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Stress Inducing Factors of Bonytail Hatchery and Stocking Practices

U.S. Department of the Interior
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Bonytail release on the Bill Williams River near Parker, Arizona.

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Lower Colorado River Multi-Species Conservation Program

Stress Inducing Factors of Bonytail Hatchery and Stocking Practices

by

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Introduction

Endangered Colorado River fishes are commonly reared in hatcheries and grow-out facilities, and repatriated under the Lower Colorado River Multi-Species Conservation Program (LCRMSCP). Wild bonytail (*Gila elegans*; Figure 1) were federally listed as endangered in April 1980 (USFWS 1980) and are now believed only to exist as scattered repatriates and extremely rare wild individuals in Lakes Mohave, and captive populations in isolated backwaters and off-channel bodies of water (USFWS 2002, Pacey and Marsh 2007). Fisheries managers have repatriated millions of bonytail larvae and juveniles in the mainstream and larger tributaries of the Colorado River with no success in the last 30 years (Johnson 1985, USFWS 2002). Under the LCRMSCP there is a long-term, multi-agency effort to conserve and work towards the recovery of endangered species, such as bonytail. LCRMSCP stocking efforts, along with habitat conservation and restoration, are the only measures hindering the extinction of bonytail on the Lower Colorado River.

Figure 1.—Bonytail (*Gila elegans*) at the Achii Hanyo Native Fish Rearing Facility.

The first bonytail captive broodstock program began in the late 1970s with as many as 10 adult fish captured from Lake Mohave to produce a cohort at Willow Beach National Fish Hatchery (NFH), Arizona (Hamman 1982, Hedrick *et al.* 2000). The captive broodstock were later moved to the Dexter NFH and Technology Center, Dexter, New Mexico, where a portion of the F1 progeny remain alive today, but all the original wild broodstock have died (Hedrick *et al.* 2000). Dexter NFH and Technology Center is responsible for the propagation and rearing of bonytail in conjunction with six other aquaculture facilities: Willow Beach NFH; Achii Hanyo Native Fish Rearing Facility (NFRF), Ouray NFH, Wahweap State Fish Hatchery, Mumma Native Aquatic Species Restoration Facility, and Uvalde NFH (Mueller 2006, Pacey and Marsh 2007, Montony 2008). Bonytail adults for LCRMSCP are hand spawned at Dexter NFH and Technology Center and raised to fingerling size where they are then transferred to Willow Beach NFH for initial rearing. Willow Beach NFH holds these fish for at least a year until they are juveniles and then transfers a portion to Achii Hanyo NFRF grow-out facilities for later stocking into River Reaches 3, 4, and 5 (Figure 2). Other fish are maintained at Dexter NFH and Technology Center for repatriation to these sections of the river, or are transferred to Uvalde NFH for growth rate assessment and site production capability. The LCRMSCP has committed to repatriate 620,000 (300-mm) bonytail into the Lower Colorado River Basin over 50 years, which began in 2005 (LCRMSCP 2004, LCRMSCP 2006, Pacey and Marsh 2007).

Long-term post-stocking survival is thought to be negligible, as few fish are recaptured after release (Pacey and Marsh 2007, Bestgen *et al.* 2008). Past stockings of smaller-sized fish were most likely lost to predation, and fish recaptured a few months post-stocking reduced biomass and infections (Pacey and Marsh 2007, Bestgen *et al.* 2008). Bonytail have been successfully cultured, held in grow-out ponds, and used in moderately long monitoring studies (Marsh and Mueller 1999, Badame and Hudson 2003). Bonytail are thought to be sensitive to handling and have a reputation for stress-related mortality (Chart and Cranney 1993, Tyus *et al*. 1999, Ward 2003, Paukert *et al*. 2005, Mueller 2006). Stressors are known to elicit corticosteroid responses and suppress bonytail immune functions, and as a result fish are susceptible to outbreaks of the parasite ich (*Ichthyophthirius multifilis*; Tyus *et al.* 1999, Montony 2008). Cortisol levels can remain elevated in fish infected with ich and have been shown to be higher than those observed in healthy fish following handling and PIT-tag insertions (Montony 2008). Tyus *et al.* (1999) observed delayed mortality of 77% after acquiring genetic tissue samples from bonytail, and many of the deaths were attributed to ich infections. Similarly, Chart and Cranney (1993) reported 75% mortality in bonytail used in telemetry studies after handling and tagging. Mueller (2006) observed dark banding associated with capture and handling stress that suggest muscular tissue damage a few days post-capture, and these symptoms usually resulted in secondary infections and death. Montony (2008) also detected black banding across the caudal peduncle after taking blood samples, but seemed to fade and disappear in surviving fish after 4 weeks. Likewise, Paukert *et al.* (2005) found bonytail that were recaptured and handled multiple times grew significantly less than unhandled fish and those less frequently handled. Stress-associated growth reductions have been seen in other fish species leading to immunosuppression and reduced reproductive success (Wedemeyer *et al.* 1990, Barton and Iwama 1991,

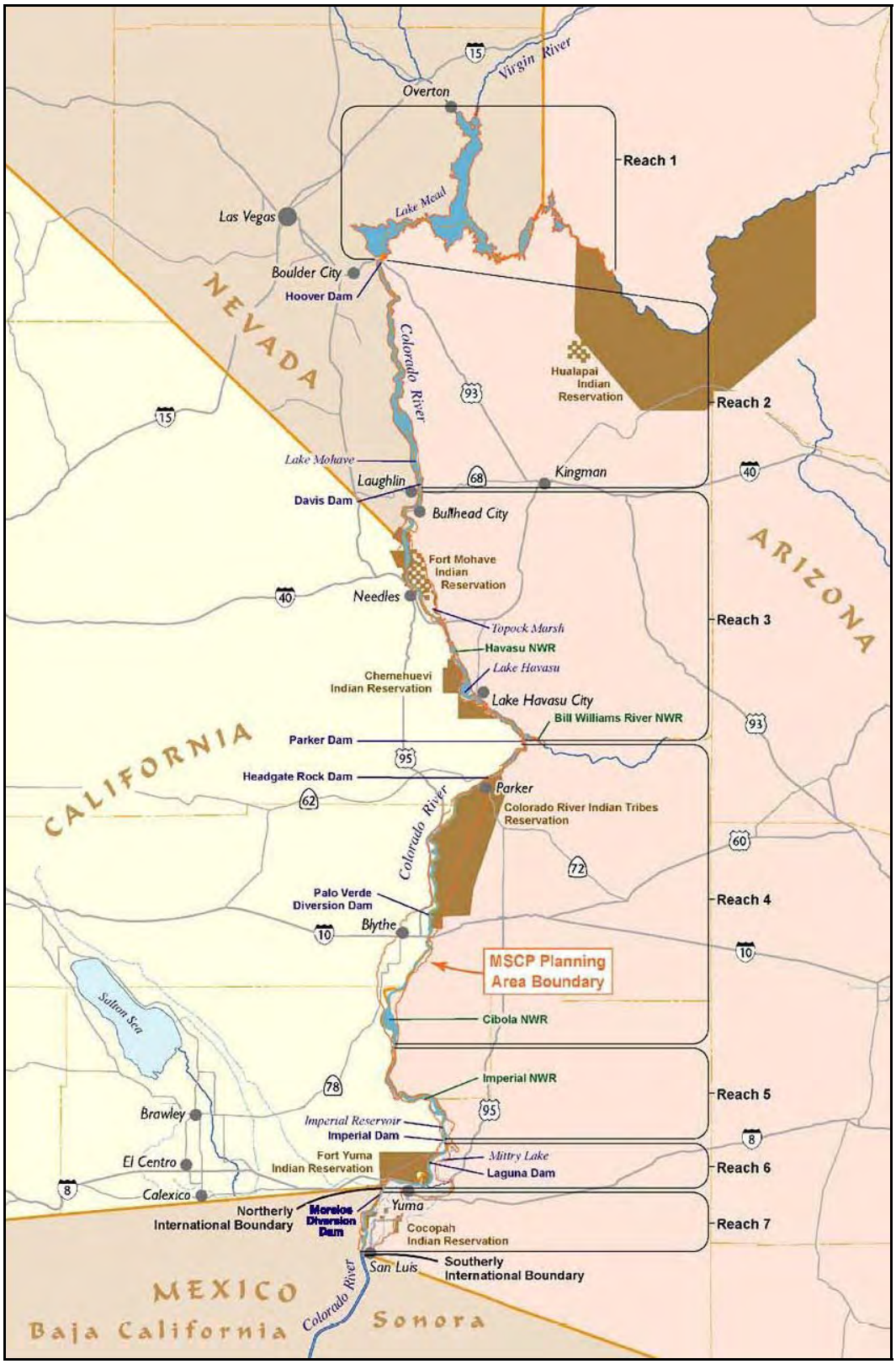

Figure 2.—Lower Colorado River Multi-Species Conservation Plan project areas in Nevada, Arizona, and California.

Mommsen *et al.* 1999). These documented cases are important to understanding bonytail demise and deserve closer study. If bonytail are sensitive to capture and handling stressors, it may help explain the very few bonytail recaptures in the wild after stocking. Little is known about the effects of hatchery-rearing, transportation, and stocking on the success and overall health of endangered Colorado River fishes. However, these practices have been shown to affect the physiological function, endocrine and cellular responses, immune system, and vulnerability to predators in other fish species, adversely affecting population size and sustainability (Portz 2007). Responses to stress are often thought to be interrelated functionally from biochemical, organismal to population and community levels of organization (Wendelaar Bonga 1997, Barton *et al.* 2002, Portz 2007). Stress prevention and alleviation of uncontrollable stressors are necessary to reduce the effects of disease and to improve health and survival of bonytail, both before and after stocking.

The consequences of stress are generally considered detrimental to fish (Wendelaar Bonga 1997, Barton *et al.* 2002) and can be dependent on whether they are acute, relatively short-term occurrences (*e.g.*, handling stress), or a longer-term chronic stressor (*e.g.*, periods of poor water quality including, but not limited to, the accumulation of ammonia, nitrite, carbon dioxide, acidification, and hypoxia). The likelihood for both types of stressors is high among fish culture practices because of the need to handle, move, grade, medicate, tag, and feed fish, and clean tanks, with the potential of poor water quality from inadequate water sources or overcrowding from intense culture or improper waterflows (Colombo *et al.* 1990, Davis 2006, Portz *et al*. 2006). The degree of stress is generally measured by the extent and duration of chemo-physiological changes (*e.g.*, cortisol, glucose, lactate, hydrominerals) and how quickly these conditions return to basal levels (Barton *et al.* 2002). However, some of these changes reflect a normal response to less extreme stressors from which a fish can easily recover, and an assessment of fish wellbeing and performance should not be restricted to an examination of chemo-physiological changes alone (Portz 2007). A combination of physiological changes in plasma constituents and the ability to perform under controlled challenge tests *(e.g.*, swimming performance, predator, and disease resistance challenges), with the assumption that stress is debilitating and influences are additively or synergistically diminishing performance capacity (Wedemeyer *et al.* 1984, Portz 2007), should assess more accurately stress-related effects of bonytail culture, transportation, and stocking, identifying the most stressful components of these practices.

Stress Response in Fish

Teleost fishes exhibit primary, secondary, and in some cases tertiary responses to stressors. The primary stress response involves the release of catecholamines (*e.g.*, epinephrine, norepinephrine) into the circulatory system from chromaffin cells (adrenal medulla homologue). This stress response also stimulates the hypothalamic-pituitary-interrenal (HPI) axis to release corticosteroids (*e.g.*, cortisol) from the interrenal tissue (Randall and Perry 1992, Wendelaar Bonga 1997, Mommsen *et al.* 1999). Catecholamine release into the bloodstream is rapid (Randall and Perry 1992), whereas cortisol synthesis has a temperature-related delay of minutes (Wedemeyer *et al.* 1990,

Lankford *et al.* 2003). Blood sampling procedures are invasive and thus stressful, and the temporal characteristics of catecholamine production make it difficult to use plasma catecholamine concentrations as a measurement of stress in small, wild fishes. In contrast, the inherent delay of cortisol formation makes it a more useful measurement. In most fishes, plasma cortisol, plasma glucose, hemoglobin, and hematocrit may increase over a 15-minute to 4-hour period in response to capture, handling, and transportation stresses (Robertson *et al.* 1988, Frisch and Anderson 2000, Grutter and Pankhurst 2000).

Primary stress responses trigger sequential secondary responses (*e.g.*, increases in plasma glucose, hematocrit, lactate, heart rate, gill blood flow, metabolic rate; decreases in plasma chloride, sodium, potassium, and liver glycogen; Pickering 1981, Mommsen *et al.* 1999, Barton *et al.* 2002). Secondary responses generally appear within a few minutes to an hour. These changes often remain for longer periods of time compared to catecholamine and corticosteroid increases, and assist in mobilizing fuels to meet increased energy demands. Hyperglycemia after stress results, in part, from glycogenolysis stimulation from catecholamines to satisfy increased energy demands for a "flight or fight" response (Mazeaud and Mazeaud 1981). Catecholamines also function to regulate some cardiovascular and respiratory functions, including increased blood flow, gill permeability, and lamellar recruitment (Booth 1979, Randall and Perry 1992, Wendelaar Bonga 1997). The resulting increase in gas exchange increases gill permeability to water and some ions. This can be observed as a gain in water and loss of small ions from the blood of freshwater fishes (Mazeaud *et al.* 1977, Wendelaar Bonga 1997).

In addition to catecholamines, cortisol is also responsible for physiological preparations to combat the effects of stressors. An elevation of plasma cortisol induces a wide range of secondary physiological responses in fishes including the stimulation of metabolic and osmoregulatory responses, and immunosuppression after recognition of an actual or perceived stressor (Barton and Iwama 1991). Cortisol is essential to the long-term maintenance of hyperglycemia after the catecholamine effects have subsided (Barton and Iwama 1991, Mommsen *et al.* 1999). However, chronic stress can be maladaptive, resulting in severely compromised immune function and/or energy stores. Non-specific immune function is extremely important serving as a first line of defense, and can be suppressed as a result of increased corticosteroids in the blood (Yin *et al*. 1995, Montero *et al.* 1999, Ortuño *et al*. 2001).

The secondary responses may ultimately cascade to tertiary stress responses, which include reduced growth rate, reduced metabolic scope for activity, decreased disease resistance, decreased reproductive capacity, as well as altered behavior and survivability (Wedemeyer *et al*. 1990, Barton and Iwama 1991, Mommsen *et al*. 1999). The extent of tertiary responses may be directly related to the severity and duration of the stressor Lankford *et al*. 2005). Physiological compensation to stress may be achieved, but chronic stress, even at low levels, may impair performance by diverting energy resources that might otherwise be used for routine activities, growth, immune function, and/or reproduction (Barton and Iwama 1991, Barton *et al*. 2002). Elevation of plasma cortisol decreases growth by increasing gluconeogenesis which is an endergonic process that

generates glucose from non-carbohydrate carbon substrates such as lactate, glycerol, and glucogenic amino acids. Increased plasma glucose necessary to combat stressors results in metabolic costs, and consequences of this action may effect somatic and reproductive growth somatic as well as increase disease susceptibility by immunosuppression (Maule *et al*. 1989, Barton *et al*. 2002).

Stress tolerance to handling, trucking, and stocking depends upon the species, life stage, genetic background, and previous exposure to stress (Woodward and Strange 1987, Barton and Iwama 1991, Portz 2007), and bonytail seem to be unusually stress sensitive (Chart and Cranney 1993, Tyus *et al*. 1999, Ward 2003, Mueller 2006). Many fish seem to survive immediately after exposure to stressors, but later die of disease, physiological dysfunction, or starvation (Mazeaud *et al*. 1977, Bestgen *et al*. 2008). Therefore, handling and transferring techniques that minimize stress and physical harm to already compromised fish should reduce debilitating effects.

Studies examining stress-related effects of crowding and confinement under high fish densities have shown conflicting results (Portz *et al*. 2006). Observed differences in the stress response can most likely be attributed to different species, life stage, size, behavior, nutritional status, amount of handling, sampling techniques, and time of sampling. In addition, direct comparisons of absolute plasma cortisol concentrations, as a measure of the primary stress response between species, are complicated by different handling protocols, husbandry, and tameness of fish that can generate substantial plasma cortisol concentration differences (Pankhurst and Sharples 1992). Woodward and Strange (1987) found that confinement of wild rainbow trout (*Oncorhynchus mykiss*) for 6 hours resulted in plasma cortisol concentrations nearly three times higher than hatchery stock under the same conditions. However, cultured rainbow trout (>2 years old) can acclimate to a confined space within 2 weeks, expressed by the return of cortisol to basal levels (Pottinger and Pickering 1992). Many authors have reported a temporary increase in blood glucose and/or plasma cortisol levels during crowding under high densities (Schreck 1981, Carmichael *et al*. 1984a, Woodward and Strange 1987, Barcellos *et al*. 1999, Ruane *et al*. 2002), while others have observed no effect (Vijayan *et al*. 1990, Kebus *et al*. 1992, Procarione *et al*. 1999). These inconsistencies may reflect sampling differences due to handling, because elevations in plasma cortisol associated with crowding stress are much less than those due to handling (Robertson *et al*. 1988, Ruane and Komen 2003). Chronic crowding under high densities appears eventually to lead to increased cortisol clearance rates and may be somewhat responsible for the disparities (Schreck *et al.* 1985). Ruane and Komen (2003) found common carp (*Cyprinus carpio*) plasma cortisol levels were highest when initially introduced to high densities, and then returned to basal levels in a few days. Pankhurst and Sharples (1992) also discovered that cortisol levels of snapper (*Pagrus auratus*) were initially elevated and remained high until after 60 minutes of confinement. Due to its relatively fast transient response as a primary stress indicator, plasma cortisol levels, alone, may be an unreliable chronic stress indicator for overcrowding.

Plasma cortisol affects carbohydrate metabolism by stimulating glucose production through gluconeogenesis resulting in an elevation of plasma glucose (Barton and Iwama

1991). Measuring fluctuations in blood glucose, in addition to cortisol levels, has become one of the most widely used techniques to monitor stress in fish (Pickering and Pottinger 1989, Barton and Iwama 1991), especially for acute stress because fish return to basal levels within 24 hours (Pickering and Pottinger 1989, Carmichael *et al*. 1984b). Fish held at high stocking densities are more likely to show increased levels of plasma cortisol and plasma glucose than those held at lower densities and may be incapable of acclimating to the crowded conditions (Clearwater and Pankhurst 1997, Vazzana *et al*. 2002). Although, other findings suggest that brown trout (*Salmo trutta*) and rainbow trout can acclimate to high densities (Pickering and Stewart 1984, Pottinger and Pickering 1992).

Only a few studies have investigated the role of life stage development on cortisol and stress responses of fish. Larval rainbow trout respond to acute stress with elevated levels of cortisol at only 2 weeks after hatching, demonstrating that the HPI axis is fully developed by this stage (Barry *et al*. 1995). Atlantic salmon parr (*Salmo salar*) are less responsive to confinement than smolts because of the physiological processes associated with the parr-smolt transformation (Carey and McCormick 1998). Due to anecdotal information of increased responsiveness to environmental stressors, care should be taken when handling, holding, and transporting larval and juvenile bonytail to minimize negative effects and mortality.

The mechanisms through which reproduction is affected by stress are not fully understood; however, there is evidence that reproduction is mediated by cortisol (Carragher *et al*. 1989, Campbell *et al*. 1992, 1994, Foo and Lam 1993, Hobby *et al*. 2000). Increased levels of plasma cortisol result in lower levels of plasma androgens and estrogens in stressed fishes (Pankhurst and Dedual 1994, Clearwater and Pankhurst 1997). There may be an effect of stress on metabolism and clearance of steroid hormones from the plasma. Whereas sex steroid binding protein protects androgens and estrogens from metabolism and aids in transport to target tissues, cortisol has been identified as a chief component of stress that changes the concentration of sex steroid binding protein and its binding characteristics (Hobby *et al*. 2000). Estradiol bound to sex steroid binding protein is not normally displaced by physiological levels of cortisol; however, it can be under extraordinary circumstances, such as, when the cortisol concentration is two orders of magnitude greater than that of estradiol (Hobby *et al*. 2000). Both rainbow and brown trout subjected to acute and chronic stressors before spawning exhibited reduced egg sizes, reduced sperm counts, and decreased survival rates of progeny (Campbell *et al*. 1992, 1994). Tank confinement (1–96 hours) initially increased plasma cortisol levels and decreased estradiol and testosterone levels. The sex hormones remained low, indicating no recovery of steroidogenesis in confined fish (Clearwater and Pankhurst 1997, Hobby *et al*. 2000). Therefore, the affects of stress on reproduction may have devastating consequences on reproductive success influencing population sustainability in repatriated bonytail.

Achii Hanyo Native Fish Rearing Facility Site Visit

A site visit was conducted on December 3–4, 2008, to observe bonytail culture, handling, tagging, truck loading, and fish-hauling procedures at the Achii Hanyo NFRF on the Colorado River Indian Tribes Reservation, near Parker, Arizona. We then continued to assist and monitor bonytail transportation via a fish hauling truck to a boat ramp on Lake Havasu at the U.S. Fish and Wildlife Service (USFWS), Arizona Fishery Resources Office, Parker, Arizona. At this location, bonytail were barge-released in the furthest navigable extent of the Bill Williams River National Wildlife Refuge with the assistance of Mitch Thorson (USFWS). The intention of the site visit was to provide suggestions indicating possible stressors that might influence overall fish health and survival during hatchery techniques, fish-hauling procedures, and stocking methods.

Achii Hanyo NFRF (Figure 3) is a grow-out facility for threatened and endangered fish species consisting of 7 earthen ponds, 4 raceways, and 16 circular tanks of varying sizes that are supplied with flow-through canal water. The ponds are used to grow fish until they are large enough to be stocked into the river. In December, Achii Hanyo Native NFRF staff (Mark Yost, USFWS) spent several nights slowly draining the ponds containing bonytail with a final rapid drain the night before harvest. Pond water depths were reduced to about 30 cm where seines were pulled through the pond to collect the majority of bonytail (Figure 4). Captured fish were removed from the seines and transferred to 1- by 1-m live cages where they could be easily dip netted and carried up

Figure 3.—Aerial photograph of Achii Hanyo Native Fish Rearing Facility on the Colorado River Indian Tribe, near Parker, Arizona, showing grow-out ponds and facilities.

Figure 4.—Seining grow-out ponds after levels have been lowered to crowd and collect bonytail.

the pond banks (Figure 5) to a live haul tank on a truck for transport and temporary holding in long, fiberglass tank raceways inside a Quonset building. After most fish were collected, remaining water and fish were drained into the pond's kettle, and remaining bonytail could be dip netted from the catch basin. Bonytail were then transported in the live haul tank to the building with the indoor raceways. Fish were again dip netted and transferred to the raceways. Once in the raceways, bonytail are crowded, measured, and sorted by size (Figure 6). Adult bonytail that had reached target size (≥ 300 mm) for stocking are then crowded and confined once more, before they are netted and placed into an anesthetic bath of tricaine methanesulfonate (MS-222; Argent Chemical Laboratories, Inc., Redmond, Washington). They are removed from the bath, measured (total length; Figure 7), and wire tagged (Figure 8) before returned to a raceway. Upon completion of tagging, bonytail are crowded and netted, once more, into the fish hauling truck (Figure 9) for the ca. 60-km trip to the boat ramp at the USFWS Arizona Fishery Resources Office near the confluence of the Bill Williams River and Lake Havasu. Fish were the unloaded with dip nets onto a barge with a live haul tank (Figure 10). Multiple barge trips were taken to the furthest navigable extent up the Bill Williams River where the fish were either dip netted out of the live haul tank (Figure 11) or released through the tank's gate valve (Figure 12). Released fish quickly swam away and did not loiter near the barge.

Figure 5.—Dip netted bonytail are carried up the pond banks to a live haul tank on a truck for transport to the temporary holding building.

Figure 6.—Bonytail are crowded, measured, and sorted by size.

Figure 7.—Measuring length of bonytail on a fish measuring board.

Figure 8.—Wire-coded tagging of bonytail before release.

Figure 9.—U.S. Fish and Wildlife Service's fish hauling truck used to transport hatchery raised bonytail to their release locations.

Figure 10.—U.S. Fish and Wildlife Service's barge with a live haul tank used to repatriate bonytail in locations other than roadside or dockside releases. Barge made it possible to repatriate bonytail in the furthest navigable extent up the Bill Williams River.

Figure 11.—Releasing bonytail by dip netting from the barge live haul tank.

Figure 12.—Releasing bonytail through the gate valves of the barge live haul tank.

Handling, Transportation, and Stocking Recommendations

The intent of the following recommendations is to reduce the potential loss of bonytail by reducing the number, severity, and duration of stressors associated with hatchery and stocking practices. Harvest from grow-out ponds is a potential initial stressor prior to temporary holding, handling, tagging, transporting, and release. Gentler, more 'fish friendly' protocols and reducing handling may provide conditions to inhibit stress levels from becoming initially high and restrict them from reaching their thresholds at the onset of harvesting and stocking-related practices. Fish can better handle the occurrence of the next stressor if allowed some time to recover from the initial stressor. Fish that are subjected to multiple stressors have fewer tendencies to respond to a stimulus and require longer recovery times than fish that are exposed to a stressor once (Sigismondi and Weber 1988, Portz 2007). Bonytail may not be able to accommodate subsequent stressors after experiencing hatchery and stocking-related stressors and leave them vulnerable to predation and/or disease. Handling and transportation may stress fish, leading to direct and indirect mortality during the harvest and transportation process and upon release (Wedemeyer 1976, Barton *et al.* 1986). Fish may suffer increased mortality rates due to predation after handling, holding and/or transportation release (Olla and Davis, 1989; Masuda and Ziemann, 2003). Further research is needed to address hatchery and stocking protocols and conditions, and to fully understand the relationship of bonytail physiological stress with disease resistance, predator avoidance, and post-stocking survival. The following recommendations and future LCRMSCP research will provide valuable information to modify hatchery and stocking operations to enhance fish survival.

Stress control in the hatchery

Fish in hatcheries and grow-out facilities are subjected to unavoidable and sometimes persistent stressors from routine operations. A fish's reaction to stressors under these rearing conditions is maladaptive and often compromises growth, reproduction, and survival. Nevertheless, there are approaches to alleviate some of the stress and limit the damage that may result. Often the approaches may be procedural changes in operations and not major design or infrastructure modifications. If changing hatcheries procedures results in greater survival of repatriated fish, the increased costs and efforts would be worthwhile.

1) **Proper training of personnel should be required and is essential to handling sensitive species**. All personnel should be properly trained in appropriate fisheries collection techniques, harvesting procedures, and stress mitigation before collecting, handling, and transporting bonytail.

2) **Limit the exposure to stressful activities**. Stress responses in fish are often proportional to the exposure frequency, severity, and duration of the stressor. Handling time must be kept to a minimum to avoid stress. Dip netting, hand-caring, measuring, tagging, marking, and time out of water can be highly stressful and may lead to delayed mortality. Minimize handling and the time that bonytail are kept out of water (≤ 30 seconds). Attempt to keep bonytail wet when

handling. All physical measurements should be taken on fish anesthetized in a solution of 50–100 mg/L MS-222 until they lose equilibrium, 0.13 mL/L Polyaqua, and 5 ppt salt.

3) **Allow for sufficient recovery time between multiple stressors**. Additive and/or synergistic effects of multiple stresses are more detrimental to fish than single events alone (Barton *et al.* 1986). Simultaneous stressors should be avoided because they might overwhelm the stress response to a point that will be unrecoverable. Short holding durations, greater than 12 hours (depending on species and intensity of stressor), are recommended and valuable in allowing fish to recover from a prior stressor to reduce subsequent stress.

4) **Avoid stressful practices when water temperatures are elevated**. Temperature affects the physical and chemical properties of water and all chemical reaction equilibria. Water temperature influences gas solubility, and, therefore, the dissolved gas content in water. Water temperature is critical to the physiological reaction rates and metabolic processes of fish. Every species has an optimal temperature range where no signs of thermal stress and/or altered behavior are present. *Bonytail water temperature should be kept the same or slightly lower than originating source water (<13 ^{o}C)* to avoid thermal shock when fish are transferred. As a fish's body temperature increases, biochemical reaction rates generally increase for enzyme reactions and membrane transport flux dynamics, even relative to minute temperature shifts of less than 0.5 ºC (Elliot 1981, Neill and Bryan 1991). Thermal stress occurs when the water temperature exceeds the optimal temperature range, thus initiating changes that disturb normal physiological functions resulting in energy expended towards stress responses, and even a potential decrease in individual survivorship (Brett 1958, Fry 1971, Elliot 1981). Optimal temperature ranges may change with ontogeny, body size (Brett 1956, Fry 1971), and the individual's health, including past thermal experiences (Elliot 1981). For example, most fishes can acclimate to gradual temperature changes over months, such as with season. However, rapid water temperature changes or exposures to sustained temperatures outside the optimal range often result in thermal stresses or lethal conditions. Allowable temperature acclimation rates are difficult to recommend because of many confounding factors, however it is generally accepted that a *conservative acclimation rate of 1 ºC per hour, not to exceed 3 ºC per day is optimal*. Many times in hot climates and transporting fish on long, overland distances, temperature differentials are unavoidable and oxygen levels should be kept at saturated levels. *Transportation can be less stressful, if it takes place during cooler months or in chilled water* (Carmichael *et al.* 1984a, 1984b, Robertson *et al.* 1987, Erickson *et al.* 1997, Pavlidis *et al.* 2003). Temperature differences between transport truck temperature and new holding tank or stocking location should be minimized. Cooler water temperatures typically decrease metabolic processes (*e.g.*, oxygen consumption rate, ammonia (NH_3) excretion rates, activity levels), while oxygen solubility increases. Lipid reserves, plasma proteins, blood osmolality and serum electrolytes may decrease due to ion- and osmo-regulatory dysfunction creating

physiological imbalances with thermal stress (Houston and Schrapp 1994, Swanson *et al.* 1996, Claireaux and Audet 2000). The additional metabolic costs from thermal stress responses subtract from immune function, reproduction, and growth (Schreck 1981, Barton and Schreck 1987, Donaldson 1990, Barton and Iwama 1991). Thermal stress-related behaviors include a reluctance to feed, sudden or erratic movements with possible collision with the tank wall, jumping, rolling, pitching, color change, and increased regurgitation, defecation, and gill ventilation (Elliot 1981, Alsop *et al.* 1999, Cooke *et al.* 2003, Smith and Hubert 2003).

Water temperature also affects swimming performance, muscular action, and movement. For example, acute temperature changes have resulted in reduced swimming performance (Kieffer 2000, Parsons and Smiley 2003). Fishes often acclimated to a range of temperatures typically exhibit the poorest swimming performance at the lowest temperature (Alsop *et al.* 1999, Myrick and Cech 2000). Additionally, a predator's ability to forage and prey's ability to escape predation, as well as general migration and spawning capacities are affected by temperature (Moore and Townsend 1998, Myrick and Cech 2000, Cooke *et al.* 2003a, Parsons and Smiley 2003, Marine and Cech 2004). With the possible additive or synergistic effects of water temperature changes associated with live transport and subsequent release, along with stressors attributed to handling, higher than expected fish mortality is likely due to decreased swimming ability, and increased predation vulnerability (Moore and Townsend 1998, Parsons and Smiley 2003).

5) **Be mindful of water quality, it is one of the most important contributors to fish health and stress level**. Fish may be able to tolerate adverse water quality conditions; however, when adverse conditions occur from a concert of environmental and biological variables, fish may be quickly overcome by the resulting physiological challenges. The effects of water quality vary considerably with species, life stage, and previous exposure to stress (Wedemeyer 1996). Therefore, effective management of fishes under confinement commands species-specific knowledge of their biological requirements with respect to water quality and an understanding of their previous exposures or condition when introduced to new bodies of water. Fishes may incur additional energetic costs associated with stress responses from physical and chemical fluctuations in aquatic systems (Barton and Iwama 1991). Poor water quality, as determined by each species, can prompt the reallocation of energy from secondary physiological processes (*e.g.*, growth, reproduction) towards primary processes (*e.g.*, metabolism, immune function). Thus, satisfactory water quality is essential for holding fish in an environment that will neither activate their stress responses nor alter their normal energy budget. Short-term exposure to poor water quality can result in permanent damage or mortality if physical or chemical variables are allowed to reach lethal levels and/or synergize in a deleterious manner (Carmichael *et al*. 1984a, Robertson *et al*. 1987, Erickson *et al*. 1997, Pavlidis *et al*. 2003).

Water quality remains the most controllable factor for survival and promoting fish health during short-term holding and transportation (Portz *et al*. 2006). Water quality can become quickly deteriorated by poor flow rates, high temperatures, low oxygen concentration, high dissolved carbon dioxide concentration, high fish loading densities, elevated amounts of detritus, total suspended solids, and anoxic benthic substrates, and high concentrations of total ammonia-nitrogen (TAN) and ammonia, and when fish are hyperactive and/or stressed. In addition, some water sources may not be adequate for fish holding without alteration. Figure 13 shows the poor water quality of the live haul tank used to transport bonytail from the rearing ponds to the Quonset building for temporary holding. The water source to fill this tank did not provide adequate clean water which was exacerbated by the addition of anoxic mud with each net of fish and the lack of an aeration system under extremely high fish loading densities.

Figure 13.—Poor water quality and lack of aeration in the live haul tank on a truck for transport to temporary holding building

Saturated dissolved oxygen (DO) levels should be maintained at >6.0 mg/L for bonytail. DO content is the most critical and limiting water quality variable (Fry 1947, 1971) because it acts as the respiratory medium for most fish. DO is essential for aerobic activity, and thus DO concentrations below the species' aerobic limit (critical pO_2) require fishes to exploit anaerobic metabolic pathways, which are inefficient and useful only for short time periods. Cech *et al.* (1979) showed that the critical pO_2 of a fish increases as temperature and metabolic

demands for O_2 increase. When the ambient pO_2 is sub-optimal, respiratory stress can occur and be lethal (Neill and Bryan 1991). Conversely, hyperoxic (supersaturation) conditions can decrease gill ventilation increasing blood pCO_2 with potentially severe consequences. The oxygen consumption rate and the degree of respiratory stress is dependent upon the species, life stage, body weight, prior exposure, health, and activity levels (Elliot 1969, Wedemeyer and McLeay 1981, Alabaster and Lloyd 1982).

Respiratory stress involves increased gill ventilation frequency and volume (Campagna and Cech 1981, Lutnesky and Szyper 1990), pituitary-interrenal activity (Wedemeyer and McLeay 1981), and hematological changes (*e.g.*, increased red blood cell capacity; Lochmiller *et al*. 1989). It also includes changes in cardiac rate, stroke volume and output, gill perfusion patterns, and oxygen consumption rates (Webber *et al*. 1998, Brodeur *et al.* 2001, Stecyk and Farrell 2002). As a result, fishes can experience tissue hypoxia (hypoxemia), stunted or retarded growth (Weber and Kramer 1983), reduced fecundity, or unconsciousness. In addition, fish exhibit altered behavioral patterns, such as erratic swimming, side-lying, aquatic surface respiration, reduced spawning activity, avoidance of low oxygenated areas, and movement into cooler water reducing their metabolic oxygen consumption (Hughes 1981, Kramer 1987, Lutnesky and Szyper 1990, Love and Rees 2002). The effects of low DO content on swimming performance are important to consider when releasing fishes from temporary holding systems. A fish in hypoxic conditions (*e.g.*, associated with sub-optimal transportation system) may exhibit reduced swimming ability or performance, impairing foraging or predator avoidance. For example, largemouth bass, *Micropterus salmoides*, and coho salmon, *Oncorhynchus kisutch*, have decreased maximum sustainable swimming speeds in hypoxic conditions (Dahlberg *et al*. 1968).

Aeration equipment or airstones attached to oxygen/compressed air cylinders need to be in place and used in all holding practices. Monitoring of TAN, nitrite (NO_2^-) and nitrate (NO_3^-) concentrations is recommended for even short-term holding (Portz *et al*. 2006). TAN accumulation occurs in holding containers that have low water exchange, high fish load densities, foraging, and/or stressed fish. Commercially available kits are available to monitor TAN, NO_2^- and NO_3^- at low levels (*i.e.*, TAN < 2.5 mg/L), which are preferable for this application to the high concentration kits. Short-term exposure to elevated TAN and NH_3 concentrations can have detrimental effects on fish, causing increased gill ventilation, erratic and quick movements, loss of equilibrium, coughing, convulsions, lack of foraging, and even mortality in fishes (Meade 1985, Russo and Thurston 1991). All holding water should contain 5 ppt NaCl (5 g/L); 0.13 mL/L PolyAqua®, and 25 mg/L MS-222 (or greater concentrations for deep sedation and loss of equilibrium). *Use dilute salt solutions (5 ppt NaCl) during stressful practices* to alleviate the hydromineral imbalance and limit the loss of ions caused by impaired osmoregulation of the gill membrane for freshwater fishes.

6) **Stop feeding fish 1–2 days prior to handling and transportation**. Food should be withheld for 24 hours in fish estimated to be less than 10 g and 48 hours for larger fingerlings and brood fish. This reduces water fouling associated with fish waste and regurgitated food, and reduces the oxygen requirements of the fish. Increased fish wastes and food also increase TAN and ammonia levels in the water.

7) **Capture gear types and techniques that require entanglement** (*e.g.*, gill and trammel nets) **and long set durations should be avoided**. Mortality rates and stress vary substantially with capture devices and practices (Kelsch and Shields 1996). Less abrasive net materials and knotless nets are preferred to reduce both physical trauma to skin and mucous layer, and physiological stress from confinement and handling. Matching the correct net mesh size to the targeted fish is also important so that gilling fish can be avoided (Figures 14 and 15). Nets should be cut from bonytail if they are severely entangled or gilled to speed removal and avoid harm.

Figure 14.—Gilled bonytail being removed from the seine after seining a grow-out pond.

Figure 15.—Capture and handling-related lesions and injuries that may result from excessive handling, improper net size, and mechanical trauma.

8) **Use anesthesia during severely stressful practices to increase ease of handling, suppress the cortisol response, and promote survival**. Be conservative with anesthetics because anesthesia itself may result in physiological stress and mortality. Summerfelt and Smith (1990) provide an overview of fish anesthetics and anesthesia techniques. Anesthesia allows for easier handling and reduces the possibility of dropping and/or injuring the fish. MS-222 is very effective, safe, and probably the most well used fish anesthetic. MS-222 is a fine crystalline powder and it forms a clear, colorless, acidic solution in water. *Because of its acidity, MS-222 should be buffered with twice the amount of sodium bicarbonate as MS-222 used* (*e.g.*, 5 grams MS-222 : 10 grams sodium bicarbonate). Use of this anesthesia does require a 21-day holding period for fish with possible human consumption in the wild, which is not a concern for bonytail due to their ESA status (Marking and Meyer 1985). Immobilization, transport, and minor surgery require some degree of tranquilization; however concentrations vary with species and temperature. A light sedation of 25 mg/L MS-222 performs well to lessen the stress response associated with transport. Greater concentrations (50–100 mg/L MS-222) should be administered for deep sedation and loss of equilibrium when measuring, sorting, and tagging fish. Caution should be taken when inducing rapid rates of anesthesia, and fish must be observed closely for signs of over exposure. If this occurs, they should be returned to freshwater or flush gills before medullary collapse often indicted by the ceasing of opercular activity. Bonytail need to be recovered from MS-222 in

freshwater prior to release until equilibrium is restored so that they can orient to any present current, escape capture by predators, and avoid entrainment.

9) **Minimizing handling and proper water quality can contribute significantly toward alleviated immunosuppression and the prevention of disease**. The type, intensity, and duration of the stressor are major determinants of the type of immune response. Susceptibility of fish exposed to acute stressors and bacterial or fungal infections is temporal. There is immunosuppression shortly after the onset of stress and immune enhancement 12–24 hours later, and another decrease in disease resistance ability about a week later (Maule *et al*. 1989). Heightened fish immune function (*i.e.*, increased concentrations of lysozymes, cytotoxic cells, and phagocytes; Demers and Bayne 1997, Ruis and Bayne 1997, Ortuño *et al*. 2001) has been observed following an acute stress. After exposure to acute stressors the fish's body mobilizes the immune system, presumably protecting itself from potential subsequent trauma. Most often, deleterious effects associated with immune system dysfunctions result from both severe acute and chronic stress (Yin *et al*. 1995, Ortuño *et al*. 2001, Vazzana *et al*. 2002). Elevated (especially, prolonged) plasma cortisol levels act as an immunosuppressant in fish, reducing the number of leukocytes in the circulating blood and phagocytic activity (Pickering and Pottinger 1989, Barton and Iwama 1991, Montero *et al*. 1999, Vazzana *et al*. 2002). The elevation of cortisol inhibits both the transformation of B-cell precursors into antibody producing cells and the synthesis of cytokine needed to stimulate antibody production. Cellular immune responses can also be affected by stress. Handling and crowding stress can elicit a decrease in the chemiluminescent response of pronephros (kidney) cells in fish, depressing the phagocytic function of leukocytes (Yin *et al.* 1995, Vazzana *et al.* 2002). Monocyte and granulocyte concentrations are mobilized to the circulating blood from the head-kidney for 48 hours after exposure to a stressor (Ortuño *et al.* 2001). Despite their significant increase in number, the phagocytic ability of these leukocytes is significantly reduced immediately after stress, and normal immunocompetence may not recover for more than 48 hours (Ortuño *et al.* 2001, Vazzana *et al.* 2002). Short-term crowding stress also depressed serum hemolytic activity, an important humoral immune parameter (Ortuño *et al.* 2001). Increases in hematocrit, hemoglobin, and erythrocyte count have been also observed and may suggest a strategy for increasing the blood's oxygen carrying ability during metabolically challenging periods (Montero *et al.* 1999). Data suggest that even short duration (*i.e.*, a few hours) confinement exposure under high fish densities elicits an immune response and might result in a reduced immunocompetence (Yin *et al.* 1995, Ortuño *et al*. 2001).

Hatchery-reared bonytail have been found to survive for only short periods in the wild after release (Bestgen *et al*. 2008). Bestgen *et al*. (2008) found that repatriated fish survived no more than 4 months and if they were able to be recaptured, many had *Lernea* infestations, fungal infections, and a 20% loss of body mass. Other studies have also shown that handling stressors suppress bonytail immune functions and as a result are susceptible to outbreaks of the

parasite ich (Tyus *et al.* 1999, Montony 2008). Fish infected with ich can have prolonged elevated cortisol levels and have been shown to be higher than those observed in healthy fish following handling and PIT-tag insertions (Montony 2008). Bonytail that were handled for tissue sampling have been observed to have a 77% mortality that was attributed to ich infections (Tyus *et al.* 1999). Fifteen bonytail mortalities from the Achii Hanyo NFRF site visit were frozen and shipped to Denver for necropsy. No *Ichthyophthirius* infections or trophozoites (white nodules) on the skin or gill epithelium were visible. *Saprolegniosis* infections were found on superficial lesions of many of the bonytail (Figure 16).

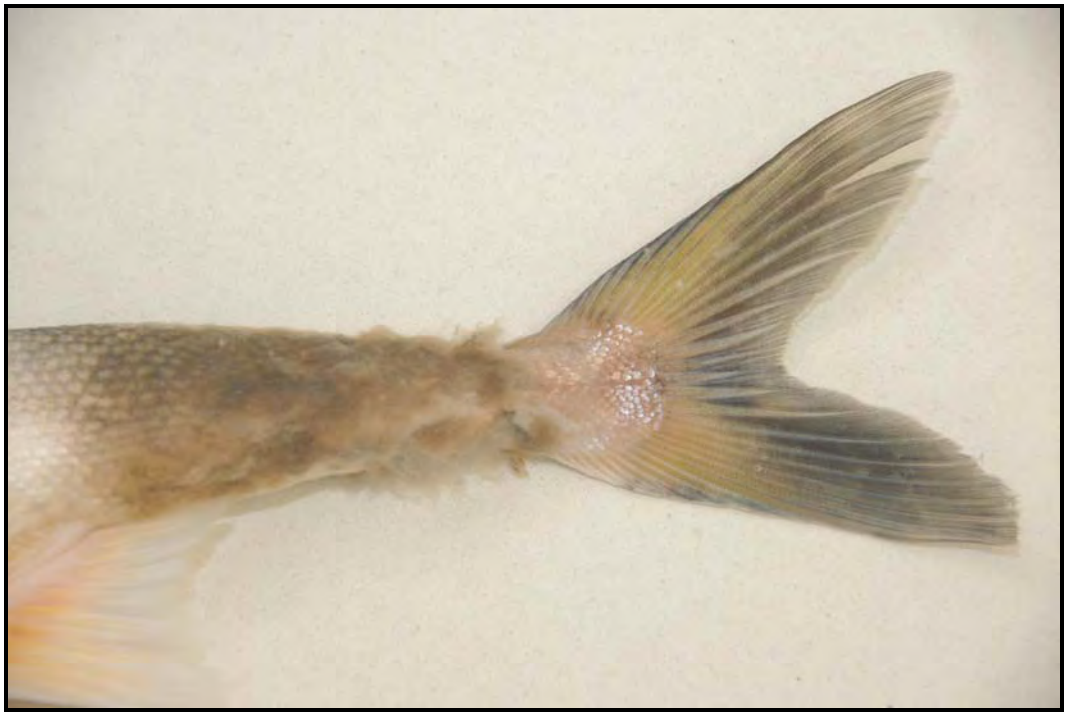

Figure 16.—*Saprolegniosis* infection of the peduncle area of a deceased bonytail that was collected from the fiberglass raceways in the Quonset building of the Achii Hanyo Native Fish Rearing Facility.

These infected regions did not exhibit inflammation but did have a loss of natural pigment. Many lesions were covered by furry looking mycelium (Figure 16) and on a wet mount, the nonseptate hyphae and zoosporangia of the mycelium can be seen under a microscope (Figure 17). The mature *Saprolegniosis* zoosporangia also had dark spores in finger-like projections. Fungal infections, such as *Saprolegniosis*, are uncommon when fish do not have a compromised immune system, physical damage, or physiologically stressed. In this case, fish were suffering from both handling-related (netting, crowding, tagging, hauling, *etc*.) physiological and mechanical trauma that caused skin and tissue wounds that provided a vector, or entry point for *Saprolegniosis* infection. The fungal infection was most likely exacerbated by a suppressed immune system as a result of physiological stress.

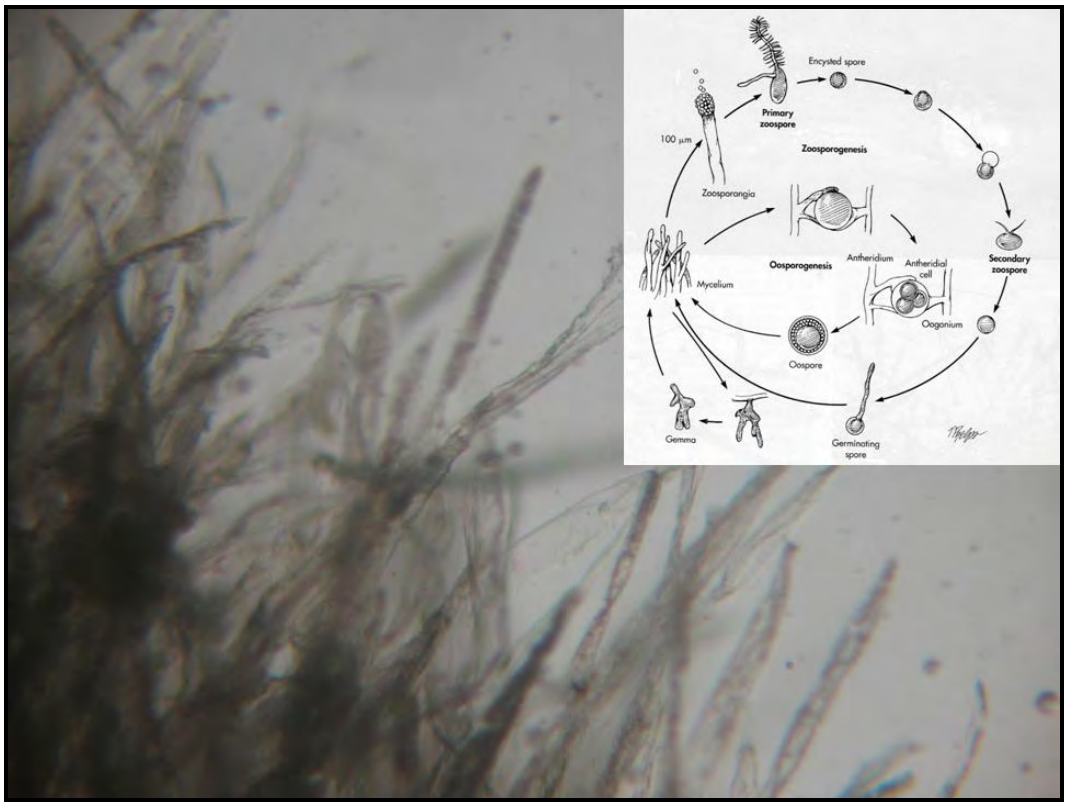

Figure 17.—Wet mount sample of a bonytail *Saprolegniosis* infection showing the mycelium with nonseptate hyphae and zoosporangia. *Saprolegniosis* zoosporangia have terminal finger-like projections with a dark spore mass when mature. Insert is the *Saprolegniosis* life cycle (Noga 1993) depicting the mycelium and zoosporangia stages in the photograph.

Prophylactic treatments may be beneficial to minimize the chance of infection from bacteria, fungi, parasites, and viruses. Skin and mucous damage and predisposing stress should be avoided to decrease the fish's susceptibility to infection. Numerous drugs and chemicals are available and can be administered directly into the water. The use of a synthetic slime coating products (*e.g.*, PolyAqua®, NovAqua®, Stress Coat®) for damaged tissue provides a barrier against loss of internal fluids and hydrominerals, and reduces the possibility of infection by bacteria and external parasites. Prolonged immersion in saltwater (> 3 ppt NaCl) is an effective prophylactic treatment for fungal and bacterial infections, and some parasites. *Ichthyophthirius* multifilis is very sensitive to saline water and cannot tolerate salinities over 1 ppt. Malachite green is the most effective remedy for fungal infections in fish. Many protozoan parasites (*e.g.*, *Ichthyophthirius* species) can also be treated with malachite green, but administering a combination of formalin and malachite green for 7 days is more effective. While these treatments are effective in tanks, they are impractical in commercial and pond-type facilities. Copper sulfate is often used to combat ich for larger aquaculture practices.

Pathogens are present in most surface water sources and design of a fish facility should aim at preventing the introduction of disease organisms (*e.g.*, UV irradiation, ozone, or chemical treatment). Ultraviolet irradiation is the safest method and poses no direct risk to fish, but requires maintenance to remain continuously effective and can be somewhat costly. Regardless of the preventative measures, fish will be exposed to pathogens while rearing in grow-out ponds. However, water supplied to outdoor tanks and tank inside the Quonset building at Achii Hanyo NFRF can be UV irradiated and monitored for water quality (*i.e.*, temperature, DO, nitrogen saturation, pH, and salinity). In addition to providing clean, pathogen-free water, tanks and fomites should be disinfected with chlorine or hyamine compounds to prevent pathogen transfer. Pathogens, such as *Saprolegniosis*, are present in almost every body of surface water throughout the world, so irradiating them is not feasible. Therefore the most effective means of controlling infections is by reducing stressors (Westers and Pratt 1977).

10) **Tagging and tissue sampling of bonytail should only occur after acclimatization to holding facilities and given at least 24 hours recovery time after harvest**. Allowing for recovery from previous stressors is recommended to reduce subsequent stressors cause by tagging or tissue sampling. These practices should only be performed after being anesthetized in a solution of 50–100 mg/L MS-222 and 5 ppt NaCl. Incisions from PIT tag insertions, fin clips, and tissue sample sites should be observed for bleeding and severed skin treated with betadine topical antiseptic to prevent potential infection.

11) **High fish densities in nets and tanks are one of the most common problems throughout aquaculture facilities and live-fish transfers** (Wedemeyer 1996). The maximum density of fishes in a confined space depends on a fish's behavioral and physiological requirements for space. Carrying capacity is greatly governed by water quality and when it is approached or exceeded, may contribute to lowered metabolism, immunosuppression, increased disease transmission, and lowered survival rates. Holding fishes, even for short periods, at high densities is known to adversely affect many biological functions including metabolism (Vijayan *et al*. 1990, Jørgensen *et al*. 1993, Montero *et al*. 1999), growth (Trzebiatowski *et al*. 1981, Soderberg *et al*. 1993, Procarione *et al*. 1999), health and immune function (Yin *et al*. 1995, Demers and Bayne 1997, Ortuño *et al*. 2001), corticosteroid responses (Schreck *et al*. 1985, Kebus *et al*. 1992, Ruane and Komen 2003), and survival (Soderberg and Meade 1987, Plumb *et al*. 1988, Holm *et al*. 1990). Despite numerous studies on the effects of stocking density and confinement on various physiological aspects of fishes, there is a lack of consensus in the literature as to how these processes are affected in fishes held in grow-out ponds, tanks, hauling trucks, and aquaria. Fish survival may differ due to species, origin and holding conditions. Domestic and wild strains of fish may not respond similarly to high densities and crowding. Confinement stress results when species specific space requirements are not met, stimulating a physiological response. Intense, short-term crowding stress that commonly occurs in

aquaculture, commercial and recreational fishing, stocking, and salvage practices, possesses characteristics of chronic as well as acute stress, including compromised immune systems resulting in disease or death (Portz *et al.* 2006). Therefore, it is important to stay within the density tolerances of bonytail, regardless of short-term cost savings or convenience. If these values are not known, conservative estimates from similar species should be used. *Hauling densities for bonytail should not exceed 30 g of fish per liter water* (USFWS 1998).

Mortality at high-densities has been commonly ascribed to poor water quality and disease. Poor water quality can place an extra burden on an already physiologically compromised fish under high densities. High fish densities can deteriorate water quality when being held in systems with inadequate filtration or waterflow (Pickering and Stewart 1984, Wedemeyer *et al*. 1990, Kebus *et al*. 1992). These holding conditions can lead to hypoxia and accumulation of toxic compound (*e.g.*, ammonia) concentrations, which can be detrimental to fish health. The risk of pathogen transmission in high stocking-density systems is elevated because of the closer proximity of fish (Bosakowski and Wagner 1994, Wedemeyer 1996). Fish biomass is just one measure of fish density for predicting water quality changes or stress effects. Presumably because of the allometric relationship between body mass and metabolism, a smaller mass of small fish can be held per volume water compared to the same mass of large fish (Piper *et al*. 1982, Wedemeyer 1996). More research is needed to help determine maximum and optimal stocking bonytail density capacities at different body sizes, life stages, and holding conditions.

12) **Stress during short-term holding and transportation should be minimized**. Hauling live fish can be accomplished without severe stress and harm to fish if the proper facilities, equipment, staff training, and conditions are present. Stressors associated with holding and hauling fish may include water quality changes, handling, physical damage, crowding, confinement, pathogens, and sloshing. See Portz *et al*. (2006) for a full review on short-term holding of fish. Many studies have shown that transporting fish can causes stress (Carmichael *et al*.1984, Robertson *et al*. 1987, Carmichael and Tomasso 1988, Erickson *et al.* 1997, Swanson *et al.* 1996, Frisch and Anderson 2000). Most fish-friendly transportation techniques include means to either alleviate the stressor or mitigate its impacts. Many of these methods have already been discussed, including reducing handling impacts, lowering fish densities, water quality treatments, *etc*.; however other means deserve some mention in decreasing transport stress. Transport tank size, shape, and design should be considered (Portz *et al*. 2006). Baffles should be installed in transport tanks or fill tank completely full to minimize sloshing. Winkler (1987) and anecdotal information from transporting salvaged fish at the Tracy Fish Collection Facility, Tracy, California, indicate that techniques used to decrease sloshing during fish hauling reduces stress.

During transportation, water quality often deteriorates at an accelerated rate (potentially within 1–2 hours after loading) due to the fishes' stress responses, physical reactions (*i.e.*, regurgitation, excretions, and mucus production), release of breakdown products from organic matter, and microbial respiration. Stress responses are typically exacerbated by poorer water quality conditions from inadequate water exchange rates (Chow *et al.* 1994; Davis and Schreck 1997; Paterson *et al.* 2003). For example, the capture, handling and hauling of fishes increases their metabolic activity and thus their oxygen consumption rates (Davis and Schreck 1997). Fish respiration enhanced by crowding reduces DO and increases dissolved CO_2 concentrations, while metabolism-related secretions increase the ammonia (NH_3), total suspended solids (TSS) and total dissolved solids (TDS). Assuming the temperature remains the same, pH will decline with the increasing dissolved CO_2. Because of previous exposure to stressors at the hatchery and already heightened stress responses coupled with deteriorating water quality, great care should be taken when transporting bonytail to minimize negative effects and mortality.

Transporting protocol should be as follows (modified from Jensen 1990a, 1990b, USFWS 1998, and Tracy Fish Collection Facility hauling procedures):

a. Before loading the fish, the transporting staff should make detailed arrangements with receiving personnel and inform them of any specific needs or requirements. Water temperature and other relevant water quality information should be shared so that transport water corresponds to receiving water. Oxygen and temperature meters should be operational on holding tanks. Drivers should follow a preplanned specific route and contact receiving personnel with estimated time of arrival (cellular phone communication is recommended). Transport and stocking arrangements should be verified before the fish transport truck leaves the hatchery.

b. Have 12 hours of back-up oxygen and nitrogenous detoxifying compounds (*e.g.*, AmQuel Plus®; removes all forms of ammonia, ammonium, nitrites, nitrates from the water) available to prevent loss of bonytail during vehicle breakdown.

c. Fish need to be quickly transferred from holding tanks to the fish transport truck. If performing net transfers to the fish hauling truck, do not overload nets because fish on the bottom will be crushed and suffocated. A water-to-water transfer using buckets is preferred to a net transfer. When emptying the nets and buckets into the transport tanks, avoid dropping fish into the water. Commercial devices (*e.g.*, Archimedes screw lifts and fish friendly pumps) may also be used to transfer fish, however they should be thoroughly tested on the target species before use. These transfer devices specifically designed for moving fish can inadvertently elicit a significant stress response (Portz 2007).

d. Do not allow the hauling density to exceed 30 g/L fish to water.

e. The DO concentration of the hauling water will be at saturation (>6.0 mg/L). Hauling water will contain 5 ppt NaCl, 25 mg/L MS-222, 0.13 mL/L PolyAqua®. The temperature should be slowly tempered to meet receiving water temperature.

f. Immediately after loading, all equipment on the transport vehicle should be re-checked before departing. Aerators or oxygen stones, oxygen concentrations, and temperatures should be checked one last time within an hour after leaving the hatchery.

g. When unloading fish, a tempering water pump should be present and functional, and thermometers should be used to compare water temperatures.

h. After confirming similar water qualities between truck hauling tank and host water. Carefully unload fish using gravity driven hoses, buckets, or nets from hauling tank to receiving water or other transport vehicle for further dispersal (*i.e.*, barge for river transport).

i. Record stocking data and properly store in database for reference.

13) **Determine an index for the optimal age (length) for stocking based on stress tolerance, disease resistance, predator avoidance, and post-stocking survival**. A mathematical model needs to be developed that estimates the size of bonytail that has the best chance for survival when factored with environmental stress, disease occurrence, and predator encounters. These risks and post-stocking survival should be compared with various sizes of bonytail to determine optimal stocking size(s) because these factors are life stage and size dependent.

14) **Provide exercise conditioning prior to release to increase avoidance behaviors**. Culturing of bonytail in ponds and tanks with low flows or standing water may hinder the development of swimming performance and potentially survival after release (Ward and Hilwig 2004). Stream orientated fish that are reared under flows are generally healthier (Davidson 1989), have increased muscular development (Young and Cech 1994), and are more likely to evade predation. Low-level aerobic exercise also promotes post-stress recovery in fish (Young and Cech 1993, Meyer and Cook 1996). Hatchery-reared bonytail that are held in ponds or tanks with low flows are more likely to experience down stream displacement (Mueller and Marsh 1998, Foster and Mueller 1999a, Mueller 2003) because they lack the stamina and skills associated with maintaining position in flows. The lack of swimming ability also makes these fish more vulnerable to predation (Webb 1982, Fuiman 1986, Marsh and Brooks 1989). Ward and Hilwig (2004) found that bonytail swimming performance can be increased by 15% in as little as 10 days. Their results indicate that bonytail

reared in non-flowing water environments have lower exercise conditioning and swimming performance compared to exercised and wild fish. These two factors, along with handling and transportation stress associated with stocking, may provide some indication why few fish are seldom recaptured during population monitoring events and have limited success from repatriation efforts.

Bonytail should be conditioned to low velocities (c.a. 0.1-0.3 m/s) for a minimum of 2 weeks before release. Research is needed to determine the optimal conditioning velocity for bonytail. Fish should not be exercised to the extent that growth is hindered by the reallocation of energy for swimming. Exercise conditioning can be performed in large circular tanks and raceways at the hatchery, or screened flow-through canals. For more information on exercise conditioning of fish see Davison (1997). Exercise conditioning at the hatcheries or grow-out facilities should be further researched and evaluated to gain a better understanding in its affects on post-repatriation survival.

15) **Expose bonytail to predator cues prior to repatriation to strengthen predator recognition, alarm responses, and escape behaviors**. Hatchery-reared fish often do not have an innate recognition of potential predators and danger. They must acquire this knowledge on the basis of experience, previous escapes, and alarm cues (both visual and chemical) from other fish and aquatic organisms (Suboski 1990, Smith 1999). Studies have shown that prey can acquire predator recognition in as few as a single exposure to the predator combined with alarm cues (Chivers and Smith 1994a and b, Alvarez and Nicieza 2003). Recent studies demonstrate that juvenile Chinook salmon (*Oncorhynchus tshawytscha*) exhibit increased antipredator responses when exposed to unfamiliar predator odors, and the response could be enhanced through acquired recognition learning (Berejikian *et al.* 2003). These studies indicate that learned responses to predator recognition can be formed through conditioning and there is potential to increase survival during future predator encounters. See Brown *et al*. (2006) for an indepth review of predator recognition and fish cognition.

16) **Selectively breed genetic strains of bonytail for a low or high corticosteroid response**. This would suggest the possibility of producing bonytail through selective breeding that would be less responsive to handling and rearing conditions, however these fish may lose survival advantages in the wild by having a less sensitive stress response (Pottinger *et al*. 1994, Pottinger and Carrick 1999). If hatchery and stocking practices are the greatest influence on the survival of repatriated bonytail then producing genetic strains that select for low stress responses might be beneficial. Selecting for reduced cortisol response seems likely to result in benefits for disease resistance and growth (Davis 2006). It is a common misconception that evidence of a stress response always indicates a physiological state that is detrimental to the fish's well being. Acute stress, that fish can easily recover, has been shown to increase the immune response and actually be helpful (Demers and Bayne 1997). Only when a fish's ability to maintain homeostasis is overtaxed, because of the severity and/or prolonged

exposure(s) to stressors, do its homeostasis and "overall health" become compromised (Barton and Iwama 1991, Portz 2007).

Conversely, robust stress responses of bonytail may serve an adaptive advantage in terms of fitness in the wild and these attributes promote survival. Nevertheless, if hatchery fish become ill from handling and stocking practices and do not survive after release, then these highly stress responsive traits are not beneficial. The use of a bonytail selective breeding program could provide a new and powerful approach towards minimizing the problems of hatchery and transport-related stress.

17) **Stocking site acclimation for bonytail should be implemented to allow sufficient time to fully recover from transportation stress and become familiar with the conditions of the new environment**. A reevaluation of stocking procedures may be necessary for endangered and/or sensitive species, such as bonytail. The lack of stocking success may suggest that current stocking protocols may expose stocked fish to unnecessary behavioral or physiological stress that could impact performance and ultimately survival. Other riverine fish species (*e.g*., razorback sucker) stocked directly into the river from the fish haul truck tended to move rapidly downstream and were displaced from suitable habitats (Mueller and Marsh 1998, Foster and Mueller 1999b, Mueller et al. 1999). It is believed that bonytail share the same tendency after being released. Rapid downstream dispersal, typically associated with hatchery-produced fish could be reduced by allowing fish a period of time to recover from stocking-associated stress in backwaters and net pens. Fish can be allowed to casually leave these acclimation waters when they have recovered from the previous stressors and have a random dispersal making it more challenging to be captured by predators. Bonytail should be acclimated at stocking sites for a minimum of 1 week prior to release and possibly longer if funding, space, and staff are available to monitor fish while they voluntarily escape.

Conclusions

Some sources of stress in aquaculture, fish transport, and repatriation programs may be unavoidable. Harvesting, handling, sorting, holding, transporting, and release are all part of the routine operations of these practices that can have significant effects on bonytail physiology and survival. Nevertheless, there are some aspects and approaches of holding fishes that are under the control of aquaculturists and biologists. The use of proper techniques for care, handling, and transportation of bonytail, along with appropriate planning, can greatly increase their health and survival. Furthermore, an understanding of the stressors affecting fish holding can lead to practices that reduce stress and its detrimental effects. Most often, mortality of fishes held for relatively short periods is not the result of a single stressor. The cumulative and physiologically interactive effects of sublethal stresses that occur during harvest, capture, handling, confinement, and transport may be lethal, even if each independently does not exceed a physiological threshold.

Water quality is one of the most important contributors to fish health and stress level during short-term holding and transportation. Fish may be able to tolerate adverse water quality conditions; however, when combined with other stressors, fish may be quickly overwhelmed by the resulting physiological challenges. Handling and physical traumas directly and indirectly affect mortality and a fish's physiological function and stress response. Lastly, high fish densities in tanks and nets are one of the most common problems throughout aquaculture facilities and live-fish transfers resulting in both the previously mentioned water quality and physical damage.

Further research is needed to address the condition leading to their lack of survival following repatriation and provide some insight into their demise. Relatively little is known about the effects of hatchery-rearing, transportation, and stocking on the success and overall health of bonytail. Assessing tolerances of bonytail to hatchery and stocking stressors will provide valuable information to reduce the impacts of stressors. Responses to stress at hierarchal levels of organization (*i.e*., biochemical, organismal, population, community, and ecological) are interconnected and many times are functionally regulated by one another (Portz 2007). To fully comprehend bonytail responses to stress at ecological levels, it is important to include whole-body stress responses, rather than solely observing potentially isolated chemo-physiological responses. Stress effects on fish cannot be adequately evaluated by measuring either a single stress response or multiple responses from one level of biological organization (*e.g.*, plasma constituents), because the sensitivity of fish to stress is not constant across all stressors, and no single variable is adequate for interpolating or predicting changes at the population level. Therefore, a reliable evaluation of fish well being and performance should not be restricted to an examination of internal chemo-physiological changes alone. An assessment of a combination of physiological changes in plasma constituents and the ability to perform under controlled challenge tests should assess more accurately stress-related effects of stocking procedures. Finally, additional information on bonytail tolerances can be used to identify the most stressful components of aquaculture, fish transport, and repatriation programs, thereby helping to design alternative strategies or equipment to increase the survival after release.

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