, it was not possible to determine which variables were most responsible for inducing the spawn. It was noteworthy that four of the seven 2003 study aquaria in which spawning occurred were control aquaria. Spawning occurred in each of the four Population III aquaria (Aquaria III-1, III-2, III-3, III-4), in one of

Table 5. Environmental variables and spawning dates for spikedace during 2002-2003.

Aquarium Identifier Code	Variables	Substrate	Number of Adults	Date of Spawn	Number of Progeny (estimate)
2002					
III-A	Food [1]	Gravel	5	ca. 12, 30 March	ca. 30
III-B	Water Temperature (24°C)	Gravel	7	na	
III-C	Control	Gravel, Cobble	11	ca. 30 March	ca. 20
III-D	Water Temperature (21°C) Light [2]	Gravel, Cobble	10	ca. 26, 28 February; 01, 12 March	ca. 200
III-E	Water Temperature (23°C) Light [2], Food	Gravel, Cobble, Plants	15	na	
III-F	Light [2], Food	Gravel, Cobble	29	ca. 26, 28 February; 01, 19 March	ca. 100
III-G	Light [2]	Gravel	16	na	
2002 TOTAL			93		>350
2003					
III-1	Control	Gravel, Cobble	17	ca.23 May; 10, 13, 16 June; 04, 10 July	ca. 100
III-2	Light, Food	Gravel, Cobble	30	ca. 16 May	ca. 50
III-3	Water Temperature (26°C), Food	Gravel, Cobble	29	ca. 25, 30 March; 11, 28 April, 04 July	ca. 300
III-4	Water Temperature (26°C), Light, Food	Gravel, Sand	18	ca. 10 July	ca. 50
IV-1	Food	Gravel, Cobble	47	na	
IV-2	Control	Gravel, Cobble	20	ca. 07, 09, 14, 21 July	ca. 300
IV-3	Water Temperature (26°C), Light	Gravel, Cobble	20	na	
IV-4	Light	Gravel	12	na	
IV-5	Water Temperature (16°C), Food	Gravel	10	na	
V-1	Food	Gravel	10	na	
V-2	Control	Gravel	20	ca. 25 March	ca. 50
VI-1	Control	Gravel	74	ca. 25 March; 11, 20, 23 30 April	ca. 150
2003 TOTAL			307		>1,000
GRAND TOTAL			400		>1,350

[1] = food: fed four-five times per day
[2] = light: photoperiod 14-h light, 10-h dark

four Population IV aquaria (Aquaria IV-2, control), and in one of two Population V aquaria (Aquaria V-2, control). Population III spikedace were age 4 at the time of the 2003 spawning event and had also spawned in 2002, although it could not be determined if the same individuals had spawned during consecutive years. Population IV and V spikedace, of which only one group of each population spawned (controls), were primarily age 2 fish. None of the 2002 year class progeny produced in the laboratory had achieved the developmental stage necessary for spawning during the 2003 environmental manipulation experiment.

The 12th aquarium used in the 2003 study was the only one to contain spikedace from a location other than the Bird Area (Gila River). That aquarium, a control, contained 74 spikedace (Population VI; Aquarium VI-1) from Aravaipa Creek, Arizona that had been provided on 10 January 2003 by researchers at the University of Arizona. Aravaipa Creek spikedace spawned on numerous occasions from late March through late April 2003.

While a large portion of the 2003 reproductive effort in spikedace occurred collectively in March and April, there were additional spawning events in May, June, and July. All four Population IV spikedace in the control aquarium (IV-2) spawned at no other time except 7-21 July. They were the only fish from that Population that spawned during the 2003 manipulation of environmental variables. The Population III control lot of spikedace (Aquarium III-1) spawned during May, June, and July while the Population III-3 lot spawned March, April, and July. The other two Population III lots of spikedace spawned only once each.

Stripping of Gametes

Numerous female spikedace from the 19 April 2001 sample had expelled eggs during the injection of hormone. Likewise, milt was occasionally observed while male specimens were subjected to injections. Given this relatively free expression of reproductive products, an attempt was made to strip gametes of selected individuals from the 19 April 2001 sample. Two female and four male spikedace were selected for stripping of gametes.

The initial step in the procedure was to over-anesthetize two male and one female spikedace. Anesthetized fish were subsequently removed from the MS-222 solution and injected with excessive amounts of a saline solution forcing the release of gametes. Injections were given to both genders simultaneously with vents of the fish being held in close proximity. Gametes were allowed to drip into a wetted 100 ml jar and swirled for about 15 seconds. A small amount (10 ml) of aquarium water was also added. Stripped eggs where placed in an incubation aquarium and observed over the next several days. While the release of female gametes was easily documented, we were not able to state definitively that this procedure resulted in the release of milt.

Eggs obtained from the stripping effort did not develop and soon become enveloped in fungus. An additional attempt to obtain fertilized eggs by stripping gametes from mature adults proved ineffectual. These two separate attempts were undertaken because the opportunity existed. Even if stripping of gametes had yielded viable progeny, this would have been the least desirable method for producing additional generations. This effort did indicate that spikedace eggs were demersal and adhesive as, soon after being expelled, they stuck to whatever surface they were exposed (i.e., fingers, side of jar, wooden stir rod) and sank rapidly to the bottom of the jar and aquarium.

Rearing

Gross assessment of larval spikedace mortality was conducted in 2002 using a series of eggs (all of which were assumed fertilized and viable) maintained in rearing chambers. Larval spikedace mortality was about 25% at 60-h post-hatching and approximately 40% seven days post-hatching.

The 227 spikedace from the March 2002 spawning events reared at differing densities (from 16-63 individuals per aquarium) exhibited differences in mean growth rate. Random subsamples of 15 individuals per aquarium showed mean length of spikedace was greatest (40.7 mm SL) in the aquarium with the fewest fish (J3) and smallest (32.8 mm SL) in the aquarium with the highest density (J6) of spikedace (Table 6). Spikedace from the J1 aquarium (n=70) were not included in the density-length portion of the study as they were provided to researchers at the University of Arizona for use in thermal tolerance experiments.

Egg and Larval Fish Ontogeny

Approximately 1,350 eggs/larvae were counted over the duration of this project from aquaria that held adult fish. A subset of 50 eggs and larval fish from the first documented spawning event (26 February 2002) were preserved at irregular intervals to provide cursory information pertaining to ontogenetic stages and appearance of developmental characteristics. Those, and additional larval spikedace produced during this study, were provided to research personnel at the Larval Fish Laboratory, Colorado State University, for their studies of Gila River Basin larval fishes.

Fertilized spikedace eggs were opaque and white, round, demersal, adhesive and about 2 mm in diameter. Hatching occurred about seven days after spawning in water maintained at a relatively constant 19°C. Recently hatched larval spikedace were yolked, about 5 mm SL, and had pigmented eyes. About one-week post-hatching, the progeny were late-protolarvae, had absorbed their yolk sac, developed a swim bladder, and increased in length about 1 mm (Table 7).

Metalarval spikedace (13 mm SL) were first observed in the sample taken 58 days post-hatching with the final sample of this batch (n=7 individuals) of progeny (taken 79 days post-hatching) being comprised of five metalarvae 13-19 mm SL. None of the 35 larval fish in the sample had progressed to the juvenile stage (*sensu* Synder, 1981) during the 86-day tenure of this portion of the project.

Feeding Trial

There were differences in percent survivorship of larval spikedace provided a diet of artificial rotifer (0% and 25%) compared with those fed bio-engineered freeze-dried cyclops (25% and 45%; Table 8). Conversely, there was little difference in mean standard length, per treatment, of individuals that survived this experiment (9.2, 10.0, and 11.4 mm SL). Survivorship data from this experiment are tenuous as an assumption of this pilot study was successful hatching of all eggs in each aquaria. Replication of the experiment should employ recently hatched larval fish (protolarvae) and multiple trials.

Table 6. Density dependent differences in laboratory spawned and reared spikedace standard length.

Aquarium Identifier Code	J3	J5	J2	J4	J7	J6
Number of spikedace per Aquarium	16	32	37	39	40	63
Random Subsample	SL (mm)	SL (mm)	SL (mm)	SL (mm)	SL (mm)	SL (mm)
1	44	34	41	34	33	32
2	37	38	37	31	38	40
3	42	41	34	43	39	31
4	38	39	32	35	39	32
5	41	37	39	30	34	34
6	43	36	32	38	41	34
7	42	31	40	33	36	32
8	40	30	32	37	40	31
9	42	33	41	36	41	30
10	42	38	39	31	36	31
11	39	35	35	29	36	28
12	43	32	34	38	32	37
13	40	33	31	41	28	30
14	37	31	33	37	37	32
15	40	37	36	41	34	39
Mean SL	40.7	35.0	35.7	35.6	36.3	32.9
Standard Deviation	2.2	3.3	3.5	4.3	3.6	3.4

Table 7. Ontogenic stages and lengths of laboratory spawned and reared spikedace in 2002. (N values are the total numbers of individuals, per ontogenic stage, used to determine lengths and age).

Developmental Stage	Standard Length SL mm	Standard Deviation mm	Total Length TL mm	Standard Deviation mm	Days Post Hatching
Egg [1] (N = 14)	1.7 - 2.1				
Hatching [2] (N = 2)	4.8 - 4.9		5.2 - 5.3		0
Protolarvae - yolked (N = 2)	4.8 - 4.9		5.2 - 5.3		0
Protolarvae - yolk absorbed (N = 6)	5.9 - 6.9	0.44	6.2 - 7.4	0.50	7 - 65
Mesolarvae - preflexion (N = 4)	6.0 - 7.1	0.50	6.3 - 7.6	0.58	37 - 58
Mesolarvae - flexion (N = 12)	7.5 - 13.0	1.98	8.0 - 14.5	2.47	43 - 79
Metalarvae (N = 11)	12.9 - 19.0	1.93	14.1 - 22.0	1.93	58 - 79

1 = diameter of fertilized eggs
2 = hatching occurred seven days after spawning

Table 8. Feeding trials, using rotifer and cyclops, of larval spikedace.

Aquarium Identifier Code	Treatment [1]	N - eggs (Start)	N - larvae (End)	% Survival	Mean mm SL (surviving fish)
A1	Rotifer	40	0	0%	NA
A2	Rotifer	20	5	25%	11.4
A3	Cyclops	40	10	25%	10.0
A4	Cyclops	20	9	45%	9.2

NA = not applicable

[1] = Rotifer: Azoo™ artificial rotifer

= Cyclops: Cyclop-eeze™ bio-engineered freeze dried cyclops

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DISCUSSION

The multiple facets inherent in research that attempts to manipulate extremely complicated physiological functions of an organism require, among other things, recognition of the need to be flexible and adaptive throughout the study while maintaining a clear recognition of the predominant objective. For this study, the overriding goal was to induce the successful reproduction of spikedace under controlled conditions in a laboratory setting. Accomplishment of that task, in and of itself, would have made the project a success with any supplemental information gleaned from the effort a valuable addition. Likewise, a clear understanding of why spikedace did not spawn would have been equally as valuable but such information (demonstrating why something does not occur) is much more difficult than the former to obtain and is often extremely speculative. The inability (in this study) to define the relative importance of selected variables should not diminish the value of the accomplishment of a primary objective which was documenting that spikedace could be induced to spawn under controlled conditions.

Problems encountered in the initial year of this study with the pathogen *Ichthyophthirius multifiliis,* provided the opportunity to address an issue and omnipresent parasite whose affects can vary markedly between species and is extremely detrimental under hatchery conditions. Discovery that spikedace were highly susceptible to *Ichthyophthirius multifiliis* infestation meant that disease control became the primary objective of this study temporarily displacing all other study goals. Fortunately, the disease treatment protocol developed in late 2001 was successful and allowed a redirecting, in spring 2002, of efforts back to attempts to induce spawning in spikedace.

A key assumption or prerequisite of an experiment to determine spawning habitat preference is an equal probability or likelihood that study subjects (spikedace) will spawn if provided the correct substrate or other variable or combination of variables being tested (i.e., photoperiod, water temperature). In other words, in such experiments one needs to be sure that failure to spawn is the result of an absence of the dependent variable (being tested) and not due to another (untested) reason. If that assumption can not be met with a high level of certainty, the results of the experiment will not have been statistically valid.

The inability to induce spawning in spikedace in 2001, either through introduction of hormones or modification of environmental variables, made it unfeasible to attempt to conduct statistically defendable tests of spawning preferences. In the absence of this scenario, the majority of the initial 2002 effort was directed towards inducing spawning by spikedace while concerns of the statistical validity of the experiment were temporarily discounted. The general protocol adopted in 2002 was to provide spikedace a wide range of habitat variables, within the constraints of the aquaria systems and that which would be encountered in the natural environment, and subsequently evaluate the results and refine the tests in 2003.

Spikedace spawned in 2002 in three of six aquaria in which variables were manipulated as well as in the control aquarium. Despite the 2002 spawning success of spikedace, there was no discernible pattern regarding environmental variables necessary or most important to spawning. Besides accomplishing the principal objective of the study, observation of 2002 prespawning behavior and discovery of egg type and deposition location led to the conclusion that plant material was not a component necessary for spikedace reproduction. Progeny from the 2002 spawns (February and March) were employed as test subjects in ancillary tests, concurrent with attempts to complete another key study objective (2F: Develop and document conditions necessary for incubation, hatching, and normal growth of young spikedace through juvenile life stage). A component

of this objective (2F) required periodic sacrifice of larval individuals so that gross rate of progression through developmental stages could be accessed. In addition, an initial attempt to gauge density dependent growth rates of larval spikedace was undertaken as that information would be valuable for future attempts to rear spikedace under hatchery conditions. When the 2002 ancillary tests were initiated, it was done under the premise that reproduction by spikedace would continue into April and possibly May (normal period of reproduction) thereby providing sufficient progeny to meet criteria needed for statistical analysis. Unfortunately, the final 2002 spawning event was 30 March.

In 2003, attempts to induce spawning in spikedace through manipulation of environmental variables were refined and effort almost doubled. As in 2002, spikedace spawned in both control and experimental aquaria but again there were no obvious patterns to identify the most important environmental parameters. Noteworthy differences between 2002 and 2003 were that spawning occurred sporadically into July 2003 and the majority of spawning was by the oldest cohort of fish (age 4). That cohort had also spawned in 2002 and had been held in captivity since October 2001. An ancillary experiment initiated with 2003 year class progeny was a test of two different types of larval fish food.

Although it was not possible to predict spawning based on adjustment of environmental variables, much general information was obtained during this study regarding spikedace reproductive activity. Study results indicated that spikedace will spawn at temperatures above 18°C and when photoperiod mimics late-spring or early summer conditions. Simulation of these conditions in the laboratory, prior to their onset in the wild, did result in spawning by spikedace.

Spikedace appeared to be communal spawners that broadcast demersal, adhesive eggs in relatively close proximity to the substrate. While the egg type of spikedace was documented during this study (demersal and adhesive), until a confirmed spawning event can be observed, the reproductive strategy of spikedace as a communal broadcast spawner remains the most parsimonious hypothesis. Spawning occurs over several days and, although not confirmed during this study, appears to involve multiple spawning events (i.e., release of gametes) by an individual fish. There was no evidence of selection of or preference towards a substrate type (i.e., sand, gravel, cobble) or structure (plants), which supports their designation as broadcast spawners. Fertilized eggs settle in the interstices of the substrate where they develop for about seven days prior to hatching.

Spikedace eggs are about 2 mm in diameter upon release and protolarvae about 5 mm TL on hatching. The yolk-sac is relatively small at hatching as most yolk was absorbed while in the egg. Any yolk remaining at hatching is absorbed relatively rapidly (1-2 days). The swim bladder inflates about two-three days post-hatching allowing the larvae to emerge from the protection afforded by the cover of the substrate and providing larval fish the ability to initiate exogenous feeding.

Filial cannibalism (eating of one's viable progeny) in spikedace should be assumed during future efforts to artificially breed this fish. Spikedace were observed nipping at material in intersticial spaces at a greater frequency and elevated intensity during spawning periods than during non-reproductive periods. In addition, fewer eggs and larvae were recovered from aquaria that held high numbers of adult fish relative to those aquaria that held fewer individuals. There were often several spikedace spawning events in a single aquarium and it was not possible to identify and track individual activity to determine if egg cannibalism was gender specific or if it was being performed exclusively by either spawning or non-spawning individuals. This behavior is well documented in freshwater fishes (Fitzgerald, 1992) and was previously observed during laboratory spawning of Rio Grande silvery minnow, *Hybognathus amarus* (Platania and Altenbach, 1998).

While pectoral fin morphology, as described by Barber et al. (1970) proved reliable for determining spikedace gender, the ability to ascertain reproductive development of adult spikedace via expression of gametes was quite variable. Female spikedace occasionally expressed eggs when inspected and when pressure was applied to their abdomen but male fish rarely expressed milt under the same handling scenario. The lack of expressed milt was often not indicative of male reproductive state as fertilized eggs were frequently recovered in aquaria that contained male spikedace that had recently (within 48-h) failed to express milt. Assessing spikedace reproductive state based on general morphology or secondary sexual characteristics was also employed. Gross female reproductive condition was obvious due to abdominal distention, but in males, only those individuals displaying breeding coloration (yellow-hue) could be easily classed as reproductively active. Expression of breeding coloration in male spikedace was rarely observed and on the few occasions that it did occur, was markedly less extreme in captive than wild specimens.

Difficulties associated with induction of spawning via hormone injection may have been due to the timing of injections. The injection of gonadotrophins such as CPE (hypophysation) stimulates ovulation, spawning in females, and spermiation in males. The procedure does not stimulate earlier stages of gametogenesis in either gender (Treves-Brown, 2000). Determining the correct time to inject hormones into spikedace is difficult for several reasons. Developmental assessment of specimens is usually based on visual external inspection and relative annual variability of environmental conditions (e.g., discharge) necessary to induce spawning in spikedace imparts considerable error in estimating spawning period.

It appears that by the time captive spikedace are able to spawn (the point at which hormone injection would be most beneficial), they will spawn without hormone injection. The aforementioned is obviously premised upon providing the necessary environmental conditions in aquaria. It may be more reasonable to use hormones to attempt to increase the overall production of individuals rather than to control the timing of actual spawning events. Unfortunately, studies to assess this specific hypothesis would be difficult to conduct given the number of individuals that would be required, variability of influence of hormone on individual fish, and difficulty in constructing a statistically valid test scenario.

Spikedace holding, rearing, and disease treatment procedures were successfully developed under this study. We were not able to induce spikedace that had been spawned, hatched, and reared in the laboratory (F_1 generation) to spawn and produce an F_2 generation. This was likely due, in part, to the age of those fish (2002 year class; age 1; ca. 14 months) at the time spawning activity was highest among wild fish held in the laboratory. It had been noted, during 2001 spikedace spawning efforts, that it was very difficult to determine gender in age 1 fish and that those individuals did not appear ready to spawn. Not surprisingly, none of the 2002 year class F_1 spikedace exhibited secondary sexual characteristics.

An additional issue regarding rearing of spikedace that arose during the tenure of the study was the loss of a source of larval fish food (BioKyowa™ A-250). This food was valuable because of its small size (250 micron) and high caloric and amino acid content. It was primarily used during the crucial period of transition from endogenous to exogenous feeding. The outbreak of bovine spongiform encephalopathy (BSE, mad-cow disease) in Europe and Japan (September 2001) resulted in implementation by government agencies of the United States of America of strict importation regulations that ultimately precluded availability of this well tested and effective food for larval fish. Various other larval fish foods were used during the spikedace rearing process but a substitute with the important specific properties of BioKyowa™ A-250 was not located.

The small size of spikedace (ca. 70 mm TL maximum) means relatively low fecundity (both annually and during their lifetime) and that increased care is required to minimize stress due to handling. In addition, these fish are short-lived (3-4 years) and have a relatively short reproductive season. In large fish, it is possible to more closely control reproduction through injection of hormones and stripping of gametes. Given the size and greater durability of large-bodied fish than small-bodied individuals, this technique (hormone injection and gamete stripping) is nonlethal. In addition, these techniques were developed almost 20 years ago and are still being refined. The short life-span of spikedace means that there is a relatively short period in which to attempt to manipulate environmental variables in an effort to induce physiological changes necessary to induce spawning.

Many aspects of the reproductive ecology of spikedace recorded during this study mirrored those reported by Blinn et al. (1998) and should be considered in regards to hatchery production of spikedace. In their work, Blinn et al. (1998) documented that flow was an important component of reproductive ecology of Little Colorado spinedace noting that all spawning occurred in the artificial stream. While early attempts to mimic stream flow conditions in aquaria in our study were unsuccessful and discontinued, such efforts should be considered in future breeding and rearing programs. Biological information on spikedace suggest that spawning in this species occurs in reaches of moderate to high velocity versus low-velocity habitats (Propst et al., 1986). A facility with holding aquaria and raceways/runs might prove valuable for spikedace spawning and rearing. Raceways could be lined with trays containing gravel and, if spawning was observed, those trays could be removed and placed in incubation systems for rearing of larvae.

RECOMMENDATIONS

Recommendations for adapting results of this work to hatchery production of spikedace

1. Specialized holding procedure: Wild collected brood stock should be assumed to be infected and must be treated for ectoparasites after arrival. The ectoparasite treatment detailed in this document was successful for ensuring the general health and high survivorship of wild caught spikedace. Although this protocol requires more effort and handling than would likely normally occur, this treatment markedly reduces the probability of large-scale mortality associated with broad spectrum treatments.

2. Holding/rearing systems: Discrete aquarium units or recirculating systems should be employed in hatchery production of spikedace. Water (municipal) should be with treated with carbon-filters and the system equipped with mechanical, ultraviolet, and biological filtration to allow for more control over water quality and pathogen exposure. In addition, such systems allow for more practical and efficacious treatments of disease and closed systems reduce the volume of potentially contaminated effluents leaving the facility.

3. Water temperature: Water should be maintained between 18°C and 22°C.

4. Photoperiod: Replication of natural season shifts in photoperiod are suggested. During this study, spikedace spawned when photoperiod was manipulated to produce 14-h light and 10-h dark photoperiod. Fish in control aquaria also spawned even though photoperiod was ca. 10-h light, 14-h dark.

5. Diet: Different foods will need to be provided for differing ontogenic stages. Adult spikedace should be fed a variety of food simulating their natural diet. In addition to feeding adult spikedace zooplankton and invertebrate larvae, they should also be provided vegetable matter to replace plant/algal material that natural prey items would normally ingest. Fish should be fed three times per day to maintain health but feeding should be increased to four or five times per day in anticipation of spawning. Larval spikedace should be fed a variety of commercially available fry feeds at a minimum rate of four-five times per day.

6. Water quality parameters: Dissolved oxygen concentrations should be >6 mg/l and pH between 7.5-9.0. Ammonia should be <0.20 mg/l, nitrite <0.50 mg/l, and nitrate <10 mg/l.

7. Spawning specifics: Aquaria should contain a gravel substrata (<64 mm) from which adults can easily be removed after spawning occurs (to minimize egg cannibalism). Another alternative is to provide gravel lined trays in raceways that can be removed and placed in another system after spawning to allow safe hatching and growth.

RECOMMENDATIONS (CONTINUED)

Recommendations for hatchery production of spikedace based on our laboratory spawning and rearing experience with other endangered and threatened fishes

1. Although there are direct benefits to fish associated with pond rearing (natural photoperiod, volume, natural light spectrum, invertebrate prey), the numerous negative effects should be considered. Holding captive fishes in ponds makes them susceptible as prey for piscivorous birds, mammals, reptiles, and invertebrates that have access to the facilities. In addition, many parasites of fishes require invertebrates or piscivorous vertebrates to complete their life cycles. Maintaining fish in ponds will not only expose them to additional parasites and pathogens, but may also increase encounter and infection rates such that individuals are not able avoid or overcome infections. It is also very difficult to accurately monitor environmental conditions and survival of stocks reared in ponds.

2. The feeding ecology of spikedace may be dissimilar to that of fishes previously held at propagation facilities. Any captive propagation effort should therefore take species specific feeding habits into consideration before attempts at holding and rearing are made. For example, adult spikedace feed primarily on mayfly nymphs. Wild individuals will adapt more readily to a captive diet that accommodates this feeding strategy. In addition, should it be decided that larval specimens will be reared in captivity, a diet that best meets the developmental needs of this species should continue to be investigated and be developed.

3. The potential for using hormone injections either to control spawning in spikedace or to increase egg production should be investigated further.

4. Development of a successful captive propagation effort requires a thorough understanding of population genetics of the study taxon. This information is necessary to provide the framework (number of individuals and paired matings, etc.) to maximize the genetic structure of propagules and increase the likelihood of their survival in the wild. Without such information, a captive propagation program may actually lead to decline in effective population size and could negatively impact the long-term survival of wild populations.

5. Although captive propagation efforts may benefit declining spikedace populations, they should not be considered a reasonable alternative to confronting the issues responsible for this decline. Hatchery production can enhance wild populations, but cannot compensate for the negative impacts that loss of habitat, altered flow regime, and introduction of predators (or competitors) have on native stream fishes.

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Alexandra M. Snyder (MSB) oversaw all aspects of collection management and incorporated retained specimens into the permanent collection of the Division of Fishes at the Museum of Southwestern Biology. Robert K. Dudley, W. Howard Brandenburg, Michael A. Farrington, and John P. Larson (all of MSB) assisted in field collection of spikedace in the Gila River, New Mexico. Corissa J. Carveth and her graduate advisor, Scott A. Bonar, both of the Arizona Cooperative Fish and Wildlife Research Unit, University of Arizona, provided spikedace from Aravaipa Creek, Arizona for use in the study. Eileen M. Corcoran, Lee E. Renfro, John P. Larson, and Jason A. Rose (all of MSB) were valuable participants in the effort to maintain and rear spikedace during the 37-month tenure of this project.

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APPENDIX I

Developmental stages of larval fish as defined by Snyder (1981)

Protolarvae Phase of larval development characterized by the absence of dorsal, anal, and caudal fin spines and rays. Transition to the mesolarval stage is based on the appearance of at least one distinct spine or ray in any of the median fins.

Mesolarvae Development stage of larval fish characterized by the presence of at least one dorsal, anal, or caudal fin spine or ray. Specimens in this stage lack the adult complement of principal fin rays in all median fins (=dorsal, anal, and caudal) and have not yet developed pelvic fin buds.

Metalarvae Larval fish developmental stage characterized by the presence of the adult complement of principal fin rays in all median fins and pelvic buds or fins.

Juvenile Transition to the juvenile requires that the finfold and atrophying fins must be absorbed beyond any recognition, the full adult complement of fin spines and rays must be formed in all fins, and segmentation must be evident in the rays of each of the fins.