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BEHAVIORAL OBSERVATIONS OF THE ENDANGERED RIO GRANDE SILVERY MINNOW IN A CONSERVATION AQUACULTURE FACILITY

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ABSTRACT

A major reason why conservation aquaculture is needed to improve the success of aquaculture-assisted fisheries is that traditional production aquaculture produces fish with mal-adaptive behaviors. These behaviors can be produced via domestication and culture techniques, and preventing these mal-adaptive behaviors requires integrating improvements in genetic management and culture protocols. The genetic protocols needed to minimize hatchery-induced genetic changes have received considerable attention, but changing the way fish are raised has received less effort. Conservation aquaculture cultures fish in environments that resemble their native habitats so that when stocked, they behave like wild fish rather than hatchery fish. A purpose built-conservation aquaculture facility can also be used to learn about a species' behavior and how it reacts to changes in the environment, something which can be difficult or expensive to study in the wild. These observations can then be used to help direct both propagation and recovery management. This paper provides the rationale for why genetic management, culture systems, and management practices need to be altered to produce fish that are behaviorally similar to wild fish for aquaculture-assisted fisheries programs. It then provides a description of some of the behaviors of the endangered Rio Grande silvery minnow *Hybognathus amarus* that were observed at the Los Lunas Silvery Minnow Refugium, a purpose-built conservation aquaculture facility, and explains how some of these behaviors can be used in culture and recovery management. Behaviors described are: schooling; predator avoidance; feeding behavior; use of vegetation for cover and predator avoidance; habitat use by bottom substrate; location in the water column; upstream movement via a fish ladder; movement upstream in a high-velocity channel; response to changes in water level; spawning behavior; seine avoidance; and Kaah-chee-nyee Srkaash, a behavior described for the first time.

How to Cite

INTRODUCTION

A fish's behavior controls all aspects of its existence—reproduction, foraging, predator avoidance, diurnal swimming activity, and interactions with conspecifics and other species. These behaviors are both heritable and learned (Brown and Laland, 2001; Brown et al., 2006; Huntingford et al. 2012a, 2012b). Consequently, all aspects of management can impact these behaviors when fish are raised in fish hatcheries (Fern et al., 2006; Huntingford et al., 2102a). In food fish production, aquaculture-induced changes in fish behavior can be beneficial, as they make the fish more suitable biologically and economically for the set of management parameters that are used to produce the fish for market.

However, when fish are raised for aquaculture-assisted fisheries programs, aquaculture-induced changes in fish behavior can be counterproductive in that altered behaviors can be mal-adaptive when fish are stocked in the wild; this can make the fish less fit, which helps explain the low survival of hatchery-produced fish after stocking (Brown and Laland, 2001; Brown and Day, 2002; Huntingford et al., 2012b, 2012c). Understanding how all aspects of aquaculture can affect a fish's behavior and produce mal-adaptive behaviors that lower fitness is critical to the success of aquaculture-assisted fisheries programs, because a major goal of these programs should be to produce fish that have the same behaviors as the wild fish (Brown and Laland, 2001).

A fish's behavior is determined by its genes, by its response to environmental conditions (learning), and by the interactions between the genes and the environment (Huntingford et al., 2012b). Therefore, management of the breeding program and the way that the fish are raised must be simultaneously addressed to ensure the success of aquaculture-assisted fisheries programs.

Aquaculture can change heritable behavior by domestication selection (Ruzzante, 1994; Metcalfe et al., 2003; Lorenzen et al. 2012; Huntingford et al., 2012d). Domestication selection modifies fish behavior by selecting for genetically controlled behavior that is beneficial in the hatchery environment. Traditional fish hatcheries and traditional production management combine to raise fish in physically constrained environments that are impoverished, and they experience unusual conditions such as high densities, abundant and predictable food, and the absence of predators. Under these conditions, selection is relaxed for behaviors such as predator avoidance and death by starvation (foraging behaviors), while selection is intensified for behaviors such as competition for resources (Huntingford et al., 2012c). Because this modification in behavior is heritable, domestication selection can fix the alleles that produce the behaviors that are superior in the hatchery but, in doing so, it produces fish that behave differently than wild fish; it is likely that this will make them sub-viable, and it can also lower the fitness of the wild stock when the hatchery-produced fish mate with wild fish (Huntingford et al., 2012d).

Learned behavior can be altered by the environment in which the fish are raised and by the management used to produce the fish, making them more successful in exploiting the hatchery environment, which is desirable in food fish production. Unfortunately, these behaviors that are learned to exploit hatchery conditions can make them less successful in the wild (Maynard et al., 1995; Olla et al., 1998; Brown and Laland, 2001, 2003; Sundström and Johnsson, 2001; Brown and Day, 2002; Ellis et al., 2002; Brown et al., 2003a, 2003b; Sundström et al., 2004; Orlov et al., 2006; Huntingford et al., 2012a).

Recognizing that traditional production aquaculture can alter a fish's behavior and thus prevent the success of aquaculture-assisted fisheries programs requires a reevaluation of the way fish are raised in these programs. This is critically needed, because these programs are an increasingly important component of fish culture endeavors and conservation efforts around the world as fish stocks decline and as the number of species that become endangered increases. Because of this and other problems, the effectiveness of traditional aquaculture programs in aquaculture-assisted fisheries program has been questioned (Schramm and Piper, 1995; Nickum et al., 2004).

Aquaculture-assisted fisheries can be divided into two major categories: "put and take" or recovery. The only goal of a put-and-take fishery is to stock hatchery-produced fish into a body of water so that the stocked fish can be harvested by recreational or commercial fishermen, not to recover a population. To accomplish this, fish are raised using traditional production aquaculture management similar to that used to raise food fish.

When the goal of an aquaculture-assisted fisheries program is to recover or to help produce a self-sustaining wild population, a new form of aquaculture is required: conservation aquaculture. Because hatchery-produced fish will mate with and compete with wild fish, the management goal of conservation aquaculture is to produce fish that are as close to wild fish as practicable. The reason for this is quite simple: This type of aquaculture-assisted fisheries will succeed only if the fish that are stocked provide a net benefit for the target population (Bowles, 1995).

This can be achieved by integrating two components of fish culture management. The first is to use conservation genetic management in the breeding program that is used to produce the fish at the hatchery; the second is to culture the fish using conservation aquaculture. Managing the fish's genetics has long been recognized as critically important, but recognizing that changing the way fish are raised is equally important has not received as much attention. The combination of hatchery-induced genetic changes and the culture environment can produce fish that are less fit, have mal-adaptive behaviors, and are even morphologically

different than their wild counterparts (Einum and Fleming, 1997, 2001; Fleming and Einum, 1997; Moore et al., 2012; de Mestral et al., 2013). Consequently, both the genetic management and culture components of conservation aquaculture must be integrated into management to make this category of aquaculture-assisted fisheries successful (Flagg et al., 2004; Flagg and Mobrand, 2010).

Genetic management and fish behavior

The genetic goal of recovery aquaculture-assisted fisheries programs is to conserve the genetics of the targeted stock; i.e., the gene and genotypic frequencies of the cultured fish and the wild fish should be the same (Tave, 1993; Doyle et al., 2001). This requires that a program of "no selection" be conducted (Tave, 1993). Unfortunately, this type of breeding program is difficult, because genetic changes are inevitable when there is control over a population's reproduction (Tave, 1993, 1995, 1999). These changes often occur because the only fish that are spawned are those that are in spawning condition and respond to hormonal injection when the annual work plan schedules fish spawning. All of these facets of spawning management produce genetic changes— they are a selective breeding program (Tave, 1993).

The number of fish that are spawned can also produce genetic changes. These genetic changes occur because most hatcheries spawn as few brood fish, especially females, as possible, because it is cost effective or because there are labor and facility limitations. This will produce a population of fish with a small effective breeding number, resulting in inbreeding and genetic drift, which changes gene and genotypic frequencies and results in the loss of alleles (Tave, 1984, 1993, 1999). Equally important, hatchery personnel seldom equalize family size; unequal family size dramatically lowers effective breeding number, so equalizing family size should be a key component of spawning protocol (Tave, 1984, 1993, 1999; Ryman and Lairke, 1991; Fisch et al., 2015).

When hatchery populations with small effective breeding numbers are stocked, they can lower the effective breeding size of the wild stock (Ryman and Lairke, 1991; Christie et al., 2012a), which makes hatchery supplementation counter-productive management in that while population size in the wild may increase, the genetic size will decrease resulting in the lowering of fitness. Araki and Schmid (2010) reviewed 266 peer-reviewed papers that examined the effects of hatchery fish in aquaculture-assisted fisheries programs; 70 of these studies examined the genetic effect that the breeding program and hatchery environment had on the fish, and none showed a positive effect.

To prevent these breeding-induced genetic problems from harming the wild stock, breeding programs must be re-designed to produce large numbers of single-pair matings (or nested factorial matings) and family size must be equalized each generation in order to produce the desired effective breeding number generation after generation. Tave (1993, 1999) described procedures that can be used to customize the desired effective breeding number for a program, based on genetic goals and number of generations of captive breeding in the program.

Other breeding techniques can be incorporated into breeding programs to minimize genetic changes. One way to minimize the loss of genetic variance and minimize inbreeding during captive propagation is to genotype parents and make directed matings in order to capture rare alleles and to prevent consanguineous matings (Doyle et al., 2001). An example of a breeding program that incorporates this genetic management is described by Fisch et al. (2013) and Lindberg et al. (2013). A second technique is to incorporate managed gene flow, which brings selected wild fish into the hatchery breeding program in order to minimize the genetic divergence of the hatchery and wild populations (Waters et al., 2015). A third approach is to use DNA fingerprinting (Doyle and Herbinger, 1994) or minimal kinship breeding (Doyle et al., 2001) to prevent the mating of closely related fish, which will minimize inbreeding and maximize genetic diversity every generation.

Finally, the culture practices and the environment in which the fish are raised can change a population's genome. It produces fish that do well under the artificial hatchery environmental conditions; this is domestication selection (Doyle, 1983; Doyle et al., 1995; Tave, 1993), and it does so by selecting rare alleles that are deleterious in the wild (Frankham, 2008). The artificial hatchery environment and management used to raise the fish, coupled with the absence of natural selection pressures, combine to alter morphology, physiology, reproduction, development, survival, and behavior by domestication; changes in behavior are often the most obvious (Hynes et al., 1981). While domestication selection produces fish that have a higher fitness in the hatchery environment, the correlation between hatchery and natural fitness is usually negative (Doyle et al., 1995; Lorenzen et al., 2012).

It is virtually impossible to avoid domestication if fish that have been cultured in a hatchery are spawned (Harada et al., 1998). Genetic problems that are produced as a result of domestication can occur as a result of a single generation of captive culture (Araki et al., 2007; Christie et al., 2012b, 2016; Milot et al., 2013; Wilke et al., 2015), and they will occur even if brood fish are captured from the wild each generation (Doyle et al., 1995).

Christie et al. (2016) found that a single generation of domestication altered the expression of over 700 genes in steelhead trout *Oncorhynchus mykiss*. Domestication can fix deleterious alleles that natural selection maintains at low frequencies in the wild (Lynch and O'Hely, 2001). Christie et al. (2012b) found that fish with the highest hatchery fitness produced offspring that performed the worst in the

wild. Christie et al. (2014) determined that inbreeding was only responsible for a small percentage of reduced fitness in hatchery-produced steelhead trout and concluded that domestication selection was responsible for most of the reduced fitness.

Consequently, when hatchery fish are stocked and breed with wild fish, the frequency of sub-viable genes and genotypes increases in the wild population and lowers its fitness. A single stocking of hatchery fish can have long-term consequences, as there is a carry-over effect that extends to subsequent generations in the wild (Araki et al., 2009), and this hatchery-induced lowering of fitness in the wild population can create a permanent problem that persists after the stocking program has ended and can decrease the probability of recovery (Lynch and O'Hely, 2001; Bowlby and Gibson, 2011). Domestication can alter behavior that is critical for post-augmentation survival. For example, Johnsson et al. (1996) found that domestication reduced anti-predator response in brown trout *Salmo trutta*. Paradoxically, while the hatchery fish have a higher mortality rate in the wild because they are less fit genetically, the gametes of fish produced under production-type management are functionally equivalent to those of wild fish (Yeates et al., 2014). The totality of the genetic changes produced by traditional breeding practices and traditional aquaculture management in aquaculture-assisted fisheries can lower the fitness (assessed by genetics, behavior, physiology, reproduction, survival, or catch) of the target wild stock, which means aquaculture-assisted fisheries could do more harm than good. This has been well documented in salmonids (e.g., Vincent, 1960; Moyle, 1969; Reisenbichler and McIntyre, 1977; Fraser, 1981; Keller and Plosila, 1981; Chilcote et al., 1986, 2011; Leider et al., 1990; Hindar et al., 1991; Doyle et al., 1995; Reisenbichler and Ruben, 1999; Einum and Fleming, 2001; Lynch and O'Hely, 2001; Heath et al., 2003; Metcalfe et al., 2003; Araki et al., 2007, 2008, 2009; Fraser, 2008; Christie et al., 2012b; Milot et al., 2013; Bellinger et al., 2014; Wilke et al., 2015). Domestication can affect a fish's behavior by altering brain size. A single generation of domestication reduced brain size in Atlantic cod *Gadus morhua* raised using intensive production aquaculture (Mayer et al., 2011). Altering a fish's behavior by domestication selection can even produce morphological changes in the fish, if the change in body conformation enhances the behavior. Kern et al. (2016) found that zebrafish *Danio rerio* selected for boldness had larger caudal regions. One component of endangered species recovery management is to produce fish that are similar morphologically to wild fish, so domestication selection can thwart this goal.

Culture environment and fish behavior

The other management component of aquaculture-assisted

fisheries that must be addressed if the goal is to augment a wild population in order to create a self-sustaining population is the type of management that is used to culture the fish. Most fish hatcheries are production hatcheries: production units are uniform systems—be they ponds, raceways, or tanks—with a uniform culture environment designed to make harvest and other aspects of management simple and efficient; high stocking rates are used; fish are fed a nutritionally complete artificial ration; great care is taken to minimize predation; high survival rates are desired; low feed conversions and fast growth rate are desired; high yields are desired. The non-genetic effects produced by the hatchery and the management used to raise the fish can be as harmful as domestication in adversely affecting the success of an augmentation program (Hynes et al., 1981). Traditional production management can be counterproductive in aquaculture-assisted fisheries in unintended ways. For example, high stocking densities can reduce the genetic size of the population by selecting for families that do well under that management and which then have decreased post-stocking survival (Thompson and Blouin, 2015). High stocking densities can also adversely affect the development of critical foraging and predator avoidance behaviors (Brockmark et al., 2010).

The key metrics by which traditional hatchery management in an aquaculture-assisted fisheries program is evaluated are high survival, rapid growth, and the number of fish released (Brown and Day, 2002). While this type of management may be economically effective for food fish aquaculture, it will make the creation of a self-sustaining wild population difficult or impossible, because the fish it produces are unprepared to live in the wild. Management efforts to improve hatchery survival rates actually increase post-stocking mortality rates (Wales, 1954; Suboski and Templeton, 1989). Because production management and traditional ponds or raceways have been shown to be counter-productive in producing fish for aquaculture-assisted fisheries programs, a paradigm shift is needed in order to produce fish that will enable these programs to succeed. Lynch and O'Hely (2001) warned that unless the hatchery environment is similar to that of the natural environment, aquaculture-assisted fisheries will create wild populations that are incapable of being self-sustaining. The impoverished culture environment in traditional production units can help explain the low post-augmentation survival rates of hatchery-produced fish (Huntingford et al. 2012c).

To accomplish this, fish must be raised in redesigned conservation hatcheries and fish culture management must be changed (Maynard et al., 1995, 1996a, 2004a, 2004b; Flagg and Nash, 1999; Flagg et al., 2004, Flagg and Mobrand, 2010; Tave et al., 2011). Conservation hatcheries should mimic the natural habitats in which the target species lives. Consequently, fish being cultured can move from one type of habitat to another depending on age of the fish, time

of day, or season. This means that the fish can interact with a complex environment that contains various substrates, different water depths and velocities, plants, etc.

Management used to culture the fish should also be modified. Fish should not be fed artificial diets, but should be able to forage on the food organisms that they will encounter in the wild; lower stocking rates should be used; and controlled predation should be allowed (Flagg and Nash, 1999; Flagg et al., 2004; Maynard et al., 2004a, 2004b; Tave et al., 2011). These changes in fish culture management have one goal: the cultured fish are as close to wild fish as practicable. The new science of epigenetics provides another reason why changing from production aquaculture to conservation aquaculture could improve success of aquaculture-assisted fisheries programs. Epigenetics is non-genetic inheritance. It is an environmentally−produced change in phenotypic expression due to a modification of the way genes are transcribed, and these changes in the way genes are transcribed can be transmitted to succeeding generations. Consequently, the fish hatchery environment and the management used to raise the fish can produce stable, heritable, non-genetic changes in the population (Salinas and Munch, 2012; Evans et al., 2014; McGhee and Bell, 2014; Shao et al., 2014; Jonsson and Jonsson, 2016). Moghadm et al. (2015) suggested that customizing epigenetic modification could be used to improve fish and/or profits in food fish farming. Their idea has great relevance to aquaculture-assisted fisheries; it is possible that epigenetic changes produced by traditional production aquaculture creates fish that do well in the hatchery environment but that do not do well in the wild environment. Conservation aquaculture could lessen the negative effects of epigenetic modification of hatchery fish. A key component of conservation aquaculture that will help improve success of aquaculture-assisted fisheries programs is to provide a hatchery environment that enables fish to develop natural behaviors before augmentation. Traditional production hatcheries excel at producing copious numbers and high yields, but production management does not produce fish that have the behavioral flexibility to adjust to the environmental variables that they will encounter in the wild (Braithwaite and Salvanes, 2005). Traditional production management produces fish with behaviors that make them sub-viable in the wild, something that has been known for over 100 years (Brown and Day, 2002).

A properly designed conservation aquaculture facility will provide fish with a complex environment so that they develop proper behavior in: predator avoidance; foraging; social interactions with conspecifics; finding and constructing nests/shelters; orientation and navigation of complex terrain, risk-taking, and moving about in complex terrain (Brown and Laland, 2001; Brown and Day, 2002; Braithwaite and Salvanes, 2005; Roberts et al., 2011). Environmentally rich hatchery environments produce fish that have more flexible behaviors, which can improve survival in the wild (Salvanes et al., 2013).

A complex hatchery environment can actually produce fish with more developed brains or increased head tissue gene transcription response (Lema et al., 2005; Kihslinger and Nevitt, 2006; Evans et al., 2015). Because fish brains can grow throughout the life of the fish, studies that show that fish brain development and gene expression is increased by hatchery enrichment makes sense, but the exact reasons for this have not been elucidated. Furthermore, even though it makes sense that a larger brain could produce more flexible behavior and advanced learning, the correlation remains undetermined. An important component of traditional aquaculture management is feeding fish to achieve good growth, high survival, and high yields, which are traditional evaluation metrics for success. Feeding is a component of management that produces domestication-induced and culture-produced increased aggression or more prolonged aggressive encounters in fish, increased risk-taking behavior, boldness, and exploratory behavior (Moyle, 1969; Ruzzante, 1994; Fleming et al., 1997; Metcalfe et al., 2003; Biro et al., 2004; Sundström et al., 2004; Härkönen et al., 2014). The increased aggression of hatchery fish can cause them to expend more time and energy to obtain the same success as wild fish during territorial encounters (Sundström et al., 2003), can adversely affected their ability to forage (Orlov et al., 2006), and can lower viability in the wild (Einum and Fleming, 2001; Metcalfe et al., 2003; Biro et al., 2004).

Increased aggression can also affect schooling behavior in fish, which is a balance between repelling behavior (aggression) and attracting behavior (anti-predator behavior). If hatchery fish school with wild fish, their increased aggression can upset this balance, making the fish in that school more vulnerable to predation (Ruzzante, 1994).

Intensive production-type management, which requires feeding, may prevent fish from developing proper foraging strategies, which is learned behavior, and can also produce mal-adaptive behavior that is developed to feed on the artificial ration (Suboski and Templeton, 1989; Furuta, 1996; Maynard et al., 1995, 1996b; Olla et al., 1998; Brown and Laland, 2001; Sundström and Johnsson, 2001; Ellis et al., 2002; Brown and Day, 2002; Brown et al., 2003a, 2003b; Metcalfe et al., 2003; Wintzer and Motta, 2005; Orlov et al., 2006). Poorly developed foraging behavior due to hatchery feeding management is a major contributor to poor survival of hatchery fish after stocking (Olla et al., 1998). For example, Florida largemouth bass *Micropterus salmoides floridanus* fed pelleted rations at hatcheries have difficulty capturing live prey when released, which helps explain the 99% mortality following release into the wild (Wintzer and Motta, 2005); in another study, Thompson et al. (2016) attributed high post-stocking mortality to a combination of inefficient foraging behavior produced by feeding the fish a pelleted ration during culture, as well as poorly developed

anti-predator behavior which made them more vulnerable to predators.

Stocking density is another component of intensive production that can have a detrimental effect on fish behavior. Brockmark et al. (2010) found that stocking densities greater than that in the wild adversely affected the ability of brook trout to find prey, to eat novel prey, and to avoid predators. The hatchery environment can develop or enhance behaviors in fish that become mal-adaptive in the wild. Raising fish in a traditional hatchery can cause them to occupy upper portions of the water column rather than the bottom, which is occupied by wild counterparts (Vincent, 1960; Moyle, 1969; Furuta, 1996); to avoid concealment, unlike their wild counterparts (Vincent, 1960); and to have an altered diurnal activity pattern (Álvarez and Nicieza, 2003). Hatchery-raised fish are bolder than their wild counterparts, which results in risk-prone behavior (Sundström et al., 2004). Hatchery-raised fish also have increased exploratory behavior (Härkönen et al., 2014). These kinds of culture-induced behaviors make hatchery fish more vulnerable to predation after stocking than their wild counterparts.

One of the most critical components of behavior is predator recognition and avoidance. A major goal of traditional production-type aquaculture is to maximize survival; it is one of the metrics used to measure success (Wales, 1954). Wales (1954) warned that high hatchery survival rates were anti-Darwinian, in that it allowed the survival of the un-fit. Program success is usually determined by number of fish produced in the hatchery (survival rate), rather than survival of the fish after stocking, which is the reason they were produced (Brown and Day, 2002). To maximize hatchery survival, considerable effort is used to keep the culture systems predator-free. This produces what are termed "naïve" animals. Even though some anti-predator behavior in fish is innate, much of it is learned (Suboski and Templeton, 1989; Brown and Laland, 2001, 2003; Griffin, 2004; Huntingford et al., 2012e), so fish raised without predators are more vulnerable to predation than wild fish (Einum and Fleming, 2001; Stunz and Minello, 2001; Brown and Day, 2002; Jackson and Brown, 2011). Not only is this a lot of effort for little return, it also raises what Brown and Day (2002) described as an ethical problem—producing and stocking fish that you know will die. One component of conservation aquaculture is to expose fish to predators or predator odors to produce predator avoidance behavior and improve post-augmentation survival, and a number of studies have shown that this produces fish that are not predator naïve (e.g., Maynard et al., 1998; Berejikian et al., 1999, 2003; Gazdewich and Chivers, 2002; Hossain et al., 2002; Vilhunen, 2006; Olson et al., 2012; Kopack et al., 2015; Fu, 2015).

A properly run conservation aquaculture program is one designed and managed as an integrated and complex holistic entity, not one managed to address various components of an animal's behavior via piecemeal management. For example, Fu (2015) found that predator training improved predator avoidance of qingbo *Spinibarbus sinensis*, but that the ability to avoid capture was greatly improved by raising the fish in a flowing-water environment, as fish raised in that environment had a faster burst speed and greater prolonged swimming ability than those raised in a static-water environment. Berejikian et al. (1999) found that raising chinook salmon *O. tshawytscha* in a complex hatchery environment in and of itself did not improve post stocking survival; antipredator training had to be incorporated into the complex hatchery environment to accomplish that.

Raising fish in a purpose-built conservation aquaculture fish hatchery should improve the success of aquaculture-assisted fisheries because it can lessen aquaculture-induced mal-adaptive behaviors two ways: First, it produces the fish in an environment that is similar to that in the wild, so fish develop the learned behaviors that will enable them to survive after they are stocked. Secondly, the environment and management used in conservation aquaculture should minimize heritable mal-adaptive changes in behavior that occur because of domestication selection, and this will not only help improve post-stocking survival, it will lessen the negative impact that aquaculture-assisted fisheries programs can have on fitness of the target stock.

Observing fish behavior at a conservation aquaculture facility

An added advantage of raising fish in a purpose-built conservation fish hatchery is that it provides the opportunity to observe fish behavior in a semi-natural environment that mimics the natural environment where the fish will be stocked. This ability to make behavioral observations under semi-natural conditions is important for management, because it is difficult and expensive to study fish behavior in the wild, particularly with an endangered species where the population may be small and where endangered species regulations limit research activities. This was a major reason for building a conservation aquaculture facility for the endangered Devils Hole pupfish *Cyprinodon diabolis* (Feuerbacher et al., 2016). Understanding how a fish behaves under a specific set of environmental conditions and how it reacts to various stimuli can be used to modify fish culture protocols and to direct management used in recovery efforts.

The Los Lunas Silvery Minnow Refugium (LLSMR), Los Lunas, New Mexico, USA is a unique purpose-built conservation aquaculture fish hatchery that raises the endangered Rio Grande silvery minnow *Hybognathus amarus*. Construction was completed in 2008, and fish have been cultured in a naturalized mesocosm (refugium) using conservation aquaculture management since 2009.

This paper describes behavior observations, including a fish behavior that is described for the first time, that have been made of the Rio Grande silvery minnow from 2009-2016 in the refugium and discusses some of the implications of these observations to improve recovery efforts.

MATERIALS AND METHODS

The key component of LLSMR is the refugium, the first purpose-built, large-scale conservation aquaculture mesocosm. The refugium is designed to mimic the Rio Grande, including its hydrology. The refugium is 0.2 ha, with 0.11 ha of interconnected water habitats: a stream with sand bars, five ponds, shelves, marshes, attached bars, and overbank areas that can be inundated to create floodplains (Figs. 1 and 2).

Fig 1. The naturalized outdoor refugium (refugium) at the Los Lunas Silver Minnow Refugium in 2012. The large island in Pond 1 has since been removed. The white "structure" in Pond 4 is two of the co-authors sampling fish with a 3.05-m (10-foot) seine. Sand Bars 1 and 2, which are discussed in relation to swimming behavior, can be seen. Sand Bar 1 is between the entrances to Ponds 1 and 2. Sand Bar 2 is downstream from the entrance to Pond 2 and is connected to the berm that separates Ponds 2 and 3.

The refugium and how it functions hydrologically were described by Tave et al. (2011). To help create a natural mesocosm, native plant species were planted in the ponds, marshes, shelves, and along the berm that surrounds the refugium (Coleman et al., 2011). Conservation aquaculture management techniques used to culture the Rio Grande silvery minnow in the refugium were described by Hutson et al. (2012). The spawning biology of the Rio Grande silvery minnow was studied in the refugium by manipulating water levels to produce floodplain habitat (Hutson et al., in press). Since 2009, we have observed Rio Grande silvery minnow behavior in the refugium during fish culture projects and the spawning study. We have also observed the fish's behavior when it was raised in fiberglass tanks using production
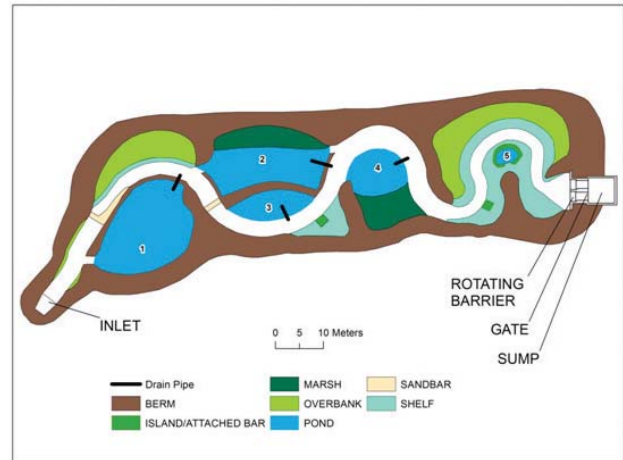

Fig 2. Schematic of the naturalized outdoor refugium (refugium) at the Los Lunas Silvery Minnow Refugium in its current configuration, showing the different habitats contained in the mesocosm.

aquaculture management for other projects (Tave et al., 2012; Hutson et al., 2013, 2017; Powell et al., 2017).

RESULTS AND DISCUSSION

In this section, we describe Rio Grande silvery minnow behaviors that we have observed during fish culture activities and a research project on spawning behavior in the refugium that have implications for management. We also describe a couple of interesting behaviors, one of which is described for the first time. We compare Rio Grande silvery minnow behavior observed in the refugium to that observed when the fish was raised in fiberglass tanks using production aquaculture management for other projects.

Schooling

One behavior that is easily and frequently observed is the fish's schooling behavior, a behavior that was also described for eastern silvery minnow *H. regius* (Raney, 1939). Technically, when fish are in a group and take up the same orientation they are said to be in a "shoal"; when fish in the shoal swim in the same direction in a coordinated manner they are said to be in a "school" (Huntingford et al., 2012e); Rio Grande silvery minnow have been observed in both shoals and in schools in the refugium; in this paper, we will refer to both behaviors as schooling. Schooling starts shortly after the fish become free-swimming. Schooling has been observed when fry are around 10-mm, which is similar to that descibed for eastern silvery minnow (Raney, 1939). Schooling might occur when fish are smaller, but fish that are smaller than 10-mm are difficult to observe in the refugium. Observations of fry in aquaria revealed that they are not good directional swimmers until they are about 8 mm and have fully developed fins, so fish smaller than that probably

cannot school. Schools that have been observed range from 5 to 4,000 fish. When there have been fish of various ages in the refugium, they schooled by age/size, a phenomenon that has been observed in other species (Couzin et al., 2006). This differs from that described for eastern silvery minnow, where schools of different sized fish were observed (Raney, 1939).
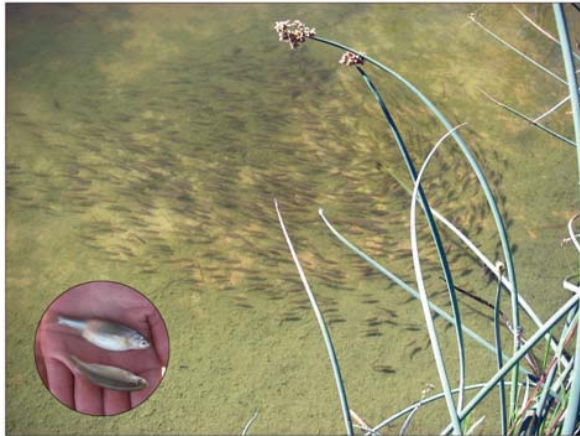

Fig 3. School of approximately 700 Rio Grande silvery minnow brood fish. The school is approximately 6 m2. The inset photograph shows gravid females. The plant in the foreground is softstem bulrush *Schoenoplectus taberaemontani*.

Fig. 3 shows a school of about 700 adults (*ca* 60-75 mm) and Fig. 4 shows a school of about 4,000 ten-mm fry. When in schools, fish density is quite high. For example, density of adults in the school in Fig. 3 is about 117 fish/m^2, and that of the fry in Fig. 4 is about 22,200/m^2. It is possible that adult fish density could be greater, but our endangered species permit restricts us to maximum of 750 brood fish in the refugium.

Fig 4. School of approximately 4,000 ten-mm Rio Grande silvery minnow. The school is approximately 0.18 m^2.

While in the schools, the fish and the school will occasionally hover and face the current, but the fish in the school usually swim. The direction that the fish in a school swim can be clockwise or counter-clockwise and the school can change from a well-defined circle to an oval to an amorphous shape and back to a circle. At times, the movement of fish in a school resembles a computer screen saver program in that groups of fish swim in several directions, move together, and break apart within the confines of the school. When the fish are startled, the school can break apart and form two or more smaller schools that reform into a single school once the perceived threat disappears.

The school can remain stationary for several hours; i.e., while the fish in the school move, the school itself remains within a small area. Adults often schooled at the base of the stream (Figs. 1 and 2) where water depth was 36 cm and velocity was 0.12 m/s (Hutson et al. in press). Throughout the day, the school would often break into several schools, move upstream, go into ponds, but would reform at the base of the stream later in the day.

The schooling behavior of Rio Grande silvery minnow has two major implications for management. The first applies to efforts to assess population size. Because of the schooling behavior, random sampling would not accurately assess how many fish are in the refugium. For example, if five random samples were taken of the fish in the refugium stream, it is likely that because of schooling, the samples would have captured no fish and the results would have indicated that there were no fish in the stream, even though a large school could be observed; conversely, if one sample captured the school, the population estimation would be greatly inflated. The schooling behavior of Rio Grande silvery minnow produces a contiguous spatial distribution, which suggests that a block design sampling program is needed to accurately assess the wild population (Elliott, 1977; Hubert and Fabrizio, 2007). Also, randomly placed fixed sampling sites are likely to miss fish concentrations and produce imprecise underestimates of the wild population due to school movement.

The other management implication is that feeding Rio Grande silvery minnow an artificial feed during hatchery grow-out could increase the vulnerability of river fish to predation following augmentation. Feeding schooling fish during culture can disrupt the balance between the repelling and attracting behaviors that are critical for schooling behavior (Ruzzante, 1994). If hatchery-fed fish merge with wild fish to form a school in the wild, the hatchery fish could cause the school to be less cohesive and more dispersed, which would increase vulnerability to predation, since the major benefits from schooling behavior is that it decreases individual vulnerability to predation by confusing the predator, enables individuals to learn about predators and other dangers to which they have not been exposed from experienced fish, and transmits information about a predator to all fish faster than the speed of the predator (Ruzzante, 1994; Couzin et al., 2006; Huntingford et al., 2012e).

Predator avoidance behavior

Exposing fish to predators so that they are not naïve when stocked is an important component of conservation aquaculture. But this must be tempered by the fact that they are often endangered and, if there is uncontrolled predation, there may not be enough fish at harvest for recovery efforts. Great blue heron *Ardea herodias* is a major avian predator at fish hatcheries in the US, especially if the culture units are shallow, as is the case with the refugium (maximum depth *ca* 0.9 m). Because the Rio Grande silvery minnow is an endangered species, we incorporated bird exclusion wires into the refugium design to minimize take (death or injury of an endangered species). The refugium is crossed by 3-mm cables that are 3-m above the refugium; cables are spaced every 0.41 m along its length (Tave et al., 2011). The long white "lines" that flank the refugium in Fig. 1 are the T-posts from which the bird exclusion wires are strung. Ironically, while the cables do an excellent job of excluding great blue heron, they provide roosting sites for another avian predator, belted kingfisher *Ceryle alcyon*. Belted kingfishers capture some fish, and we have found fish remains that were clearly the results of bird strikes. This exposure to belted kingfisher strikes has created anti-avian behavior in the fish. When a belted kingfisher roosts on a wire, the fish respond to its presence by moving, and there is fish-free zone that encompasses about one fifth of the refugium as long as the belted kingfisher perches, indicating that the fish are not naïve and have learned avian avoidance behavior. This avoidance behavior is similar to avian avoidance behavior observed in pumpkinseed *Lepomis gibbosus* (Gallagher et al., 2016).

The anti-avian behavior that the fish in the refugium have acquired can be demonstrated by simulating a bird attack via a shadow puppet when we quietly approach a school while keeping our backs to the sun. A shadow puppet is made by locking the thumbs and spreading the fingers of both hands, and by quickly raising the locked hands above the head a shadow that resembles a bird in flight is created. As soon as the bird puppet shadow hits the school it "explodes," with fish darting out of the school in all directions; the splitting and rejoining of the school is done to confuse predators (Huntingford et al., 2012e). The sudden movement of fish in all directions also has a dazzle effect as many of the fish roll a bit which produces flashes of silver; this silvery dazzle gives the fish its common name. The dazzle effect that we have observed is a well-known phenomenon that schooling species use to confuse predators (Ruxton et al., 2007; Hogan et al., 2016). The fish don't swim far from the school (*ca* 0.5 m) during this "explosion," and if the hand shadow is removed the school reforms. If the puppet shadow follows the fish, they keep swimming. This anti-avian response to a hand puppet shadow of a bird is not observed in fish that are raised semi-intensively or intensively in tanks, suggesting that predation by belted kingfisher changed refugium fish from naïve fish to fish that had learned avian predator avoidance behavior.

The avoidance behavior of the Rio Grande silvery minnow that has been observed when a belted kingfisher roosts on a wire could have implications for sampling in the Rio Grande. If birds are flying over or are roosted near a sampling site, the anti-avian dispersal response at that locale could adversely affect the ability to capture fish and could produce an underestimation of the river population. The anti-predator behavior that is produced by the presence of an avian predator is markedly different to the behavior produced by encounters with another refugium resident. The refugium is home to at least one muskrat *Ondatra zibethicus* every year. On several occasions we have inadvertently startled a muskrat that was on the lower overbank, and it dove into the stream and swam underwater to escape. During its underwater escape, the muskrat swam through a school of adult Rio Grande silvery minnow. The fish in the school showed no signs of panic or predator avoidance as the muskrat swam towards and through the school. The school parted slightly to enable the muskrat to swim though it and then quickly reformed. The lack of anti-predator behavior by the school indicated that they had previous encounters with the muskrat and recognized that it was not a predator.

Feeding behavior

An important component of our conservation aquaculture management is to minimize our contact with the fish; we do not want them to become accustomed to humans or to develop mal-adaptive human-related behaviors. To prevent the development of these mal-adaptive behaviors, a critical component of management is to avoid the use of artificial feed in the refugium. Because the fish are not fed, they do not associate our presence with a food reward, and do not approach us.

When Rio Grande silvery minnow are raised semi-intensively or intensively in tanks and are fed an artificial feed, the fish respond to our presence by moving up to the surface before and during feeding. If we dangle our fingers in the water between feedings, the fish will rise to the surface and nibble them. If we put our hands over the tank water, the fish move towards them expecting to be fed, unlike refugium fish, which exhibit the dispersal predator fright response that was described in the previous section. Feeding-induced surface hovering (either just below the surface or in the upper portions of the water column) is a common sight at fish hatcheries (e.g., Olla et al., 1998; Uchida et al., 1989; Maynard et al., 1995; Furuta, 1996). This learned hatchery behavior is advantageous in a food fish hatchery since it maximizes a fish's ability to exploit floating feed, producing lower food conversions and faster growth. But in aquaculture-assisted fisheries, this behavior is mal-adaptive because it increases a fish's risk to predation in the wild.

Location in water column

In the refugium, Rio Grande silvery minnow spends most of its time at the bottom or in the bottom 5-10 cm of the water column. Fish will dart to the surface, but quickly move back to the bottom. One reason why they reside on the bottom is they graze on benthic diatoms, which are a major component of their diet (Cowley et al., 2006; Shirey et al., 2008; Watson et al., 2009; Bixby and Burdett, 2013, 2014). If a large school has remained in an area for several hours, when it moves the bottom has a grazed look, similar to that observed in a pasture where horses have been feeding. Raney (1939) noted similar feeding behavior in eastern silvery minnow. Their preference for residing on the bottom and their bottom feeding behavior is one reason why floating feed should not be used when culturing this species. If the fish have to come to the surface to feed, they are developing a mal-adaptive behavior that can reduce viability after they are stocked in the wild (Olla et al., 1998; Maynard et al., 1995; Furuta, 1996). Rio Grande silvery minnow that are raised intensively and fed in tanks occupy a greater portion of the water column than those in the refugium; fish in the tanks occupy the bottom 20 cm of the water column in addition to the bottom. This behavioral shift in water column occupancy has been observed in other species that have been fed (Vincent, 1960; Moyle, 1969; Uchida et al., 1989; Furuta, 1996). When stocked, this feeding-induced shift in water column occupancy could make them more susceptible to avian predation or less effective at bottom substrate foraging.

Use of vegetation for cover

Ponds 2 and 4 have marshes (Figs. 1 and 2) that are composed of nine species of rushes and sedges (Coleman et al., 2011); the predominant ones are: Torrey's rush *Juncus torreyi*, common spike rush *Elocharis palustris*, and softstem bulrush *Schoenoplectus taberaemontani*. Swim-up fry are often found at the edge of these vegetated areas. Fry and juveniles move in and out of these marshes during the day. Schools of about 400 juveniles that were in the open water of Ponds 2 and 4 were observed darting into the rushes when they were startled by our sudden appearance, which was also observed for eastern silvery minnow (Raney, 1939). Creating shallow vegetated area along the edge of the Rio Grande to provide slow-moving water where juvenile Rio Grande silvery minnow can hide from perceived predators might be one way to improve survival and, ultimately, recruitment.

When observing fry, they were found only along vegetated, edge habitat. Fry would swim in between vegetation, in small schools, and disappear into shallow water areas within the vegetation. As habitat restoration is planned and completed within the Middle Rio Grande, planting is not always a priority as it is expensive and can require irrigation until established. This may be a missing component of our habitat restoration sites if they are constructed to provide nursery grounds for Rio Grande silvery minnow.

These behavioral observations are excellent examples of why fish in aquaculture-assisted fisheries programs need to be raised in conservation aquaculture facilities. Rooted aquatic macrophytes are discouraged in traditional production facilities because they can interfere with harvest. This behavior was observed only because installing emergent rooted aquatic macrophytes and creating marshes are a component of our conservation aquaculture program.

Habitat use by bottom substrate

When the refugium was built, it was excavated to an average depth of about 1.2 m, and a 60-mil high-density polyethylene liner was installed to eliminate seepage. The liner was back-filled and the refugium was constructed on the back fill (Tave et al. 2011). A geotextile fabric was installed in the ponds, and pebbles (16-64 mm; modified Wentworth classification; Murphy and Willis, 1996) were placed over the fabric; the pebbles were then covered with fine silt and sand. During the first couple of years of operation, fish used Pond 3 regularly. After a few years of filling and draining, the silt was washed out of Pond 3, and the bottom was composed of pebbles that ranged in size from *ca* 2 x 4 x 1 cm to 3 x 5 x 2 cm. Fish stopped using Pond 3 when the bottom was composed of pebbles. During 2016 winter maintenance, most of the stones were removed and the bottom of Pond 3 was covered with 1-2 cm of silt and sand. When fish were stocked in 2016, they once again started using Pond 3 and were found in there throughout the growing season.

It is known that Rio Grande silvery minnow prefers silt and sand bottoms (USFWS, 2003, 2010); a likely reason is that sand and silt provide habitat for the benthic diatoms that are a major component of the fish's diet (Cowley et al., 2006; Shirey et al., 2008; Watson et al., 2009; Bixby and Burdett, 2013, 2014). The observation of how Rio Grande silvery minnow responded to a rehabilitated bottom in Pond 3 suggests that more habitat in the Rio Grande could be created for the fish by covering sites that are composed of pebbles and cobbles, that would be otherwise suitable, with silt and sand. This is important, because the more suitable habitat that exists in the Rio Grande, the more fish that can survive, which will increase population size.

Kaah-chee-nyee Srkaash (dancing fish)

An interesting behavior that has been observed repeatedly is how a small school moved downstream across a submerged sandbar (Sand Bar 2) when the fish were surprised by our sudden appearance at the edge of the

stream. Sand Bar 2 is downstream of the entrance to Pond 2 (Figs. 1 and 2), and is used to raise stream water level to produce good flow into Pond 2. The central 60 cm portion of the sand bar is about 10 cm below water level. We often observed a small school of 20-50 fish (ca 20-70 mm) above Sand Bar 2. When the fish in the school were startled by our sudden presence, the fish swam rapidly in a ca 60-cm hollow circle (i.e., fish were only on the edge of the circle), and fish left the circle one at a time and darted over the sandbar at intervals of about 1-3 s and moved downstream. The direction that the fish swam in the circle (clockwise vs. counter-clockwise) seemed to be the direction chosen by the first fish that started swimming. To the best of our knowledge, this is the first description of this behavior. We have named this behavior Kaah-chee-nyee Srkaash. (This is pronounced kah-g-knee sh-gosh, and is Keres for dancing fish; Keres is the language spoken by the people of the Acoma, Cochiti, Laguna, San Felipe, Santa Ana, Santo Domingo, and Zia Pueblos of New Mexico).

Upstream movement

Initially, when culture level at the base of the stream was 56.3 cm, four sand bars made out of sand bags were constructed annually before filling the refugium and were then removed at harvest. This was done for two reasons: It was needed to fill Ponds 1 and 2 to capacity and it was thought that the sand bars would add habitat complexity that could be exploited by the fish. Building and removing the four sand bars was exacting and labor-intensive. To reduce the labor needed to operate the refugium, in 2014 culture level was raised to 63.7 cm, which eliminated the need for Sand Bars 3 and 4 (the lowermost two). The increased depth also allowed us to transform Sand Bar 2 (the sand bar needed to fill Pond 2) from one that reached the surface to one that could be a submerged.

Sand Bar 1 (Figs. 1 and 2) is the largest because it is used to raise water level upstream by 15 cm in order to fill Pond 1. This 15-cm difference in water level above and below Sand Bar 1 is an obstacle to upstream migration of the fish and would prevent fish from migrating to Pond 1. To enable fish to move upstream past Sand Bar 1, this sand bar is a combination of dam and fish ladder. One side of the sand bar is the dam and most water is directed to that side, which creates a 15-cm waterfall. The other half is a 7-m-long fish ladder, where water flows along its length in a 1-2 cm riffled sheet. Because the fish ladder is made from sand bags the fish ladder is very uneven with raised spots, sunken spots, cavities, small standing waves, and small eddies. Water velocity on the fish ladder has not been determined empirically.

We have observed fish as small as 15 mm move up the Sand Bar 1 fish ladder and enter the stream above the sand bar and move into Pond 1. Even though there are places on the fish ladder where velocity is such that we thought it would prevent fish movement, the fish are able to find water paths where they can avoid these places and traverse the fish ladder. The fish dart up the ladder moving from one sand bag to another, from one side of a sand bag to the other, and pausing in small cavities during their journey. Bestgen et al. (2010) observed similar movement behavior by Rio Grande silvery minnow in laboratory fishway tests.

These observations of fish movement upstream on a fish ladder have important implications for management of the Rio Grande. The Middle Rio Grande, which houses the only remnant of the original population, is divided into three reaches by irrigation diversion dams. Fish can move downstream, but upstream movement is blocked. To enable upstream movement at this dams, one component of river management that is being considered is the construction of fish ladders at these irrigation dams. Our observations about how fish moved up the Sand Bar 1 ladder can be used to help design these ladders.

Moving upstream through a high-velocity channel

In 2016, we stocked brood fish in the refugium and decided to see what would happen if the dam side of Sand Bar 1 was removed (the fish ladder side had been built). This created a high-velocity channel that was ca 30 cm wide X 9-23 cm deep X 10 m long; no water moved over the fish ladder. Pumping rate (volume of water entering the refugium at the inlet; Fig. 2) was 1,700 L/min, and this created velocities in the high-velocity channel of 0.27-1.25 m/s. A 1.2-m-long section (30 cm wide x 15 cm deep) had velocities of 1.23-1.25 m/s. Adults swam though this high-velocity channel and were able to enter Pond 1.

The Rio Grande silvery minnow's ability to swim upstream against a current was assessed up to velocities of 1.14-1.18 m/s (Bestgen et al., 2010); some fish were capable of swimming for short periods at these velocities, and it was suggested that velocities of 1 m/s could make fish passage difficult. Our observation suggests one of two things: either the fish were able to find mini-channels of slower moving water in this high-velocity channel (the boundary layer was almost certainly slower but it is difficult to measure this accurately), or they are able to burst upstream through the small sections of water that have velocities greater than those that have been assessed. These observations can be coupled with those of fish moving up the Sand Bar 1 fish ladder when designing the proposed fish ladders in the Rio Grande. The ladders should be built and operated so that there are variable velocities along its entire length and breadth along with areas of low-velocity water so fish can rest as they move upstream. These observations also suggest that further research into the fish's ability to swim upstream is needed to help design fish ladders.

Response to change in water level

Rio Grande silvery minnow from swim-up fry to adult responded to changes in water level. Adults, which had been confined to the stream during the early portion of the spawning study, entered the off-channel floodplains as they were inundating (Hutson et al., in press). This behavior suggests that when given a choice of deeper moving water or shallower static water, the fish will choose the latter. It also suggests that when floodplains are inundating during the spring snowmelt runoff, the fish will respond to the change in stage as it occurs.

Swim-up fry that were about 2.5 weeks old, moved with water as it was receding from the floodplain when the flood was drawn down (Hutson et al., in press). This response to a change in water level is critical for management. The age at which fry can respond to this change in water management must be quantified, because it will enable Rio Grande water managers to know how long floodplains must be inundated to avoid stranding fry.

These observations are important pieces of life history data that can be used to help manage water in the Rio Grande. It is critical to further refine the response of sexually mature adults and fry to changes in water stage, and the age/size at which fry can react to these changes. This will enable water managers to work with biologists to adaptively manage water supplies to meet the needs of both the Rio Grande silvery minnow and New Mexico water users.

In the fall, the water in the refugium is drained so the fish can be harvested. Initially, we followed the traditional rules of filling and draining ponds: fill slowly and drain quickly. When we dropped water level 15-25 cm in about an hour and quickly disconnected the ponds from the stream, many fish were stranded the ponds, making harvest difficult. When we changed the draining procedure and lowered water level gradually over two weeks, almost all fish left the ponds and entered the stream before the ponds became disconnected from the stream. This slow-drain process reduced the time needed to harvest fish by two days.

Sections of the Rio Grande often dry in the summer. During river drying, 12.9 km are allowed to dry every day (USFWS, 2003). Obviously, this drying strands fish and, in a dry summer, river drying can be a major contributor to mortality. Observations of how the fish responds to water level change in the refugium show that if drying can be managed so that the area that is drying is connected to the river at one end of the drying segment, fish can move with the water and swim away from the area that is drying. One management technique that could be used to reduce drying-induced mortality is to construct deep water (*ca* 0.5-1 m) refugia along the Rio Grande to enable fish to swim to a safe haven when water level drops during river drying.

Preferred spawning habitat

A multi-year spawning study was conducted in the refugium to determine the preferred spawning habitat of the Rio Grande silvery minnow (Hutson et al., in press). Rio Grande silvery minnow spawns in the spring during the spring snowmelt flooding. It was known that the species uses floodplains as nurseries (Pease et al., 2006), but there was disagreement about whether the fish spawns in the main channel and eggs and fry drift onto the floodplains (Platania and Altenbach, 1998) or whether the fish spawn on the floodplains which then serve as nurseries (Medley and Shirey, 2013). Gonzales et al. (2014) conducted a presence/absence study of constructed floodplains during spring flooding in the Rio Grande to see if Rio Grande silvery minnow occupied them during inundation; they found that gravid Rio Grande silvery minnow were present, producing indirect evidence that the floodplains were used as spawning grounds.

To determine spawning habitat preference during spring inundation, floods were produced in the refugium in 2012 and 2013 to determine where the fish spawned: the stream, floodplains, or both. Fish spawned in response to floods in both years, and all spawning occurred on the floodplains; no spawning occurred in the stream, supporting the Medley and Shirey (2013) life-history floodplain-spawning hypothesis. This is somewhat similar to that observed for the eastern silvery minnow (Raney, 1939). They moved out of the stream and into small lake coves with low velocity water to spawn. When given a choice, Rio Grande silvery minnow spawned in deeper (>20 cm) water than shallower (14-18 cm) water (Hutson et al., in press). Raney (1939) found that eastern silvery minnow spawned in water that was 30-70 cm deep.

Knowledge about the preferred spawning habitat is critical for the Rio Grande silvery minnow recovery program. The Rio Grande and its floodplain have been separated by flood control levees and dams and by channelization which has created a single-channel, incised system (Crawford et al., 1996; Phillips et al., 2011; Magaña, 2012; Medley and Shirey, 2013). This study shows that one reason why this species is endangered is that flood control management has eliminated breeding grounds. One component of recovery management is to build artificial floodplains within the river levee system. The behavioral response to flooding in the refugium shows that building floodplains that inundate annually are important not just as nurseries, but for recruitment and eventual recovery of the species. This behavior observation demonstrates the importance of raising fish for aquaculture-assisted fisheries programs in purpose-built conservation aquaculture facilities. It shows that conservation aquaculture facilities can be used to answer critical questions about a species behavior that are needed to help direct management activities.

Seine escapeability and burrowing into the mud

Eight years of sampling and harvesting Rio Grande silvery minnow in the refugium has revealed that Rio Grande silvery minnow is an expert at avoiding capture by seining. Seining in the refugium is done with 3.06-mm (1/8 inch) mesh minnow seines that are 1.82, 3.05, and 6.1 m long X 1.82 m tall. The burst speed of this species has not been quantified, but it is probably close to 1.18 m/s, based on the maximum swimming speeds documented at 1.18 m/s by Bestgen et al. (2010). The burst speed of the Rio Grande silvery minnow is faster than our ability to move a 3.06-mm mesh minnow seine through the water, and most fish easily out-swim our efforts to capture it by seining with a single seine. One way to improve capture is to double seine, using a technique similar to that described by SWCA Environmental Consultants (2015) for Rio Grande silvery minnow and by Scheurer et al. (2003) for brassy minnow *H. hankinsoni*. Either one seine is moved towards a stationary seine or two seines are moved toward each other and the bottoms are allowed to overlap before they are raised. While this improves capture, many fish still escape. When the refugium has been drained and the only water left in the ponds are 8- to 10-cm-deep puddles that are *ca* 14 m², we have to seine the puddles dozens of times before we get five consecutive hauls of zero fish.

Another reason why Rio Grande silvery minnow are difficult to capture is that they swim under the seine. To counter this, we have increased the number of lead weights on the seine to keep the seine bottom line closer to the pond/stream bottom. A standard minnow seine is "single-shotted," in that the bottom line has a 62-g lead weight every 30.5 cm. We now use a "double-shotted" bottom line, which has a lead weight every 15.2 cm to make the bottom line heavier and keep it from rising off the bottom during seining.

A final reason why this species is hard to capture is that some dive into the sand and silt substrate to avoid capture. These fish quickly re-emerge into the water. There is a local myth that claims Rio Grande silvery minnow burrow into the Rio Grande river bottom when the river dries, and when the river re-wets the fish re-emerge and this re-colonizes the river. The escape behavior that we have observed in the refugium might explain the origin of this myth. Rio Grande silvery minnow were once a commonly used bait fish, and this burrowing behavior may have been observed in the river when fishermen and bait dealers harvested the fish, and they turned this behavior into a legend.

Table 1. . Behavior of the Rio Grande silvery minnow raised using both conservation aquaculture and production management at the Los Lunas Silvery Minnow Refugium and implications for recovery management.

Behavior	Implications for recovery management
Schooling	Block design sampling is needed to assess river population since the population is not randomly distributed. Feeding can disrupting schooling in river, increasing vulnerability to predation.
Predator avoidance	Exposure to predators produces fish that are not naïve; incorporating anti-predator training into culture can increase post-augmentation survival. Installing emergent vegetation along river edge habitat may improve survival because juveniles used it for shelter when startled.
Feeding behavior	Avoiding use of artificial feed during culture enables fish to develop proper foraging strategies before augmentation. Feeding produced learned behavior where fish were attracted to humans and promotes hovering behavior at the surface, increasing vulnerability to predators.
Preference for silt/ sand substrate	Rock-covered habitats were avoided. Covering rocky substrate with silt and sand in the river can increase desirable habitat, supporting a larger.
Upstream movement	Juveniles and adults moved up a 7-m fish ladder and adults swam up-stream though channel where velocity was 1.23-1.25 m/s. These behaviors can be used to help design fish passage structures in river.
Response to change in water level	When water stage increased and fish were given a choice of spawning habitat they spawned on floodplains. Constructing floodplains that inundate annually can maximize recruitment. 2.5-week-old fry on floodplains moved with water when it receded. Quantifying the minimum age/size when fry can actively leave floodplains can improve water management and recruitment.
Seinability	Double seining with double-shotted lead-line seines is a more effective than using a single seine with a single-shotted lead line. This sampling technique can improve capture efficiency in river.

Implications of these behaviors for management of Rio Grande silvery minnow

Culturing Rio Grande silvery minnow in the refugium using conservation aquaculture has enabled us to observe behaviors that are difficult to observe in the Rio Grande. Understanding behavior can help direct both fish culture management and management activities in the Rio Grande to improve recovery efforts (Table 1). The most important are: spawning biology and fry behavior, schooling, use of plants to avoid predation, and upstream movement.

When given a choice of in-channel or floodplain habitats, fish spawned on the floodplains, and chose habitat that was deeper than 20 cm. These observations support the Medley and Shirey (2013) life history hypothesis that Rio Grande silvery minnow is a facultative floodplain spawner. This observation provides guidance in building constructed floodplains for the species. Our observation that 2.5-week-old fry can actively leave the floodplain by swimming with the receding water gives some guidelines about how long the floodplain needs to be inundated to enable young-of-year to swim to the river; further research is needed to determine minimum inundation time needed for this. The observation that young fish were often found at the edge of stands of rushes, and used these areas for shelter when startled suggests that emergent vegetation along river edge habitat could improve survival of young-of-year, which would improve recruitment.

The schooling behavior that was observed is important for two reasons. First, this behavior should be considered in sampling design when trying to estimate the river population, because the fish are not randomly distributed throughout the river. Second, the schooling behavior is also important to help guide fish culture guidelines since feeding schooling fish during culture can disrupt schooling behavior in the wild when hatchery-produced fish mix with wild ones, decreasing post-augmentation survival.

Our observations that fish as small as 15 mm could move up a 7-m-long fish ladder and that adults can move upstream though a 10-m long high-velocity channel can be used to help design fish ladders that would enable fish to move upstream past irrigation diversion structures.

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SAŽETAK

PROMATRANJE PONAŠANJA UGROŽENE VRSTE *Hybognathus amarus* U POSTROJENJU ZA KONZERVACIJSKU AKVAKULTURU

Glavni razlog zašto konzervatorska akvakultura poboljšava uspjeh ribarstva je taj što tradicionalna akvakultura proizvodi ribe slabo prilagođenog ponašanja. Do takvih obrazaca ponašanja dolazi zbog domestikacije i uzgojnih tehnika, a prevencija slabo prilagođenog ponašanja iziskuje integraciju poboljšanja genetičkog menadžmenta i uzgojnih protokola. Mnogo pažnje pridodaje se genetičkim protokolima potrebnim za smanjene genetskih promjena izazvanih umjetnim mrijestom, no mnogo se manje truda ulaže u načine uzgoja riba. Konzervatorska akvakultura uzgaja ribe u uvjetima nalik na njihova prirodna staništa, pa se nakon poribljavanja ponašaju poput divljih jedinki svoje vrste, a ne poput riba iz uzgajališta. Postrojenja građena za konzervacijsku akvakulturu mogu se koristiti kako bi se naučilo više o ponašanju riba te njihovim reakcijama na promjene u okolišu, što se teško proučava u otvorenim vodama te je predstavlja veliki trošak. Ta promatranja mogu pomoći u upravljanju prirodne oplodnje i oporavka vrsta. Ovaj rad daje obrazloženje zašto je potrebno promijeniti genetički menadžment, sustav uzgoja te prakse upravljanja, kako bi se proizvodila riba ponašanjem slična divljoj ribi za ribolovne programe poribljavanja iz akvakulture. Zatim daje opis nekih obrazaca ponašanja ugrožene vrste *Hybognathus amarus* koji su promatrani u Los Lunas Silvery Minnow Refugium, postrojenju izgrađenom u svrhu konzervacijske akvakulture te objašnjava kako se neki od tih obrazaca ponašanja mogu primijeniti u menadžmentu uzgoja i oporavka. Opisana ponašanja su: koordinirano plivanje jata riba, izbjegavanje predatora; hranjenje; uporaba vegetacije za sklanjanje i sakrivanje od predatora; korištenje staništa dna supstrata; pozicija u vodenom stupcu; uzvodno kretanje preko ribljih staza; uzvodno kretanje u području brzog toka; odgovori na promjene razine vode; mriještenje; izbjegavanje mreže; i *Kaah-chee-nyee Srkaash*, ponašanje koje je opisano prvi put.

Ključne riječi: konzervacijska akvakultura, ponašanje riba, *Hybognathus amarus,* ugrožene vrste

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