Passive Integrated Transponders in *Gila elegans*: Location, Retention, Stress, and Mortality

by Andrea Dee Montony

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ABSTRACT

Despite large-scale propagation and rearing efforts few of the critically endangered bonytail, Gila elegans, are recovered following release from hatcheries. This study tested the role of passive integrative transponder (PIT) tag loss and tagging associated stress and mortality in these low recoveries. Bonytail exhibited 98-100 percent tag retention regardless of insertion direction (ventral toward anterior and anterior toward ventral). Plasma cortisol levels were measured at 0, 0.5, 3, 6, 12, and 24 hours following PIT-tagging in fish held at three temperatures (12, 16, and 20°C) as an indicator of stress. Bonytail tagged at 16°C had significantly lower plasma cortisol levels than those of fish tagged at 12°C and 20°C. At all three temperatures cortisol levels returned to baseline with in 24 hours. Mortality associated with PIT-tagging was only observed at 20°C. These data suggest that PIT tag handling for bonytail should occur at 16°C.

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CHAPTER 1

INTRODUCTION

Bonytail, *Gila elegans*, are large cyprinid fish originally described by Baird and Girard in 1853 (Marsh 2004). Historically bonytail were widespread within the Colorado River Basin as well as parts of Arizona, California, Colorado, Nevada, Utah, Wyoming, and the Salton Sea Basin in the United States, and Baja California Norte and Sonora in Mexico (Minckley and Deacon 1991, USFWS 2002, Muller 2006, Pacey and Marsh 2007). Historic wild bonytail populations were estimated to be in the hundreds of thousands (Pacey and Marsh 2007). Currently, wild populations are few and their numbers are unknown (Muller 2006, Pacey and Marsh 2007). The decline of bonytail has been attributed to the change of an extreme riverine habitat to a dependable reservoir system as a result of damming of the Colorado River. Damming caused this warm river system to cool and temperatures to become constant. The turbid red waters for which the river was named became clear. In addition, introduced non-native fish such as striped bass, *Morone saxatilis*, common carp, *Cyprinus carpio*, channel catfish, *Ictalurus punctatus*, and bluegill, *Lepomis macrochirus,* compete with native fish for available food and space and increased predation pressures on native fish (Muller and Marsh 2002, USFWS 2002). Thus, bonytail were listed on

April 23,1980 as endangered under the endangered species act of 1973 (USFWS 1980).

Propagation and stocking measures are in effect to prevent extirpation of bonytail from the Colorado River basin. Establishment of a captive broodstock program began in the late 1970s with six adult female bonytail captured from Lake Mohave, in Arizona and Nevada. The broodstock was completed in 1981, with the addition of five adult males captured from Lake Mohave to produce a cohort at Willow Beach National Fish Hatchery (NFH), Willow Beach, Arizona (Hedrick et al. 2000). The captive broodstock is now located at Dexter National Fish Hatchery and Technology Center (NFHTC), Dexter, New Mexico (Hedrick et al. 2000). Dexter NFHTC is responsible for the propagation and rearing of bony tail in conjunction with six other aquaculture facilities: Willow Beach NFH; Achii Hanyo Native Fish Facility (NFF), Parker, Arizona; Ouray NFH, Vernal, Utah; Wahweap State Fish Hatchery (SFH), Logan, Utah; Mumma Native Aquatic species Restoration Facility, Alamosa, Colorado; and Uvalde NFH, Uvalde, Texas (Muller 2006). In the lower Colorado River basin, two major stocking commitments are in place for bonytail: 1) the U.S. Fish and Wildlife Service Biological Opinion requires the production of 25,000 (250-300 mm) bonytail for release into Lake Mohave, and 2) the Lower Colorado River Multi-Species Conservation Program is committed to stock 620,000 (300 mm) bonytail into the Lower Colorado River Basin over 50 years, beginning in 2005 and ending in 2055 (LCRMSCP 2004, Pacey and Marsh 2007).

Aquaculture dates back to 4,000 BC, when the Chinese began cultivation of the common carp, *Cyprinus carpio* (Rabanal 1998, Stickney 2000). In the United States, aquaculture and fish stocking programs were designed with emphasis on profitable species with high export demand to compensate for overfishing and dam building, which limited wild populations of fish (Royce 1972, Stickney 2000, Rabanal 1998). However, many aquaculture facilities are beginning to take an active role in captive propagation of threatened and endangered species through hatchery rearing and broodstock development (Stickney 2001). Regardless of the facility, and cultured species, exposure to stressors such as variable temperatures, poor water quality, crowding and confinement, handling, grading, and sorting are inevitable (Conte 2004, Davis 2006, Jentoft et al. 2005, Barton and Iwama 1991, Portz 2007). This exposure is known to initiate a primary stress response which elevates plasma cortisol levels (King et al. 2006, Barnett and Pankhurst 1997, Banden and Leatherland 1997).

Hans Selye (1973) defined stress as “the nonspecific response of the body to any demand made upon it”. Stress is simply a state in which homeostasis is threatened, requiring a behavioral or physiological response from an organism to manage the situation. The physiological stress response in teleosts is divided into three phases: primary, secondary, and tertiary. The primary stress response involves the release of catecholamine’s (i.e. epinephrine, and norepinephrine) from chromaffin cells into the circulatory system (Randall and Perry 1992, Reid et al. 1998) and the release of corticosteroids (i.e., cortisol) from the interrenal tissue in response to the stimulation of the hypothalamic-pituitary interrenal (HPI)

axis (Iwama 1998, Reid et al. 1998, Mommsen et al. 1999, Wendelaar Bonga 1997). Within minutes of exposure to the stressor, release of cortisol begins with the release of corticotrophin-releasing hormone (CRH) from the hypothalamus in the brain; this in turn stimulates release of the major secretagogue adrenocorticotrophic hormone (ACTH) (Chrousos 1998, Sumpter et al.1997, Mommsen et al. 1999, Iwaman 1998). ACTH results in the production of cortisol by stimulating the interrenal cells (Barton 2002, Mommsen et al. 1999, Wendelaar Bonga 1997).

Primary stress responses trigger sequential secondary responses, which to a large extent are mediated directly by the catecholamines and corticosteroids. These secondary responses include increasing levels of plasma glucose and, lactate, plus increases in gill permeability, heart rate, and metabolic rate. A decrease in plasma chloride, sodium, and potassium may also occur (Iwama 1998, Portz 2007, Barton 2002, Mommsen et al. 1999). If exposure to stressors continues, tertiary responses occur including decreases in growth, disease resistance, swimming capability, feeding, reproductive activity, and survivability (Portz 2007, Barton 2002, Iwama 1998, Wedemyer et al.1990)

The stress response presumably evolved to overcome the immediate effects of stressors and to allow for quick recovery. Although it is commonly thought that the stress response is detrimental to the organism, this is true only when exposure to a stressor is prolonged or the severity of the stress is great, thus, inhibiting the fish's ability to reestablish homeostasis (Barton and Iwama 1991). Cortisol secretion induces protein catabolism and mobilization of amino

acids for protein synthesis to maintain homeostasis during stressed conditions. In fishes, catecholamine's target the gills creating detrimental ionic imbalances through increased permeability. Cortisol compensates by increasing the number of chloride cells and reestablishing ionic balance (Randal and Perry 1992, Wendelaar Bonga 1997).

The physiological response of fish to handling stressors can be evaluated by measuring cortisol, lactate and, glucose in the plasma, and blood osmoliality (Pickering 1981, Barton and Iwama 1991). For this study, I used plasma cortisol levels to determine physiological responses in bonytail following exposure to a handling stressor. Cortisol is the most commonly used indicator of stress in fish; it is easily measured using radioimmunoassay (RIA) or enzyme-linked immunoassay (EIA, ELISA). Baseline levels can be measured by proper sampling procedure, and with continued exposure to stressors levels will continue to increase (Barton 2002, Mommsen et al. 1999, Wendelaar Bonga 1997). Because synthesis of cortisol has a delayed response of a few minutes, blood sampling procedures will not themselves interfere with desired measures (Wedemyer et al. 1990).

Crowding, netting, grading, and transporting are common hatchery practices that can stress fish. Effects of hatchery related stressors have been observed in many species such as the Atlantic cod and haddock. Upon exposure to a netting stressor; where fish were netted and held out of water for 30 seconds both species responded with an increase in plasma cortisol (King V et al. 2006). It is common for fish to be graded at aquaculture facilities to separate out different

size classes. Grading requires fish to be crowded, chased with a net, captured, and transferred. Greenback flounder, *Rhombosolea tapirina*, exposed to a grading stressor showed significant increases in plasma cortisol levels post-grading (Barnett and Pankhurst 1998). Transportation of fish has also been investigated in many species. White suckers, *Catostomus commersoni*, had increased plasma cortisol levels during an 8-hour truck haul and immediately after removal from the truck (Banden and Leatherland 1997). Similarly largemouth bass, *Micropterus salmoides*, also exhibited an increase in plasma cortisol levels in response to truck transport (Carmichael 1984). Many of the common aquaculture techniques appear to stress bonytail as well, resulting in their reputation for experimental mortality “issues” (Sowka and Brunkow 1999, Tyus et. al 1999), stress related outbreaks of disease (Muller 2006), and low recapture rates after release (Muller 2006, Pacey and Marsh 2007).

Bonytail were brought into aquaculture facilities in 1974 to serve as a genetic refugia population (Hedrick et al. 2000). Over the past 35 years, they have successfully spawned and larvae have been distributed to rearing facilities where they are raised to a size in excess of 250 mm total length before being stocked into the lower Colorado River. Bonytail are harvested annually in winter from Achii Hanyo NFF for supplementation of the lower Colorado River population. Achii Hanyo is located in Parker, Arizona on Colorado River Indian Tribe land. This facility has seven earthen ponds, four raceways, and 16 circular tanks of varying sizes, supplied with flow-through canal water. In December, water to the ponds is lowered to approximately 2 feet, at which time seines are pulled through

the pond to collect the majority of bonytail, after which the pond is drained into a catch basin to collect any remaining fish. Bonytail are loaded onto a truck and transported to raceways. Once in the raceways fish are crowded, measured, and sorted by size. Fish are again crowded and confined before they are netted and placed into an anesthetic bath of Tricaine Methanesulfonate (MS-222). After the fish loose equilibrium, they are removed from the bath, total length (tip of snout to tip of longer lobe of caudal fin) is measured, and each fish is tagged. Two tag types are used to mark these fish: wire tags and passive integrated transponder (PIT) tags.

PIT tags were invented in the 1980s, and are composed of a computer chip and antenna that are encapsulated in a glass tube (12.5 mm x 2.07 mm diameter, Biomark, Boise, Idaho). Tags require external energy sources for activation and produce a unique alpha numeric code that allows for individual identification without sacrificing the specimen (Guy et al. 1996). Therefore, PIT tags are an indispensable technique for biologists to study growth and movement of species (Paukert et al. 2006, Achord et al. 1996), and create survival, mortality, and growth estimates (Muir et al. 2001, Paukert et al. 2005). Tagging appears to be a rather invasive process. Hence, many studies have been conducted in salmonids with regards to tagging location (Prentice et al. 1990) confirming high tag retention and survivorship (Gries and Letcher 2002). Few PIT tag studies have been conducted in bonytail.

The low recapture rates could be due to either loss of the PIT tag of low survivability following release. Thus, the objectives of this study were to

determine: 1) whether there is an optimal tagging direction in this species, and associated levels of tag retention, and 2) whether bonytail exhibit a stress response to PIT tagging, crowding and confinement, netting, anesthesia, measuring, and tag insertion, with particular attention to the time course of recovery. We examined stress responses as indicated by cortisol secretion in fish held at three temperatures characteristic of those observed at the hatchery: 12°C, 16°C, and 20°C. This work was completed to address the potential role of handling methods of bonytail prior to release into the lower Colorado River in their low recovery with the goal to increase success of bonytail augmentation.

CHAPTER 2

MATERIALS AND METHODS

Experiment 1: Direction and Retention of PIT tags

To test for effects of the insertion direction of tags placed below the pelvic fin on PIT tag retention in bonytail we tested two directions and the use of biological glue. Bonytail were provided by and experiments were conducted at Willow Beach NFH, Willow Beach, Arizona. One-hundred bonytail were collected from the A1 raceway and moved to two 2,300 L circular fiberglass experimental tanks equipped with a regenerating pump and air stones. Each tank was stocked with 50 bonytail (tank 1, adult bonytail, 207.2 ± 19.7 mm; tank 2, juvenile bonytail, 147.0 ± 14.2 mm). Tanks were supplied with Colorado River water at a flow rate of 75 L/min, and warmed with solar tubing to maintain a temperature range of 16°C to 18°C. Bonytail were randomly assigned to one of five treatments in each tank: control (n = 10); ventral toward anterior tag insertion (n = 10); ventral toward anterior tag insertion with use of Nexaband (Raleigh, North Carolina) biological glue (n = 10); anterior toward ventral tag insertion (n = 10); and anterior toward ventral tag insertion with use of biological glue (n = 10; Figure 1).

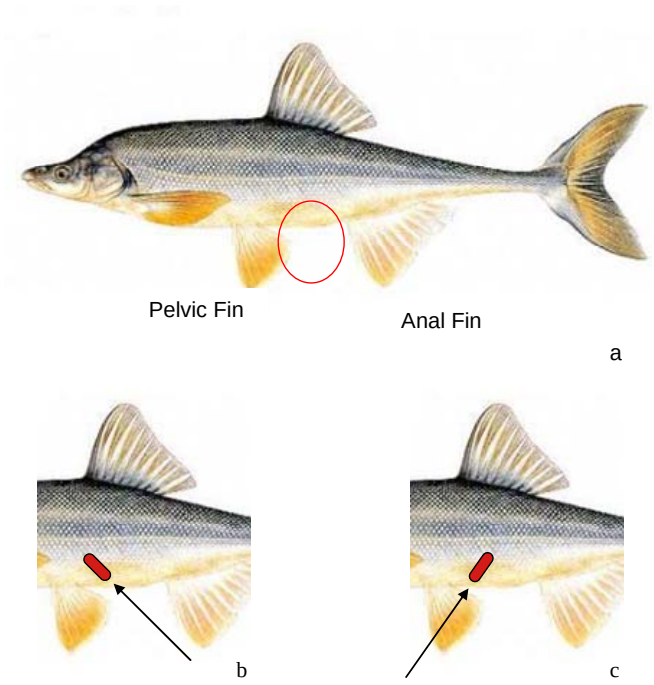

Figure 2.1: Schematic of PIT-tagging location. PIT tags are placed posterior to the pelvic fin and anterior to the anal fin (a). For anterior to posterior tag placement, the needle orientation is toward the anal fin (b). For posterior to anterior tag placement, the needle is oriented toward the pelvic fin. Bonytail drawing by Joseph R. Tomelleri.

Prior to tagging, bonytail were crowded, netted and placed into a 20-L anesthetic bath (2.2 g Tricaine Methanesulfonate [MS-222], 15 mL stress coat, and 4.12 mol sodium chloride; Figure 2). Stress coat® (Aquarium Pharmaceuticals, Inc., Chalfont, PA) is used to form a synthetic slime coating on the skin of the fish replacing the naturally secreted slime coat lost during handling. Addition of sodium chloride allows for salinity to equal osmolality of plasma which reduces both the osmotic and ion gradients so fish do not experience the severe ion and water imbalances that are characteristic of fish

stressed by handling (Colt and Tomassos 2000). Once fish exhibited a loss of equilibrium, they were removed from the bath and measured (TL mm). Bonytail were then PIT tagged on the right side, anterior to the pelvic fin with an anterior to posterior needle orientation, or posterior to anterior needle orientation, for tag insertion according to treatment. Tags were inserted using a spring loaded syringe (Avid 12G vet needles, Monoject 12cc syringe, utility comp spring (1/2 x 1-1/2 x 0.041), hog ring, and 14 gauge copper dowel, Figure 3). Fish in treatments that received biological glue were quickly wiped down at the PIT tag insertion point using a cotton swab followed by application of a single drop of glue applied. Control fish were handled similarly to PIT tagged fish, anesthetized, and measured but not tagged.

Figure 2.2: PIT-tagging process, fish are crowded, netted and anesthetized before receiving a PIT tag.

Figure 2.3: Picture of a PIT-tagging needle and PIT-tag in relation to a penny.

Bonytail were monitored for four weeks after tagging and were fed an *ad libitum* diet of a pellet feed designed the razorback sucker, another endangered fish species which occupies the same habitat as bonytail (bonytail-specific feed is currently under development) during holding. Once a week fish were netted and scanned with a Destron/IDI PIT tag scanner (Biomark, Boise, Idaho), and tag insertion points were examined for visible irritation resulting from the PIT tagging wound or glue. Handling was unavoidable. Handling was uniform for all groups with a mean handling time of 42.1 ± 2 sec.

Experiment 2: Tagging-Associated Handling Stress

In order to asses tagging associated handling stress we measured plasma cortisol levels over 24 hours post handling in bonytail held at three hatchery typical temperatures. This research was approved by the UNLV animal Care and Use Committee (R701-0106-204) on March 15, 2006. Bonytail (n = 576) were provided by and experiments were conducted at Achii Hanyo Native Fish Facility, located on Colorado River Indian Tribe land in Parker, Arizona. Fish were

maintained on site in flow-through (4.9 L/min) circular fiber glass tanks (840 L, 96 fish/tank). As in experiment 1, fish were fed an *ad libitum* diet of a specially formulated feed for razorback suckers during holding. Bonytail measured at the time of tagging averaged a total length of 260 ± 18 mm. Fish were held from December 15, 2006 – April 12, 2007 and December 20, 2007 – April 16, 2008. After the experiment fish were released into a sanctuary pond in 2007 or into Lake Havasu, Arizona and California in 2008.

Experiments were conducted at three water temperatures 12°C, 16°C, and 20° C. Temperatures' were chosen according to average temperatures above the thermocline in Lake Mohave during Bureau of Reclamation fish sampling. The lowest temperature, 12°C, is an average of monthly mean temperatures from November, December, January, and February. The mid-range temperature of 16°C is the average of monthly mean temperatures from March, April, September, and October. The highest temperature 20°C is the average water temperature in Lake Mohave in May. Fish are not handled during the months of June, July, and August due to water temperatures exceeding 22°C. Bonytail from 2007 were not sampled at 12°C; due to loss of fish during the acclimation process. All tanks were covered with netting but gaps were left on either side of the water inflow pipe, and after two weeks of acclimation approximately 60% of the fish were missing. We observed fish jumping out of the gaps into the incoming water. Bonytail from 2007 held at 16°C were not sampled due to weather problems; severe thunderstorms moved into the area during sampling resulting in unsafe conditions for the crew, and loss of electricity to equipment.

Groups of 12 fish (260 ± 18 mm, mean ± standard error, total length, TL) were transferred to eight flow-through fiberglass circular tanks (840 L) with a net cover. Each tank represented a different treatment: 1) control, not exposed to the handling stressor or blood sampling (n = 12); 2) baseline, not exposed to the PIT tag handling stressor but with a blood sample collected (n = 12); 3-8) PIT tag handling stressor fish, exposed to a handling stressor with a blood sample collected within 24 hours post-handling (n = 12, for each post-handling time; Figure 4). Sample times included post-handling times 0, 0.5, 3, 6, 12, and 24 hours. Each fish was exposed to the stressor and sampled only once. Fish were acclimated for two-weeks once they were divided among the tanks, during which time they were fed an *ad libitum* diet until 48 hours prior to the PIT-tag handling.

The PIT-tag handling stressor consisted of fish being crowded using specially designed screens in the circular tanks, for ease of netting. Fish were netted out and placed into a 40-L anesthetic bath (4 g MS-222, and 4 g baking soda). Between experiments 1 and 2 additional information led to a change in the anesthetic bath. MS-222 affects pH and baking soda is now required by the hatchery as a buffer when using MS-222. Once fish exhibited loss of equilibrium they were removed from the bath, placed on a wooden board, and measured (TL, mm). A PIT tag was inserted into each fish posterior to the pelvic fin at a 45° angle with posterior to anterior tag placement (Figure 1c). PIT-tagging needles were sterilized between fish using ethanol. Bonytail were then placed back into the tanks with fresh water.

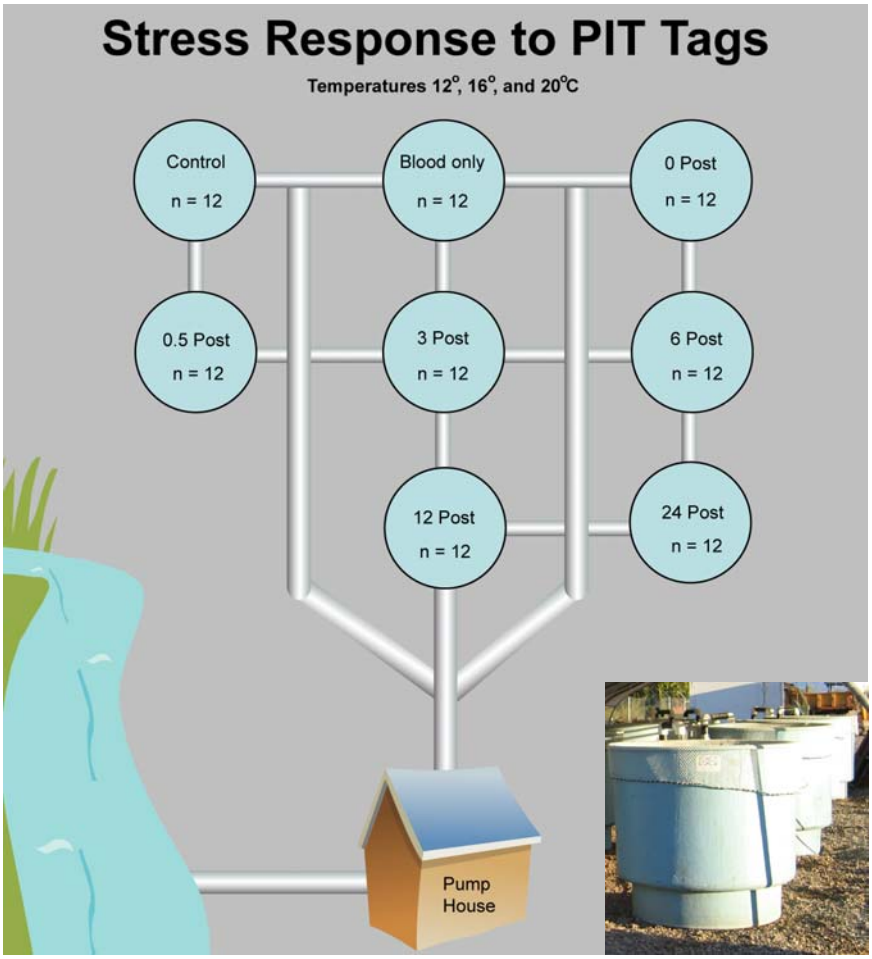

Figure 2.4: Schematic of experimental tanks at Achii Hanyo. Canal water was supplied from the canal. Water is pulled into the pump house and distributed to each individual tank. Actual tanks in bottom right corner.

Blood was collected from the caudal vessel via a syringe (25 gauge 5/8 needle, 1 cc syringe) at times 0, 0.5, 3, 6, 12 and 24 hours post-handling, and from baseline fish. The needle was coated with an ammonium heparin solution (Sigma-Aldrich, United States) to prevent coagulation of the blood. Ammonium heparin was dissolved with distilled water at the ratio of 10 U/µl of heparin. To sample the caudal vessel the needle was inserted along the ventral midline posterior to the anal fin. Blood was drawn slowly (61 ± 2.4 sec, mean ± standard

deviation) from each fish. Blood was transferred from syringes to heparinized tubes (2 mL) and placed on ice. Samples were centrifuged at 1,000 x for 15 minutes within three hours of obtaining the samples, after which the plasma (supernatant) was removed and aliquoted into sample tubes (0.5 ml) using a Hamilton syringe. Samples were frozen on dry ice and transported to the University of Nevada, Las Vegas to be stored in a -20°C freezer until assayed. All assays occurred with in six months of collection.

Additional Stressor: *Ichthyophthirius multifiliis*

By chance two tanks, one from the 2007 samples (16°C) and the second from the 2008 samples (12°C) treatment, were infected with the parasite ich, *Ichthyophthirius multifiliis*, one of the most ubiquitous diseases in teleosts. Ich is a ciliated protozoan; sufficiently large in size (0.5–1.0 mm when mature) that it is visible with the naked eye (Piper et al. 1982). The life cycle of Ich is highly dependent of temperature with optimal reproduction temperatures of 24-26°C (Post 1987). However, Ich occupies a wide range of temperatures from 2-30°C (Nigrelli et al., 1976). Ich has a relatively simple life cycle. Tomites, the immature form, are the infecting agents in water, and will burrow into the flesh of freshwater fishes. After tomites penetrate the skin, they develop into trophozoites, which continue to mature into trophonts. Once the trophont has left the host it can produce 250-1,000 tomites by multiple fissions (Post et al. 1987, Nigrellie et al. 1976).

Ich is a cause of massive mortalities in fish (Royce 1972). In response to the infection, the gill epithillium reacts by thickening, and thereby restricts the flow of oxygen into the gill blood vessels, eventually hindering respiration (Durborrow et al. 1998).

The life stages of Ich that develop within the dermal tissues of the fish are usually untreatable. Those occurring externally of the fish are susceptible to multiple chemotherapeutic agents: baths of formalin, malachite green, malachite green combined with formalin, methylene blue, potassium permanganate, quinine hydrochloride, and sodium chloride (Post 1987).

Fish with Ich that survived the two-week acclimation period were sampled to determine whether diseased fish were more stressed than those that were exposed to the PIT-tag handling stressor. Estimated cortisol levels were then adjusted by this dilution factor.

Cortisol Enzyme Immunoassay Analysis

Plasma cortisol was determined using an enzyme immunoassay kit (Assay Designs Inc., Ann Arbor, Michigan). Plasma samples were diluted 1:200 with the kit-provided assay buffer prior to assay, as pilot data indicated that cortsiol levels in undiluted plasma fell outside the assay range.

The assay was conducted per manufacturer's instructions. All samples were run in triplicate on a 96-well plate. Cortisol measurement is based on competition of unknown amount of cortisol in the sample and kit provided cortisol conjugate for a limited number of binding sites relative to the known amounts of cortisol in

the standard curve. The standard curve was increased to include eight standards to allow for use of an existing Excel template for calculation of the results. The plate included wells for measurement of background absorbency, the total enzymatic activity of the conjugate (total activity, TA), the binding of the conjugate to the wells (non-specific binding, NSB), and the maximum amount of conjugate that the antibody can bind (maximum binding, Bo).

Assay buffer of Tris-buffered saline preserved with proteins and sodium azide was added to the NSB and Bo wells. Standards one through eight were added followed by plasma samples. Alkaline phosphatase conjugated with cortisol, conjugate, and mouse monoclonal antibodies to cortisol were added to all wells with the exception of blank, TA, and NSB wells. The plate was incubated for two hours at room temperature on a plate shaker. The plate was then rinsed with a wash solution of Tris-buffered saline and detergents and wells were aspirated. Conjugate was added to the TA well, and a solution of p-nitrophenyl phosphate in buffer was added to every well. The plate was incubated for one hour without shaking. A stop solution of trisodium phosphate in water was added to every well. The optical density was then read using a microplate reader with a wavelength filter of 405 nm. Cortisol concentrations were analyzed using an Excel workbook created by Cayman Chemical (Ann Arbor, Michigan).

Enzyme Immunoassay Kit Validation

Cortisol quantification is most frequently determined using a radioimmunoassay (RIA) due to its high sensitivity and specificity for many

hormones (Andoh 2006; Sink 2007). Unfortunately, RIA has limitations including: potential health hazards due to, handling of radioisotopes, short stability of labeled ligands, disposal of materials, and licensing required for purchasing and performing the assay (Andoh 2006; Sink 2007). Most aquaculture settings are not conducive for such operations, hence, the appeal of non-radioisotopic immunoassays such as enzyme immunoassays (EIA), and enzyme-linked immunosorbent assays (ELISA).

Validation practices followed those of Sink (2007) and Barry et al. (1993). Samples were run in quadruplicate on two separate kits. Assays were preformed on separate days to allow for consideration of environmental conditions and procedural time for an accurate interpretation of the interassay variation. Sensitivity was tested by calculation of average optical density of maximum binding wells compared with average optical density of standard number seven. The detection limit was set at two standard deviations from zero on the standard curve. Precision was determined by multiple assays of the same sample on the same plate by calculating the coefficient of variation (% CV). The acceptable percent coefficient of variation was set at 20.0 or less for intra-and interassay coefficient of variation (Sink 2006, Pifat 2006). Linearity was tested using serial dilutions of the sample using the provided ELISA buffer. The limit set for the linearity test was an R^2 of >0.90 (Sink 2006). Sample recovery was determined from samples with a spiked known amount of cortisol compared to the un-spiked sample.

Survivorship

Survivorship of bonytail was investigated for four weeks following the PIT-tag handling stressor. Tanks were monitored daily and deceased bonytail were removed from tanks and placed into 1-gallon Ziploc bags. Bags were labeled with the date, and tank number, and fish were placed into a freezer until data could be recorded. Survivorship data were only collected from 2008 fish from 16°C and 20°C, as deceased fish collected at 12°C were accidentally discarded by the hatchery before data were recorded.

Statistical Analysis

Statistical analyses were performed using SPSS 15.0 (SPSS Inc., Chicago, Illinois). Data were log-transformed to meet assumptions of normality and homogeneity of variance. Baseline values and mean plasma cortisol levels for zero hour post handling fishes were compared using a one-way ANOVA. Time (0, 0.5, 3, 6, 12, and 24 hours post-handling) and temperature (12°C, 16°C, and 20°C) groups were compared using an ANOVA (two-way) with Tukey's HSD post-hoc tests. Survivorship data were assessed using Kaplan Meyer survival risk assessment for each group (control, blood only, treatment) and temperature (16°C and 20°C). Analyses were completed at 28 days post-handling, mean number of days between experiments, and at 10 days post-handling, maximum number of days mortality associated to PIT-tagging occurs. Two groups of samples were from fish infected with Ich at 12°C and 16°C; thus, mean plasma cortisol levels were evaluated and compared to baselines levels at each

temperature using Student's t-test. Survivorship data were collected for fish infected with ich acclimated to 16°C. Significant differences in survival between Ich infected fish and uninfected fish were assessed using Kaplan Meyer survival risk assessment.

CHAPTER 3

RESULTS

Our study investigating PIT tag retention with regards to insertion direction and use of biological glue found juvenile bonytail (147 ± 19.7 mm total length) exhibited 99% PIT-tag retention. Tag loss occurred in fish tagged with anterior to posterior tag insertion with the use of biological glue, and in fish with posterior to anterior tag insertion with the use of biological glue (Figure 3.1). Adult bonytail (207 ± 14.2 mm total length) had 100% PIT-tag retention among all groups (ventral anterior, ventral anterior with biological glue, anterior ventral, and anterior ventral with biological glue; Figure 3.2).

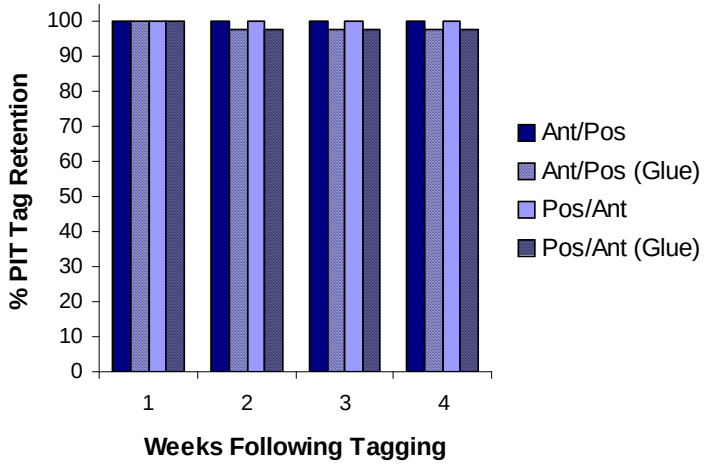

Figure 3.1: Tag retention in juvenile bonytail (147.0 ± 14.2mm). Tag loss was nonexistent in fish with anterior to posterior (Ant/Pos) and posterior to anterior (Pos/Ant) tag insertion. Fish with anterior to posterior and posterior to anterior tag insertion with glue both had 99% tag retention.

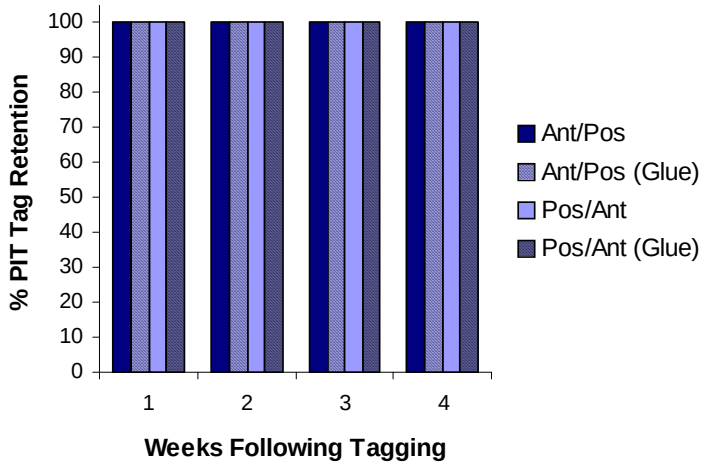

Figure 3.2: Tag retention in adult bonytail (207.2 ± 19.7mm) was 100% for all groups.

In our experiments investigating PIT-tag handling stress we ran all statistics on log-transformed data to meet the ANOVA assumption on normality. All data presented graphically are untransformed data.

Baseline plasma cortisol levels in bonytail that were only bled, and bonytail that were handled and had a blood sample immediately drawn (time 0) were not significantly different in fish held at 16°C ($F_{1,19} = 2.989$, $P = 0.101$), or at 20°C ($F_{1,11} = .395$, $P = 0.544$; Figure 3.3).

Analysis with a two-way ANOVA found a significant interaction, between time post-handling (i.e. 0, 0.5, 3, 6, 12, and 24 hours) and temperature (12°C, 16°C, and 20° C) ($F_{11,149} = 2.30$,$P = 0.012$).To further explore this interaction we conducted Tukey's post hoc test on data for each individual temperature (Table 1). No significant differences were reported at 20°C ($F_{5,37} = 1.902$, $P = 0.117$;

Figure 3.4). At 16°C mean plasma cortisol levels varied with time post handling. There is a significant decrease of mean plasma cortsiol levels from 0 hours and 0.5 hours post handling to 12 hours post handling ($F_{5,44} = 4.248$, P = 0.003; Tukey's HSD, $p<0.05$; Figure 3.5). At 12°C mean plasma cortisol levels also varied with time post handling. Bonytail had significantly higher plasma cortsiol levels at 0.5 and 3 hours post PIT-tag handling than at 0 hours post PIT-tag handling. At 6 hours post PIT-tag handling there was a significant decrease in plasma cortisol levels from 3 hours post PIT-tag handling ($F_{4,38} = 6.14$, P = 0.001; Tukey's HSD, $p<0.05$; Figure 3.6).

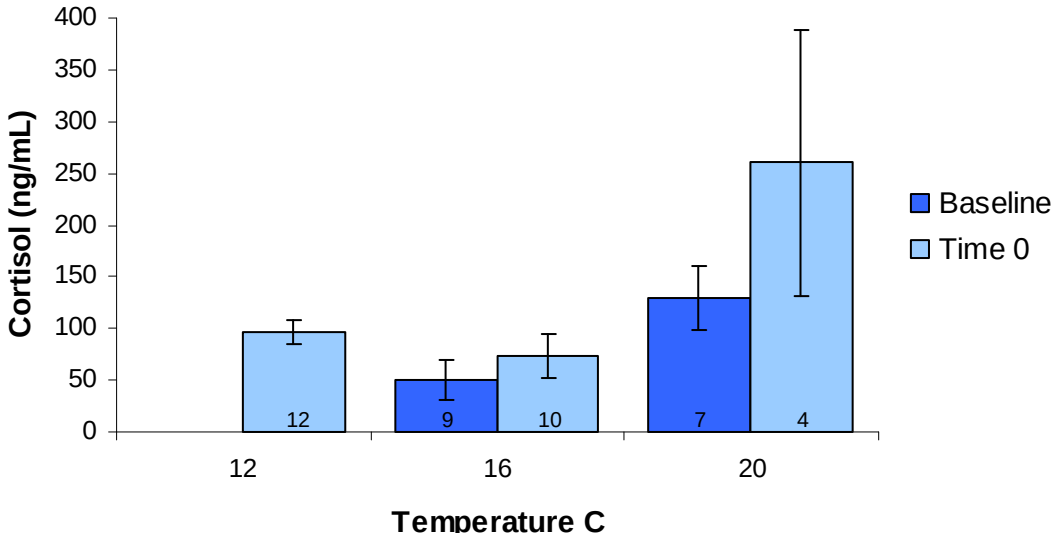

Figure 3.3: Mean plasma cortisol levels ± standard error. Baseline cortisol levels and those that were handled and bleed immediately are not significantly different at 16°C and 20°C. Baseline mean at 12°C is not presented due to sampling issues.

Source	df	F	p
Time post handling	6	2.779	*0.014*
Temperature	2	19.782	*0.001*
Time post handling x Temperature	11	0.302	*0.012*
Error	149		

A

12°C	0	0.5	3	6	12	24
0	1.000					
0.5	*0.001*	1.000				
3	*0.027*	0.885	1.000			
6	0.998	*0.016*	0.150	1.000		
12	Samples were not included in analysis					
24	0.574	0.157	0.648	0.850		1.000

B

16°C	0	0.5	3	6	12	24
0	1.000					
0.5	0.753	1.000				
3	0.482	0.095	1.000			
6	0.940	0.332	0.945	1.000		
12	*0.016*	*0.004*	0.639	0.144	1.000	
24	0.991	0.513	0.895	1.000	0.140	1.000

C

20°C	0	0.5	3	6	12	24
0	1.000					
0.5	1.000	1.000				
3	0.293	0.226	1.000			
6	0.998	0.950	0.053	1.000		
12	1.000	1.000	0.228	0.949	1.000	
24	0.999	1.000	0.284	0.931	1.000	1.000

D

Table 2.1: The ANOVA table is depicted in table A, the significant differences are italicized. Tukeys post hoc test were run at all three temperature 12°C (B), 16°C (C) and at 20°C.

At 16°C, fish exposed to the PIT-tag handling stressor had significantly lower plasma cortisol levels than fish exposed to the PIT-tag handling stressor at 20°C ($F_{11,149}$ = 2.30,P = <0.001) and at 12°C ($F_{11,149}$ = 2.30,P = 0.017). Plasma cortisol levels of fish held at 20°C and 12°C ($F_{11, 149}$=2.30, P=.644) were not significantly different.

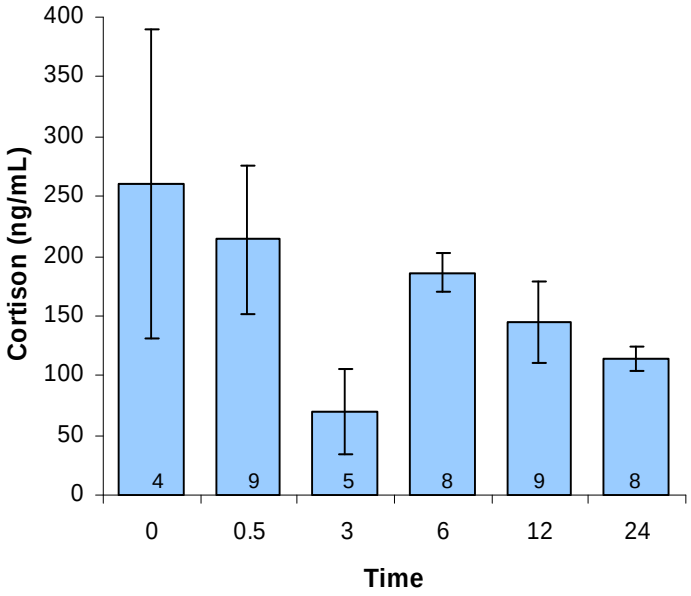

Figure 3.4: Post-stressor plasma cortisol level in bonytail acclimated to 20°C. Vertical brackets represent the standard error of the mean. There were no significant differences (p < 0.05) in post-handling plasma cortisol.

However, at 16°C plasma cortisol levels of fish sampled at 0 and 0.5 hours had significantly higher cortisol levels than fish sampled at 12 hours after exposure to the PIT-tag handling stressor ($F_{5,44} = 4.25$, P = 0.016 and $F_{5,44} = 4.25$, P = 0.01; Figure 3.5). At 12°C, cortisol levels in fish sampled at 0 hours were significantly lower than levels in fish sampled at 0.5 and 3 hours following exposure to the PIT-tag handling stressor ($F_{4,}38 = 6.13$, P = 0.001 and $F_{4,}38 = 6.13$, P = 0.027) Cortisol levels in fish handled at 0.5 hours were significantly higher than levels in fish sampled at 6 hours following exposure to the PIT-tag handling stressor ($F_{4,}38 = 6.13$, P = 0.016; Figure 3.6).

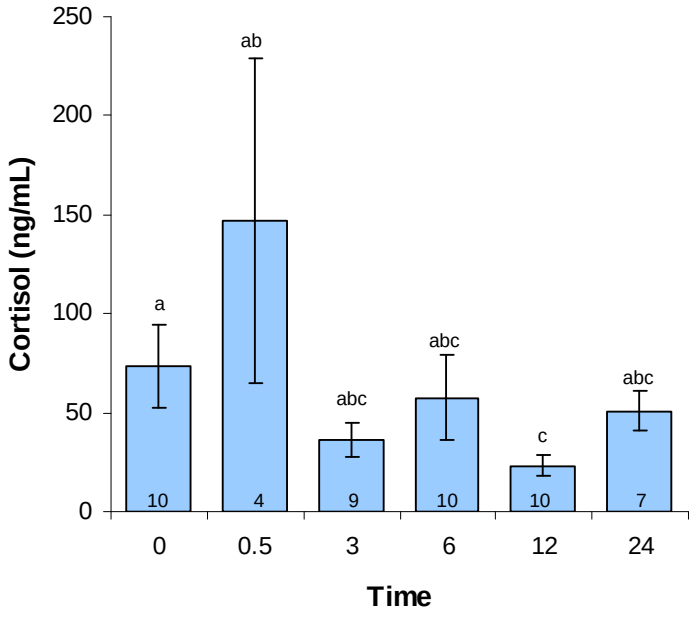

Figure 3.5: Post-stressor plasma cortisol level in bonytail acclimated to 16°C. Vertical brackets represent the standard error of the mean. Different letters above bars indicate a significant difference (P<0.05) between sample times with in the same temperature.

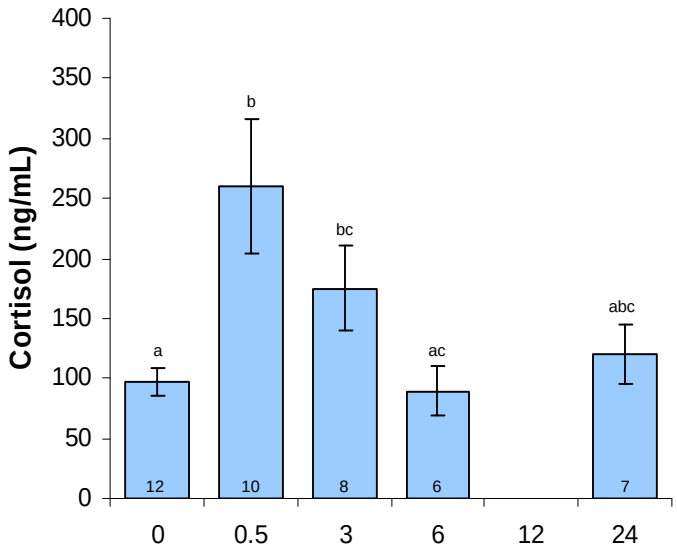

Figure 3.6: Post-stressor plasma cortisol level in bonytail acclimated to 12°C. Vertical brackets represent the standard error of the mean. Different letters above bars indicate a significant difference (P<0.05) between sample times with in the same temperature.

Bonytail infected with Ich had significantly higher plasma cortisol levels than maximum values reported at 16°C ($t_{14} = 3.91$, $P = 0.002$) and 12°C $t_{14} = 2.45$, $P = 0.03$; Figure 3.7). Bonytail with Ich did not have significantly different cortisol levels ($t_{10} = 1.13$, $P = 0.28$) at the different temperatures. However, all other groups sampled at 16°C had significantly lower cortisol levels than fish sampled at 12°C.

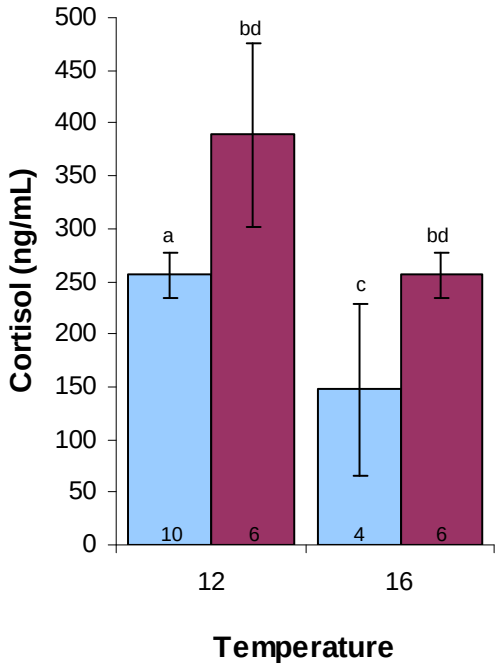

Figure 3.7: Maximum plasma cortisol levels (0.5 hours post handling) ± standard error at 12°C and 16°C compared to plasma cortisol levels in bonytail infected with ich. Mean plasma cortisol levels were significantly higher than those of maximum plasma cortisol levels reported at both 12°C and 16°C.

Survival was determined for control, baseline (bled only), and fish exposed to the PIT- tag handling stressor at 16°C. There were no significant differences in the percentage of observed mortality over the 28 day observations ($\chi^2 = 0.057$, df = 1, P = 0.81; Figure 3.8). Survival for control fish, baseline (bled only), and fish exposed to the PIT tag handling stressor, and Ich groups at 20°C were determined. Survival assessments for control, baseline and all groups sampled after PIT-tag handling stress exposure were not significantly different ($\chi^2 = 2.017$, df = 1, P = 0.156; Figure 3.9). Survival of control fish was significantly higher than survival of fish infected with Ich ($\chi^2 = 26.549$, df = 1, P = <0.001); this was

also true for control fish and fish infected with Ich (χ^2 = 23.808, df = 1, P = 0.81; Figure 3.10). A snap shot at 10 days produced 100% survival in all groups of fish held at 16°C, but fish held at 20°C pit-tagged fish exhibited mortality associated to PIT-tagging.

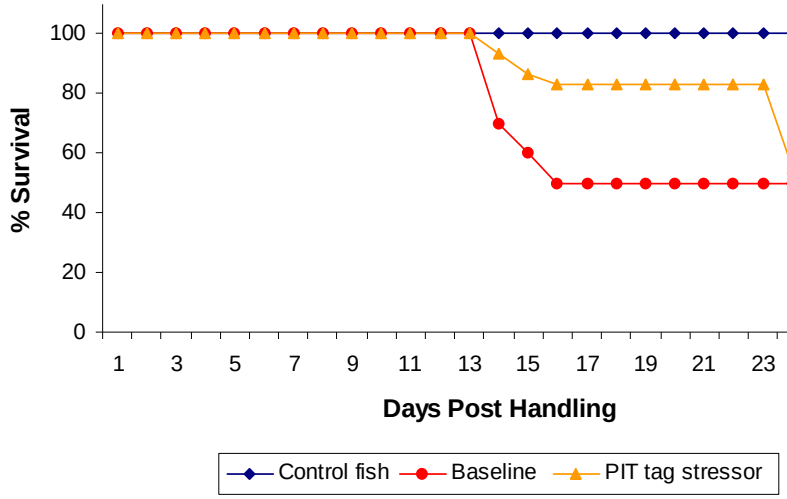

Figure 3.8: Percent survivorship of control, baseline, and treatment (all fish exposed to the PIT-tag handling stressor) Bonytail at 16°C.

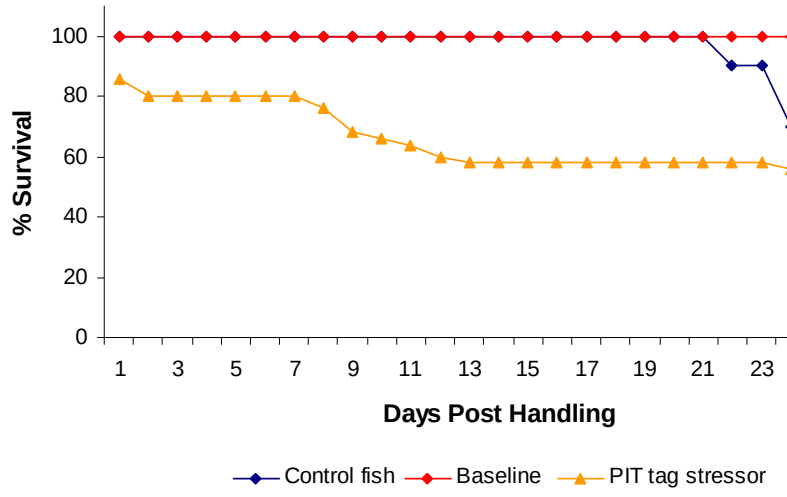

Figure 3.9: Percent survivorship of control, baseline, and treatment (all fish exposed to the PIT-tag handling stressor) bonytail at 20°C

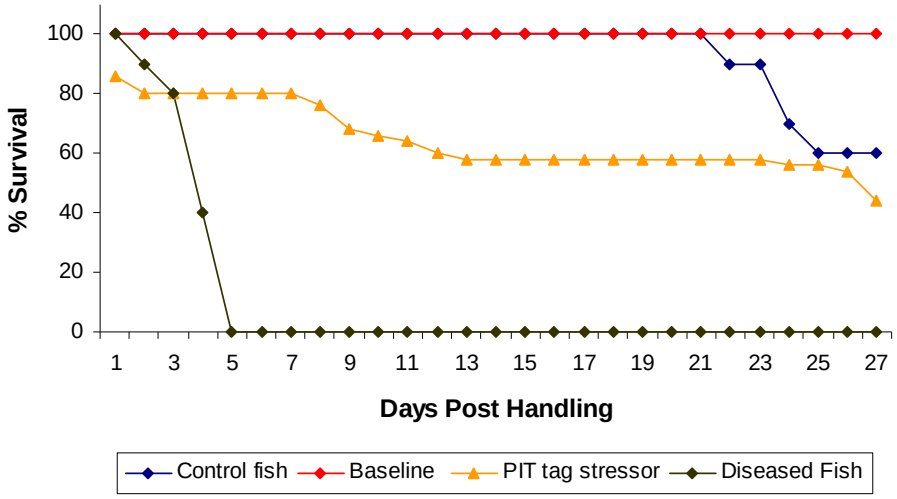

Figure 3.10: Percent survivorship of bonytail infected with ich at 16°C compared with healthy fish at time of blood collection.

CHAPTER 4

DISCUSSION

Tag retention was high in both adult and juvenile bonytail. Adult bonytail had 100% retention in all groups, while juvenile bonytail had 99% retention. Loss was associated with the use of Nexaband biological glue. However, needle orientation did not have any affect on tag retention when glue was used. Our results are consistent with those from a study conducted by the Arizona Game and Fish Department to determine effective tag locations and methods by evaluating tag retention and fish mortality. In that study two groups of bonytail were PIT tagged; the first group was not fed prior to PIT tagging and the second group was allowed access to feed for 12 hours prior to PIT tagging. Both groups had high tag retention (>98%). Tag orientation was also evaluated in Gila chub, *Gila intermedia* (Ward et al. 2008). Fish were tagged in the abdominal cavity anterior of the pelvic fin and posterior to the pelvic fin, and also in the dorsal musculature. All three PIT tagging locations had high PIT tag retention (>92%) (Ward et al. 2008). Our study and all of these suggest that PIT-tag location and orientation do not have an effect on PIT-tag retention

Biological glues have been used by veterinarians for surgical procedures in dogs and cats (Pavletic 2000), PIT-tagging big brown bats, *Eptesicus fuscus*, (Wimsatt et al. 2005), and PIT-tagging of adult common terns, *Sterna hirundo*

(Becker and Wendeln 1997). We thought that the addition of biological glue may help tag retention during PIT-tagging events. However, use of biological glue appears to be an unnecessary addition to the bonytail PIT-tagging process.

This study also investigated changes in plasma cortisol concentrations as an indicator of a stress response of bonytail subjected to a PIT-tag handling stressor at three different temperatures. Water temperatures were maintained through seasonal variation in canal temperatures. Seasonal variation in plasma cortisol levels has not been investigated in bonytail and cortisol levels have been shown to be variable among species. For example, in brown trout, *Salmo trutta,* seasonal variation is evident, and the timing of peak cortisol levels varied 2400 to 0800 hours depending on season (Pickering and Pottinger 1983).

Bonytail exhibited an increase in plasma cortisol levels in response to the PIT-tag handling stressor at all three temperatures. Increases in plasma cortisol levels in response to handling stressors have been investigated in many species. Barton (2002) compiled his previous research of 12 different species from five families (Acipenseridae, Cyprinidae, Percidae, Polydontidae, and Salmonidae) exposed to an identical 30-second aerial emersion handling stressor. He found a wide range of changes in plasma cortisol levels in response to handling (0.7 ng/mL - 218ng/mL). Changes in plasma cortisol levels in this study fell within the ranges reported by Barton (2002). Bonytail showed the greatest increase of 163 ng/ml plasma cortisol from baseline levels to peak levels at 12°C, followed by 131 ng/ml at 20°C, and 98 ng/ml at 16°C.

Temperature has also been shown to influence recovery time in response to a handling stressor. Atlantic cod, *Gadus morhua,* exposed to a 30-second aerial emersion had different recovery times at different temperatures. Recovery was significantly slower at 4°C; and plasma cortisol levels remained elevated for 24 hours after the handling stressor. In contrast at 10°C and 14°C cortisol levels were only elevated for 6 hours after the handling stressor (King V et al. 2006). In sunshine bass, *Morone chrysops* × *Monrone saxatili,* plasma cortisol levels were lower and changes in concentration were delayed at temperatures below 20°C. Sunshine bass had the fastest recovery at 10°C to 15°C (Davis 2004). Our study observed quickest recovery, 3-hours post handling, at 12°C. Plasma cortisol levels were also returned to baseline with in 24 hours at all three temperatures.

At 12°C and 16°C, plasma cortisol levels peaked within 30 minutes of exposure to the PIT-tag handling stressor. Eurasian perch, *Perca fluviatilis*, rainbow trout, *Oncorhynchus mykiss*, greenback flounder, *Rhombosolea tapairina*, and other teleosts (Jentoft et al. 2005, Barnett and Pankhurst 1998, Barton and Iwama 1991) exposed to handling experiments showed maximum plasma cortisol levels within 30 minutes to 1 hour post-handling. At 20°C we did not see a cortisol peak at 0.5 hours post-handling rather than at 0 hours post-handling. For three of our sample times there was a rather small sample size as a result of escapees, issues with obtaining a blood sample, and mislabeled samples. At 20°C, time 0, and 16°C, time 0.5 hours post-handling the variation was large and sample sizes were small. Perhaps we would have had more representative values if not for the small sample size, resulting in peak plasma

cortisol levels occurring at 0.5 hours post handling at 20°C. However, our study did not include a 1 hour-post handling sample. Such a sample should be considered in future studies as many species reach their maximum levels at 1 hour-post handling; thus we may have missed the peak at 20°C.

Plasma cortisol levels returned to baseline levels within 24 hours of exposure to the PIT-tag handling stressor at all three temperatures. While there was a response to the PIT-tag handling stressor, these fish were capable of recovery. Bonytail exposed to the PIT-tag handling stressor at 12°C appeared to have the quickest recovery time; cortisol levels returned to baseline within 3 hours of PIT tagging, whereas fish acclimated to 16°C required 12 hours of recovery time before reaching baseline cortisol levels. At 20°C, baseline levels were not observed again until 24 hours post-handling. However, at three hours post-handling, cortisol levels were lower than those at baseline. There is nothing to suggest why such a drastic decrease in plasma cortisol levels occurred at this time in this temperature group.

Water temperature has a profound effect on metabolism, growth, digestion, and food consumption rates of fish (Adams and Breck 1990). As temperatures increase fish must cope with decreasing dissolved oxygen levels, and increasing metabolic rates that increase the demand for oxygen (Hoar et al. 1969). At lower temperatures many physiological functions in fish begin to slow and a reduction in stress may occur (Davis 2006). Bonytail prefer warmer temperatures with a reported maximal thermal tolerance of 38°C to 40°C (Carveth et al. 2006). Our data showed that mean plasma cortisol levels were significantly lower at 16°C

than those of fish held at either 12°C or 20°C. There were no significant differences in plasma cortisol levels between fish held at 12°C and 20°C. It is reasonable to expect that fish at 12°C would have the most minimal response to the PIT-tag handling stressor yet this was not true in this study.

Fish commonly exhibit higher cortisol levels at warmer temperatures, while cooler temperatures are often thought to be less stressful; however, fish can suffer from coldwater stress. Many coral reef fishes exhibit coldwater stress conditions when shallow waters reach temperatures below 16°C (Roberts et al. 1982). Because bonytail favor warmer water temperatures, 12°C may possibly be low enough to induce coldwater stress, whereas 16°C may be a low enough temperature to slow many of the physiological functions so as to show a reduction in stress (Davis 2006).

Baseline plasma cortisol levels were obtained quickly by netting the fish, holding the fish on a fish measuring board, and bleeding them. Fish that were exposed to PIT- tag handling and immediately bled were also representative of baseline levels as cortisol synthesis is known to have a delayed response of a few minutes (Wedemeyer et al. 1990, Lankford et al. 2003). There were no differences detected in cortisol levels in fish that were only bled compared to fish that were exposed to the PIT-tag handling stressor and bled immediately at 16°C and at 20°C.

Baseline values at 12°C were not included for statistical analysis in the results as sampling time was compromised due to scheduling difficulties. However, when samples were analyzed baseline fish at 12°C exhibited higher levels of

cortisol than those of fish that were exposed to the PIT-tag handling stressor and bled. Samples for baseline fish were collected at 1050 hours and fish that were exposed to the PIT-tag handling stressor and then bled were sampled at 1430 hours. Fish that were sampled at 16°C and 20°C for baseline levels, and fish that underwent the PIT-tag handling stressor and immediately bled were both handled between 1400 and 1600 hours. The higher levels of cortisol in baseline fish at 12°C could reflect dial patterns of cortisol release rather than a temperature effect. Dial rhythmicity of cortisol has been extensively studied in freshwater fish species with consensus that it is variable between species. Peak cortisol values have been reported in goldfish, *Carassius auratus*, Gulf killifish, *Fundulus grandis,* and rainbow trout, *Oncorhynchus mykiss,* during daylight hours (Spieler and Noeske 1984, Garcia and Meier 1973, Boujard and Leatherland 1992), whereas common carp, *Cyprinus carpio*, and brown trout, *Salmo trutta*, exhibit peak levels during the night (Redgate 1974, Pickering and Pottinger 1983). Dial cortisol patterns in bonytail should be determined before future stress studies occur and this knowledge may be important for choosing when to tag these fish.

We looked at percent survivorship over 28 days post-exposure to a PIT-tag handling stress at 16°C and 20°C. Mortality associated with PIT tagging is frequently observed within the first 10 days subsequent to tagging (Gries and Letcher 2002, Dare 2003, Navarro et al. 2006). Observed mortality of bonytail exposed to a PIT-tag handling stressor at 16°C did not occur until 12 days post-tagging, suggesting that mortality was not associated with PIT tagging. Bonytail

exposed to the PIT-tag handling stressor at 20°C did exhibit a decrease in survivorship within the first 10 days, characteristic of previously reported PIT-tag associated mortalities in gilthead seabream*, Sparus auratus L*. (Navarro et al. 2006). In contrast, survivorship of Chinook salmon, *Oncorhynchus tshawytscha,* continued to decrease after 10 days (Dare 2003). Mortality was also observed in baseline sampled fish at 16°C after 12 days post-sampling, and in control fish 23 days post-experiment at 20°C. We analyzed the mortality data at 10 days post-handling, the time associated to PIT-tagging mortality. At 16°C there was 100% survival in all three groups, whereas at 20°C there was 100% survival in the control and baseline fish with observed mortality in fish exposed to the PIT tag handling stressor. This data suggest that at 20° handling of bonytail should be avoided or limited.

However, treatment fish not only were exposed to a PIT-tag handling stressor, but they were also exposed to a blood sampling handling stressor. We were able to obtain plasma cortisol values to evaluate the stress response to the PIT tag handling stressor without concern for a stress response to the blood sampling because of the delayed response of cortisol secretion (Reid et al. 1998, Iwama 1998). We, however, did not account for a delayed stress response to blood sampling when deciding to measure survivorship.

A fish exposed to a single stressor will display plasma cortisol concentrations that plateau and then decrease rather than increasing indefinitely, as seen with these bonytail data (Frisch and Anderson 2000). This decrease is in response to the negative feedback of cortisol on the hypothalamus, suppressing release of

ACTH from the pituitary (Barton and Iwama 1991). A cumulative response will cause this feedback mechanism to be less effective when fish are allowed short recovery periods between stressors (Barton et al. 1986). Delayed mortality could be caused by exposure to two handling stressors:1) PIT-tagging stressor, and 2) the blood-sampling stressor with a cumulative response occurring. If this is the case and bonytail are unable to adapt to such stressors then tertiary responses may occur in response to the redistribution of energy by changing the energy substrates in order to manage the increase demands of stress (Iwama 1998). This could result in decreased disease resistance and survivability, possibly resulting in delayed mortality.

Often stressed individuals show a full recovery (Barton 2002, Mesa et al. 1994). However, fish that no longer show signs of stress may still die from the cumulative effects of stress (Wood et al. 1983, Wedemyer et al. 1990). Death associated with cumulative stressors does not always occur immediately but may take place sometime after exposure to cumulative stressors. Juvenile salmon that were stressed from exposure to increased total gas pressures were shown to take as long at 50 days post-stressor for half of the population to die off (Jensen et al. 1980). The delay and continuation of mortality in our data suggests that the observed mortality is not specific to the handling stressor but may be a combination of PIT-tag handling stressor and blood sampling.

While this does seem to be a plausible explanation for fish exposed to the PIT- tag handling stressor, this does not explain the mortality observed in control fish which were neither bled nor PIT tagged, suggesting something unrelated to

the experiment may have affected survivorship of these fish. Achii Hanyo is a remote site where many unseen problems may occur such as short-duration water shutoffs resulting in decrease dissolved oxygen levels, which decrease the fishes ability to efficiently convert energy into a usable form. Disturbances from wildlife, specifically birds perching on tanks may also affect survivorship. When birds are present fish show a fright response avoiding birds by rapidly swimming away. Water temperature data were logged with a hobo data-logger, and no extreme temperature changes (>2°C) were recorded, suggesting mortality was not due to temperature extremes. Temperature mortality could be investigated in this species looking at several acclimation water temperatures and rapidly cooling or heating the water to determine how the fish will respond.

After sampling, a number of fish developed black banding across the caudal peduncle. Resembling a horse-riding saddle, dark pigmentation discoloration has been observed in rainbow trout after electrofishing, and is a result of temporary external symptoms of spinal injures (Reynolds 2008, Snyder 2003). Bonytail that exhibited banding were observed for four weeks after handling banding was observed to fade and disappear. It is suspected that banding developed where the spine was contacted during blood sampling, causing minor injury resulting in external bruising. These fish were not individually identified as we did not want to expose them to another handling stressor to obtain PIT-tag numbers. Some of these fish did die; they were usually found a few hours after death and pigmentation had faded making if difficult to confirm that the fish had the banding. Mortality could be associated with internal damage from blood sampling; the

caudal vessel was contacted on the first attempt in very few fish. It is plausible that intestines may have been punctured and damage may have occurred to the spine. This could be minimized through practicing blood drawing techniques, or adjusting sampling point, such as a lateral insertion vein puncture, or dorsal aorta puncture.

Ich was confirmed in two tanks by examining fish skin scrapes where a trophont was removed and the characteristic crescent moon nucleus was observed. Fish also exhibited characteristic white spots on their skin. Instead of discarding these fish from this study we examined cortisol levels of bonytail infected with the Ich parasite. Our findings suggest infected bonytail did indeed exhibit significantly higher cortisol levels then those not infected. In fact, cortisol levels were much higher than those observed in healthy fish following the PIT-tag handling stressor. There were no significant differences in plasma cortisol levels of fish with Ich at different temperatures. Fish that were infected with the Ich parasite exhibited much higher plasma cortisol levels than the peak levels reached by uninfected fish, showing that the PIT-tag handling stressor does not elicit the maximal response.

Achii Hanyos' water is supplied by canal water. Preventative measures were taken to reduce infection by using low bonytail tank densities, and adjusting water flow rates to flush tanks eight times daily. Other tanks not associated with this study were also infected and we suspect that there was an accidental cross contamination with nets from the infected tanks to the study tanks. The prevention of Ich is difficult in systems that use water sources such as pond,

river, and canal water (Hoffman 1970). Facilities can control Ich by filtering and sterilizing water using ultraviolet light (Hoffman 1970). However, these systems can be costly and ineffective depending on the water flows into the facility.

These results indicate that bonytail responded to the PIT-tag handling stressor relatively quickly within 0.5 hours post-PIT-tag handling. These data suggest that when bonytail are exposed to a PIT-tag handling stressor at the hatchery, treatment should begin immediately to alleviate stress. Fish could be placed immediately into a sodium chloride bath, a common prophylactic at hatcheries to aid in the alleviation of stress and prevention of disease outbreak. If possible, handling should be avoided at 12°C and 20°C as fish tagged at these temperatures exhibited much higher cortisol levels than in fish at 16°C. Mortality was observed in control fish, baseline fish, and treatment fish after 10 days in both temperatures suggesting that something else is occurring in this system that is harmful to this species and they would benefit from quick releases.

This species offers many opportunities for continued research on stressors and the responses. I would suggest before proceeding with future stress studies we gain an understanding of dial and seasonal patterns of plasma cortisol secretion in bonytail. As 16°C was found to be an appropriate temperature to handle bonytail, and 20°C was more stressful 18°C should be investigated to confirm 16°C was most optimal. Also 18°C is the temperature in which this species spawns which may affect plasma cortisol levels. PIT-tagging is only a part of the harvest process before bonytail are released. The cumulative effects of harvest handling; seining, transporting, sorting, tagging, and transport should

be investigated. This should be accompanied by evaluating the use of common hatchery treatments following stress such as salt bath, formalin baths, stress coat and no-treatments for their ability to alleviate stress and speed recovery as indicated by circulation levels of cortisol and effects on survivorship.

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