GENERAL FISH HEALTH ASSESSMENT AND AGE EVALUATION OF IMPINGED FISH AT STEAM GENERATING POWER PLANTS

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GENERAL FISH HEALTH ASSESSMENT AND AGE EVALUATION OF IMPINGED FISH AT STEAM GENERATING POWER PLANTS

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A Thesis

Submitted to

the Graduate Faculty of

Auburn University

in Partial Fulfillment of the

Requirements for the

Degree of

Master of Science

Auburn, Alabama
December 19, 2008

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THESIS ABSTRACT

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Master of Science, December 19, 2008
(B.S., Auburn University, December 2004)

110 Typed Pages

Directed by J.S. Terhune

Fish health assessments were made between impinged and reference fish collected at and in the vicinity of Steam Plant Gorgas (Black Warrior River, Walker County, Alabama) during three seasons: spring/summer, fall, and winter/spring as well as two additional plants during the fall sampling season: Steam Plant E.C. Gaston (Lay Lake, Coosa River, Shelby County, Alabama) and Steam Plant Greene County (Black Warrior River, Greene County, Alabama). The three species investigated for fish health assessments included gizzard shad (*Dorosoma cepedianum*), threadfin shad (*Dorosoma petenese*), and freshwater drum (*Aplodinotus grunniens*). Assessments were based on body weight-length, external lesions, and pathogen detection. Freshwater drum were aged using otoliths at Plant Gorgas. Impinged gizzard shad, threadfin shad, and freshwater drum weighed significantly less when compared to reference fish during all three seasons at Plant Gorgas.   Lesions were significantly more common in impinged compared to reference gizzard shad and freshwater drum during the spring/summer season and in impinged compared the reference threadfin shad during the winter/spring season at Plant Gorgas. *Aeromonas hydrophila* and internally isolated and lesion associated *Flavobacterium columnare* was detected in significantly higher percentage of impinged compared to reference gizzard shad during all seasons, and in impinged compared to reference threadfin shad during the winter/spring season. *Aeromonas veronii biotype sobria* was detected in significantly higher percentage of impinged than in reference gizzard shad during the fall season. The protozoan parasite, *Ichthyobodo necator*, was observed in higher prevalence in impinged gizzard shad compared to reference during the fall season and in impinged freshwater drum compared to reference during the spring/summer and winter/spring seasons. It was determined that there was no significant difference in age between impinged and reference freshwater drum at Plant Gorgas.

Length-weight analysis and lesion associated and internal *F. columnare* was determined to be significant in impinged compared to reference freshwater drum at Plant Greene County. None of the components of the overall disease analysis were concluded to be significant at Plant E.C. Gaston. This could be attributed to small sample size. The results of this study indicates that intake screens at steam generating power plants may be selectively impinging sick, dead, and dying fish.

ACKNOWLEDGEMENTS

I would like to thank Alabama Power for funding this research. I am grateful to my advisory committee for the opportunity to conduct this investigation as well as for their continued support and advice. Sample collections and processing could not have been possible with out the help of Justin Mitchell, Larry Craft, John Sieweke, and Jon Ponstein of Alabama Power and Karl Hayden, Darrin Durham, Square Foree, Tyler Thomas, Chase Katechis, Sara Rademaker, and Chris Sayles of the Auburn University Southeastern Cooperative Fish Disease Lab. I would also like to thank my friends, especially Gus Katechis, Patrick and Mary Jo Thompson, Whitney and Jason Hester, Katina Dunkerly, Brittany Nelson, and Grant Thomas for all the support, encouragement, and shoulders to lean on over the years. Most of all I would like to extend my utmost gratitude to my parents and brother, Richard, Angie, and Scooter Knight. They have always believed in me with unwavering support in any aspect of my life, education, and career. It is without a doubt that I would not be where I am today if I did not have all of these wonderful people helping to hold me up along this journey.

Style manual or journal used: Journal of Aquatic Animal Health

Computer software used: Microsoft Word

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

The Clean Water Act (CWA) first began as the Federal Water Pollution Control Act (FWPCA) in 1972. After amendments in 1977, the FWPCA became commonly known as the CWA (Veil 2000). This act established the basic structure for water pollution regulations with authority to enforce regulations given to the United States Environmental Protection Agency (USEPA). Section 316(b) of the CWA states that the best technology available (BTA) be implemented in location, design, construction, and capacity of cooling water intake structures (CWIS) associated with steam generating power plants for reducing adverse environmental impacts (AEI) (Dey et al. 2000; Veil 2000). Since the enactment of the CWA, the definitions and measurements of BTAs and AEIs have been highly debated. These debates have a general emphasis on CWIS designs, biological criteria, and scientific judgment. The main purpose of Section 316(b), as determined by the USEPA, is to minimize impingement and entrainment of fish and other aquatic organisms taken in the plant's CWIS (Dunning et al. 2000).

According to a 1996 study, almost half of all United States steam generating power plants use the once through cooling water process (Veil 2000). This process involves removing water from a rivers, lakes, estuaries, or oceans and diverting it into the plant at the CWIS. Eventually, the water is discharged back into the same or a nearby water body (Veil 2000). CWISs have vertical traveling screens, usually set behind trash racks that keep large debris and animals from entering the plant with the cooling water. Screens are usually composed of mesh wire with approximately 1 cm openings (Kelso and Milburn 1979). Entrainment occurs when small organisms pass through the intake screen openings and enter the plant along with the cooling water (Dey et al.2000, Veil 2000). Most entrainment studies have focused on fish larvae and fish eggs. Impingement, however, occurs when larger organisms become trapped against the screens by the velocity of the incoming water (Dey et al. 2000, Veil 2000). As the screen rotates, debris and organisms that have become impinged against the screen rotate to the top of the screen housing and are removed by high-pressure water jets that spray impinged objects into a sluiceway. The sluiceway then empties into a concrete basin where impinged organisms are collected in a basket for disposal or are discharged downstream with the flow through water. It has been reported that impinged organisms may incur physical injury and become stressed, that may result in death due to contact with the screen during the process of impingement (Lohner et. al. 2000, Fletcher 1994, White et al. 2007).

When Section 316(b) was first passed, all of the major components of the ecological community involved with impingement and entrainment were investigated. Early research efforts focused mostly on shellfish and finfish (Dey et. al. 2000). Attempts to develop technologies to decrease entrainment and impingement have also been made. The ability of these developmental technologies to meet BTA requirements is very specific to each plant, its CWIS design, and a variety of biological and environmental factors that influence each plant and their CWIS (Taft 2000). Therefore, the assessment and regulation of the environmental impacts of entrainment and impingement requires new and innovative research, development, and implementation. Historically, the USEPA has enforced Section 316(b) regulations on a plant-by-plant basis. Plant-by-plant regulations have led to enormous variability in compliance requirements among plants. To decrease compliance variability and increase universal requirements, the EPA divided the rule making related to CWA Section 316(b) into three phases in 2005.

The three phases of Section 316(b) established by the USEPA categorize power plant requirements and almost eliminate compliance variability among plants. Phase I includes all new plants. Phase II includes all existing plants that withdraw 50 million gallons of water a day or plants that use 25% or more of the water withdrawn for cooling purposes. Phase III includes small power plants and other industrial facilities. All plants included in this study fall under phase II. Requirements established for all Phase II plants include reducing impingement mortality by 80 to 95%. This reduction is based and measured against a calculation baseline, defined as the amount of mortality due to impingement that would occur at a plant with the intake positioned on the shoreline, pulling water from close to the water's surface, with a traveling screen with 1 cm openings to reduce impingement mortality.

On January 25, 2007, the three-phase ruling of the CWA Section 316(b) was rescinded by the second circuit court. By March 20, 2007, administrators were instructed to revert to establishing National Pollutant Discharge Elimination System (NPDES) permit conditions based on best professional judgment (BPJ) by the USEPA. The rule was officially suspended by the USEPA through a Federal Register (Volume 72, Number 130) notice on July 9, 2007. Therefore, as suggested by previous studies, research and monitoring must continue to be conducted at each plant due to plant specific variability. Existing plants are therefore presently required to establish the rate of fish impingement, species composition, temporal variability, and waterbody characteristics and other factors. Factors associated with impingement must be fully investigated to ensure that administrators have reliable information to base their BPJ in the implementation of the BTA for minimizing AEI.

Impingement rates can vary depending on numerous environmental, chemical, physical, and biological factors. Factors potentially correlated with impingement rates include fish age, seasonal movement, geographic range, hydrology and, fish health status. General water quality parameters such as temperature, dissolved oxygen, and pH have previously correlated to impingement as well as fish diseases. Industry and government officials agree that an evaluation of the health of the fish populations affected is the first step in assessing potential AEI of impingement (Lohner et. al. 2000). If there are mitigations for any negative impacts on fish populations, we must first understand the possible correlations between these factors and impingement rates before mitigations can be proven successful.

The overall goal of this study was to gain a better understanding of how the health of fish is related to impingement. Very few studies have been performed to evaluate the general health of impinged fish. In 2002, Baker (2007) found impinged fish had significantly higher disease prevalence than fish collected from the river in the vicinity of

Plant Barry. Furthermore, it was concluded that many of these diseases may have existed prior to impingement. Further studies need to be conducted to confirm this phenomenon at other facilities and to determine if seasonal patterns are involved.

This study has three main objectives: (1) to examine the prevalence of diseases in impinged fish versus reference fish from the river around the plants of interest, as well as fish collected from areas within the intake, (2) to assess the age of impinged freshwater drum relative to the age the reference freshwater drum (*Aplodinotus grunniens)* from Plant Gorgas, and (3) to evaluate disease prevalence in impinged fish versus reference fish over multiple seasons and geographic regions. Ultimately, a better understanding of the age and the prevalence of disease in impinged fish will help to improve the litigation of regulations concerning the issue of fish impingement.

II. REVIEW OF THE LITERATURE

Fish Health

Previous studies have suggested a potential correlation between individual fish health parameters and impingement rates (Bamber et. al. 1983, Rodgers 1995, Rohlwing et. al. 1998, Sprengel and Luchtenberg 1991). Yet, to date there is only a single study that has attempted to comprehensively investigate, by examining several parameters including bacterial pathogen and parasitic pathogen load, the variation in general fish health status between impinged fish and reference fish collected from surrounding river populations (Baker 2007). More research is needed to definitively link general fish health and impingement rates (Rodgers 1995, Baker 2007). An understanding of the published literature concerning the fundamentals of fish health, as well as the previous studies that suggest correlations between fish health parameters and the impingement rates of fish, is needed to support ongoing research. A complex relationship exists between fish, the diseases that infect them, and their environment. All of these factors must be examined before correlations between general fish health status and impingements rates can be concluded.

Biotic factors affecting fish health include infectious fish pathogens as well physiological stress. Physiological stress can occur as a response to a threat or a perceived threat. This physiological stress and/or physical injury can cause fish to become more susceptible to bacterial infections (Hawke 1974). It has also been noted that physiological stressors often lead to mortality and increase the vulnerability of fish to impingement (Lifton and Storr 1977). Under certain environmental stress conditions fish become more susceptible to virulent pathogens, and infectious diseases develop (Sneiszko 1974). The fact that stress is a highly relevant factor in infectious disease outbreaks in fish is widely acknowledged (Sneiszko 1974). Stress can also be caused by changes in abiotic variables.

Abiotic variables affecting fish health include water temperature and dissolved oxygen (Austin and Austin 1999). Water temperatures have been correlated with epizootic events, and certain temperature intervals have been associated with pathogens during such events (Austin and Austin 1999; Becker and Fujihara 1978; Pacha and Ordal 1970; Roberts 2001). Water temperatures have also been previously correlated with impingement rates. Some impingement studies have correlated increased impingement with decreased temperatures which can be attributed to cold shock. However, most of the fish pathogens in the Southeastern United States tend to be associated with high water temperature.

Bodensteiner and Lewis (1992) hypothesized that low water temperatures had an adverse affect on freshwater drum, which caused an increase in impingement numbers at a Mississippi River power plant. In a laboratory experiment, fish were collected from the river near the plant, acclimated in tanks, and given a prophylactic antibiotic treatment. The fish were then subjected to decreasing water temperatures. As the temperature approached 1°C, loss of swimming equilibrium and increased mortality of the fish were observed. Similar results have been found in laboratory experiments conducted with other species of fish including channel catfish (*Ictalurus punctatus)*, striped mullet (*Mugil cephalus)*, spot (*Leiostomus xanthurus)*, and pinfish (*Lagodon rhomboids)*. Decreased swimming ability is linked to a decrease in water temperature (Hocutt 1973, Mathur et al 1977, Rulifson 1977). Other fish species, such as threadfin shad (*Dorosoma petenense)*, are known to experience high mortality rates due to low temperatures. This correlation may be accountable for some of the variation in impingement rates.

Threadfin shad are an important prey or forage fish in its native and introduced range, but undergo massive winter mortality in the northern parts of its range. According to Loar et al. (1978), threadfin shad comprise 90% of fish impinged at steam generating power plants in nine states of the southeastern United States. Impingement rates of threadfin shad have been shown to increase with decreasing water temperatures in the southeastern United States (McLean et. al. 1985). Mortality of the fish in the reservoir coincides with the impingement and mortality of fish at the intake screens on the power plant. From October 1976 to April 1977 at Watts Bar Reservoir and the Kingston Steam Plant (Knoxville,Tennessee), McLean et al. 1985 identified cold stress as an important factor influencing both impingement and reservoir wide mortality of threadfin shad. Threadfin shad kills were evident in late December and January by the presence of large numbers of dead shad on the shoreline, deceases in the number of shad caught in the gillnets, and the large increase in numbers of shad caught on the intake screen at the power plant. These threadfin shad kills were thought to have been a result from the effects of declining temperatures on swimming and schooling ability (McLean et al.

1985). Other studies have come to similar conclusions for different species (Bodensteiner and Lewis 1992).

A study conducted by Mathur et. al. (1977) at Peach Bottom Atomic Power Station in York County, Pennsylvania, observed that the highest rates of impingement occurred in channel catfish, white crappie (*Pomoxis annularis*), and bluegill (*Lepomis macrochirus*) from November 1973 through December 1975. Impingement increased with several factors including decrease in water temperature. The authors refer to “cold-induced sluggishness” as a possible explanation for increasing impingement rates at decreased water temperatures. Since a positive correlation between swimming ability and water temperatures exist, decreased ability to swim due to decreased water temperatures could ultimately lead to increased impingement. Increased impingement would occur due to the fish’s inability to avoid the hydraulic zone of influence (HZI) in front of the CWIS.

A fishes swimming ability can be affected by other factors. Several studies have investigated the swimming ability of fish in a compromised state. Tierney and Farrell (2004) examined the swimming ability of adult sockeye salmon (*Oncorhynchus nerka*). Critical swimming speed test were performed on both fish with injury or abnormalities and apparently healthy fish. It was observed that fish infected with *Ichthyophonus* spp. and *Saprolegnia* spp. were not able to achieve critical swimming speeds equivalent to the apparently healthy fish. Fish with cutaneous lesions were classified as sick and moderately sick if lesion was $> 1 \text{ cm}^2$ or $< 1\text{cm}^2$, respectively. It was observed that fish classified as sick could not recover from initial critical swimming speed test and had slower swimming speeds (Tierney and Farrell 2004). Swanson et al. (2002) reported similar results about delta smelt (*Hypomesus transpacificus*) infected with *Mycobacterium* spp. Infected smelt had a significant reduction in swimming speed compared to uninfected smelt. A reduction in swimming speed due to infectious disease could ultimately lead to increased impingement rates due to fish being overcome by the velocity of the HZI, which has previously been negatively correlated with time to impingement (Rulifson 1977, Fletcher 1994, White et al. 2007).

Correlations between water velocity and dissolved oxygen also exist with impingement. Low dissolved oxygen concentrations are usually correlated with slower water velocities and higher water temperatures. Low dissolved oxygen levels have been linked to increased impingement rates, large fish kills, and high parasite prevalence on the gills. The role of dissolved oxygen in the survival of freshwater drum from the Mississippi River was examined by Bodensteiner and Lewis (1992). They noted that as the water temperature decreased the freshwater drum migrated into the warmer backwaters. As the dissolved oxygen decreased in the backwaters the freshwater drum moved back into the main channels. In the main channel, the fish were exposed to lower temperatures, which resulted in an increased number of moribund freshwater drum and eventually were impinged. Winter impingement rates and analysis at this plant indicated that 90% of the fish were moribund or dead at the time of impingement (Bodensteiner and Lewis 1992).

The methods used to investigate the health status of impinged and reference fish has varied, but there is a universal hypothesis: decreased health status or infection increases the likelihood that a fish becomes impinged. It has been suggested that fish with diplostimiasis, caused by an infectious parasite, *Neodiplostomum* spp., would be more susceptible to impingement due to decreased ability to escape the HZI. Bamber et al. (1983) compared diplostimiasis prevalence in impinged and reference sand smelt (*Antheria presbyter*) at Fawley Power Station (Hampshire, England). Diplostimiasis was chosen as the pathogen for evaluation because it had been previously observed in sand smelt within the vicinity of Fawley Power Station. It was concluded that no correlation exist between fish with diplostimiasis and impingement. The lack of correlation could be due to the fact that diplostimiasis is not a debilitating disease in most fish (Post 1983). A high prevalence of *Sacculina* and *Lernacera* spp. was also noted on pout (*Trisoptrerus luscus*) impinged at Fawley Power Station. However, no direct comparison was made between prevalence rates of impinged versus reference fish (Bamber et al. 1983).

Other studies have attempted to compare impinged fish versus reference fish. A study conducted from 1984 until 1985 at Quad Cities Nuclear Station (Cordova, Illinois) investigated the health factors affecting the impingement and moribundity of freshwater drum (Bodensteiner and Lewis 1992). Several factors were used to evaluate the health status of the fish. These factors included ascites, distention of gall balder, color of bile, texture of muscle, color of liver, liver weight, and presence of material in the gut. These factors were used to calculate a condition factor for the drum. The indices were used to compare reference drum collected with barrier nets at the CWIS and drum collected by seine nets in adjacent sloughs. No difference was observed in the mean body condition of the two populations sampled, and therefore it was concluded that starvation was not significantly correlated with impingement. The authors noted the presence of two fish pathogens oomycetes and *Philometra* spp. Although a 33% prevalence of oomycete growths on the impinged fish was reported, no prevalence rate was reported for the reference fish. *Philometra* spp. were found on both impinged and reference fish, however, no significant difference in prevalence rate was observed. Based on the size distribution the authors concluded that first and second year drum were more susceptible to becoming moribund and impinged. Moribundity of the freshwater drum was correlated with low temperature and low dissolved oxygen. In 1985, 98% of impinged drum were live or recently dead. In contrast, the previous winter up to 90% of impinged drum were dead (Bodensteiner and Lewis 1992).

A histopathological evaluation of alewives from potentially impinged populations collected from in front of the intake screens and reference populations was conducted in June 1986 at Pickering Nuclear Generating Station on the northwest shore of Lake Ontario (Rogers 1995). Multiple organs from 25 fish of each population were examined histopathologically. A significantly higher prevalence of nephrocalcinosis was observed in the potentially impinged fish sampled from in front of the intake screen. Nephrocalcinosis is known to be caused by an imbalance in cation intake and high carbon dioxide levels. Rodgers (1995) noted that it was unlikely that fish encountered these conditions in the intake channel and the natural occurrence of the lesion has been documented in wild fish populations. However, it was observed that the potentially impinged fish had lower liver and body weights compared to reference fish of the same size. Body weight of an impinged alewive was 10% lower than the weight of a reference alewive of approximately the same length. Rodgers (1995) explained that the decrease in liver and body weight between the two populations could be due to the depletion of energy reserves caused by exhaustion from maintaining swimming velocities that would be required to avoid impingement. It was concluded that it would be unlikely for a healthy fish to be unable to leave the intake environment prior to impingement (Rodgers 1995).

Baker (2007) conducted the most comprehensive study of fish health as it relates to impingement rates of fish to date. This 2005 study was conducted at Plant Barry (Bucks, Alabama) located on the Mobile River. Threadfin shad, channel catfish, blue catfish (*Ictalurus furctatas*), and freshwater drum were designated for investigation. Impinged fish were collected every 1-2 h and reference fish were collected within 5 km upstream and downstream of the plant's intake. Impinged and reference fish were collected during both the spring and fall of 2005. After collection, length, weights, and parasitic and bacterial observations for each fish were recorded. Compared to the reference fish, all four species investigated had decreased weight for a given total length. Two parasite species, *Ichthyobodo necator* and *Chilodonella* sp., were present on significantly more impinged fish than reference fish. Other parasites were observed but no significant difference between impinged and reference fish was observed. Over a dozen potentially pathogenic bacteria were isolated from impinged and reference fish, but only *Aeromonas* sp., *Flavobacterium columnare*, and *Plesiomonas shigelloides* were determined to be present on significantly more impinged fish than on reference fish. Compared to reference fish, the impinged fish of all four species were more likely to have a bacterial pathogen of some type. This difference was true in both the spring and fall sampling seasons (Baker 2007).

Epidemiological Studies

Columnaris disease, caused by the bacterial pathogen *F. columnare* was first described by Davis (1922), and is known to infect most freshwater fish species (Roberts 2001). Bowser (1973) conducted a study at Clear Lake, Iowa, in 1971, to determine the incidence of both symptomatic and asymptomatic columnaris infections among black bullheads (*Ictalurus melas*). The prevalence of columnaris infections was found to be seasonal, occurring primarily in the spring. Prevalence rose sharply to 60% by mid-May and then decreased to 0% as the summer approached. The highest prevalence being observed during the warming of the lake in the spring. The high spring prevalence of the pathogen is not unexpected. It has been reported that high virulence *F. columnaris* becomes a problem at a temperature of 15°C. This corresponds with the warming water temperature in the spring. Also, when water temperatures are low, below ideal metabolic rates, fish do not produce antibodies, so therefore, depending on the fish species, immunologically fish in moderate climate zones are at their weakest in early spring (Bowser 1973). It has also been concluded that high fish densities can contribute to the levels of infection (Fujihara and Olson 1962).

Fujihara et al. (1964) conducted a study comparing the prevalence of *F. columnare* in fish collected in 1963 from three sampling sites on the Columbia River to fish collected from the Yakima River. *F. columnare* detection was divided into two categories based on the number of bacterial colonies isolated. Plates cultured with tissue smears with only a few colonies growing were considered to be incidental (“incidence”). While on the other hand, the growth of a “host of colonies” on the cultured plate was considered to indicate “infection” or disease. It was concluded that 3.3% of the fish from the Columbia River were considered to be “incidence” compared to the < 1% rate of considered infection. A 93% rate of incidence was found in the Yakima River with a 15% rate of infection. It was further concluded that the presence of skin lesions or gill necrosis did not indicate the presence of *F. columnare* because it was not frequently isolated from these organs (Fuijhara et al. 1964).

Becker and Fujihara (1978) conducted an epizootiological study of *F. columnare* in fish from the Columbia River from 1964 to 1973. Sampling was conducted at four sites in 1965 and from 1969 to 1973 (n=7,000). The study was performed to investigate the possible effect of operations of the Hanford reactor on the pathogenesis of *F. columnare*. Study sites were located on the main stem of the Columbia River at 9.6 km below McNary Dam, 6.4 km below Bonneville Dam, near the town of Hanford, and at Wenatchee just above Rock Island Dam. Numerous species were sampled including channel catfish and largemouth bass (*Micropterus salmoides*). They noted a percentage of bacterial isolations from lesions as low as 10% and the successful isolation of bacteria from gill and head kidney cultures in fish presenting no external clinical signs of the disease. The detection of *F. colmunare* was divided into two categories, “exposed” and “infected”. Cultures yielding one to ten colonies were considered “exposed”, while cultures with more than ten colonies were considered “infected”. Fish were grouped by site and year and summarized as percent “exposed” and percent “infected”. Sampling efforts from May to November 1965 indicated a prevalence range of “exposed” between 4.0 to 7.5% compared to “infected” group that range from 1.9 to 4.3%. Sampling efforts from 1969 to 1973 revealed that the “exposed” group ranged from 1.7 to 34.9% compared to the “infected” group that ranged from 3.3 to 22.7%.

Various other bacterial pathogens have been responsible for epizootic events occurring in Alabama water bodies as well as other parts of the United States. During the spring of 1973 and 1974, *Aeromonas hydrophila* was determined to be the etiological agent in 90% of the diseased fish from seven impoundments located in Alabama (Hawke 1974). *Aeromonas hydrophila* is a ubiquitous bacterial pathogen. It is a long proven pathogen of reptiles, amphibians, snails, fish, and humans. Several studies have suggested the correlation between *A. hydrophila* densities in water and epizootic events in fish (Haley et al. 1967, Hazen 1979). Hazen et al. (1978) published a prevalence and distribution of *A. hydrophila* in the United States. In this study the abundance of *A. hydrophila* measured in 147 aquatic habitats in 30 states and Puerto Rico. Water samples, along with temperature, pH, conductivity, salinity, and turbidity measurements, were taken at each site. It was shown that higher densities of *A. hydrophila* were often found in lotic habitats and saline compared to freshwater habitats. *Aeromonas hydrophila* was found in a wide range of salinity, conductivity, temperature, pH, and turbidity, but could not be isolated from extremely saline, thermal, or polluted waters (Hazen et al. 1978). The detection of *A. hydrophila* did not necessarily indicate disease in the examination of apparently healthy fish collected by electrofishing from lakes

Martin and Logan Martin in 1973 and 1974. The fish collected showed a carrier rate of 10% and 25%, respectively for each lake (Hawke 1974).

The results of wild fish health surveys such as these are valuable when applied to the comparison of pathogen prevalence between impinged and reference fish. The detection of pathogens in impinged fish may be an indicator of decreased health status and thus an increased susceptibility to impingement. The chronic effects and epizootiology of these diseases also need to be considered in conjunction with many of the previously discussed factors when attempting to explain the variability associated with impingement rates.

Fish Age

Fish age and growth has long been known to be correlated with the environmental conditions in which they live. The growth of a fish can be influenced, both positively and negatively, by numerous biotic and abiotic variables. These variables can include such things as nutrient and prey availability, competition, predation, and flow (Putman et al. 1995; Bestgen and Bundy 1997; Aday et al. 2005). Yet, to date no research has been conducted to conclusively correlated fish age based on otolith aging with impingement. Examination and estimation of the age of impinged fish could lend some useful insight on how and why fish become impinged.

III. STATEMENT OF RESEARCH OBJECTIVES

The purpose of this study was to determine if an increased pathogen prevalence or decrease in general health status exists in impinged fish relative to reference fish at Plant Gorgas (Walker County, Alabama). Three species of concern: gizzard shad, threadfin shad, and freshwater drum were evaluated due to high impingement rates and/or high replacement cost in terms of potential mitigation. Aging of freshwater drum, based on otolith annuli, from Plant Gorgas was performed to determine if age was potentially correlated to impingement rates. In addition to the sampling conducted at Plant Gorgas, preliminary sampling was conducted at two other power plants that are subject to 316(b) regulations, Plant EC Gaston (Shelby County, Alabama) and Plant Greene County (Greene County, Alabama). At these two additional sites sampling was performed to begin collection of health status of impinged compared to reference fish at other geographical sites.

IV. METHODS

Study Sites

Alabama Power Company (APCO) operates five power plants affected by CWA Section 316(b) Phase II rulings and regulations. Of these five power plants, this study conducted sampling primarily at Plant Gorgas (Walker County, Alabama) with preliminary sampling occurring at Plant E.C. Gaston (Shelby County, Alabama) and Plant Greene County (Greene County, Alabama).

Plant Gorgas– Plant Gorgas is located in Walker County, Alabama on the Mulberry Fork of the Black Warrior River at the headwaters of the reservoir created by the Bankhead Lock and Dam (N 33.6443, W -87.20027) (Figure A.1). The six independent generating units have a combined capacity of 1,281 MW. Cooling water for these generating units is drawn from the river through an inverted skimmer weir at the mouth of the intake canal. The water flows down a 1.3 km intake canal where it provides cooling water to the Units 6-7 CWIS before it travels underneath the power plant discharge canal and terminates at the Units 8-10 CWIS (Figure 1).

Due to historically low impingement rates at the Units 6-7 CWIS, our sampling efforts focused on the Units 8-10 CWIS. The traveling screens at the Units 8-10 CWIS have 1 cm square openings and are located behind trash racks, (Figure 2). Debris and organisms trapped against the screen are removed by high-pressure water jets spraying into a sluiceway and empties into a concrete basin. The calculated though screen water velocity through the trash racks at full load is 0.31 m/s for Units 8-10.

Plant E.C. Gaston – Plant E.C. Gaston in located in Shelby County near Wilsonville, Alabama (N 33.24286, W -87.78196) (Figure A.1.). The power plant is on Lay Lake, an impoundment of the Coosa River System near the confluence of Yellowleaf Creek. Four independent generating units operate on a once through cooling system with one CWIS and have a combined capacity of 1,000 MW. The CWIS is located on Yellowleaf Creek, which is an open channel for withdrawal of water from the Coosa River. The CWIS has ten traveling screens and are located behind trash racks as described above. The calculated through screen water velocity at full load (all pumps operating) is 0.18 m/s.

Plant Greene County – Plant Greene County is located near the headwaters of the reservoir formed by the Demopolis Dam on the Black Warrior River in Greene County, Alabama (N 32.60194, W -87.20027) (Figure A.1.). This plant has two independent generating units that have a combined capacity of 500 MW. A 183 m canal from the Black Warrior River brings the cooling water to a single CWIS as described above. The calculated through screen water velocity is 0.33 m/s at full load (all pumps operating).

Temperature

Daily average temperature was recorded by a stationary gage located within the intake canal at Plant Gorgas. Temperature data was downloaded and seasonal averages were calculated.

Fish Sampling

Fish sampled in this study were collected from as many as four areas within the vicinity of the plants by several different methods. Fish impinged by the plant's CWIS were the primary sample source. A metal basket with 1 cm wire mesh was placed in the concrete basin to catch debris, and fish washed off the traveling screens. The basket was checked every one to two hours during sampling events. This time interval was chosen to prevent the basket from overflowing with debris, to optimize the number of fish suitable for necropsy, and to minimize postmortem change and cross contamination between fish and the environment. Fish deemed suitable for necropsy were bagged, labeled, and placed in ice immediately until the time of necropsy. Examination of the integrity of the body cavity, and gill color and state of decomposition was used to determine if fish were suitable for necropsy (Whitman 2004). Length frequencies of impinged fish by species were used as a basis for the size of reference fish collected. Reference fish were collected from three areas in the vicinity of Plant Gorgas (Figure 1).

At Plant Gorgas, the three sources of reference fish were 1) the river within 1.5 km of the intake canal, 2) the intake canal, a 1.25 km man made canal that passes underneath the discharge canal before entering the intake structure, and 3) the intake forebay, a cement impoundment directly in front of the intake screens, that is fed by the intake canal after it passes underneath the discharge canal (Figure 1). Fish from each of three reference areas were collected by electroshocking, gill netting, or snagging. Sampling of all sources of fish was conducted concurrently throughout the sampling periods. Sampling was conducted for three seasons, spring/summer (May 29, 2006 until

June 21, 2006), fall (October 2, 2006 until November 2, 2006), and winter/spring (March 14, 2007 until April 14, 2007), throughout one year to reflect potential changes in pathogens or their prevalence.

At Plant Greene County and Plant EC Gaston, sampling for fish health assessment was conducted during the fall season, (October 2, 2006 until November 2, 2006), due to extremely low impingement numbers during the two other seasons. Collection and selection of impinged fish for necropsy were performed as above. Reference fish at these two sites, however, were only obtained from the river within the vicinity of the respective plant.

General Necropsy Procedure

All fish collected were processed on site within 0-12 h of collection. A general necropsy was performed on all impinged and reference fish deemed suitable for necropsy. Total length (mm) and total weight (g) were recorded. A gross examination of the external features of the fish was conducted and any lesions or abnormalities were noted. A gill clip was taken and wet mounted for parasitological examination. Two additional gill clips were obtained: one for histology and another was smeared on a Hus-shotts agar plate (Bullock et al. 1986) supplemented with 4 μg/ml Tobramycin. The peritoneal cavity of the fish was opened aseptically and a sterile disposable 10 μl loop (Apogent Company, Portsmouth, New Hampshire) was used to streak samples of liver and trunk kidney onto Brain Heart Infusion (BHI) and Hsu-shotts agar (Bullock et al. 1986) supplemented with 4 μg/ml Tobramycin. Any external lesion, not appearing to be mechanical in nature, was also sampled and streaked on BHI and Hsu-shotts agar as above. After all aseptic samples were taken, organs were excised for histological preservation and otoliths removed.

Parasitology

The necropsy procedure included an abbreviated parasitological work up. Parasites detected were identified to the lowest taxonomic level possible (Noga 1996). A gross external examination of skin and gills was performed to detect any macroscopic external parasites present. A wet mount of a gill clip was examined to identify any microscopic parasites. A brief examination of the peritoneal cavity was also performed to detect any macroscopic parasites present. All parasites including location on the fish were recorded.

Bacteriology

Bacterial agar plates were incubated at 30˚C and growth was initially recorded at 24 h. Bacterial colonies were counted and colony morphology was recorded. If three or more colonies of similar colony morphology were present on the BHI culture, a representative colony was selected and streaked for isolation. The growth of fewer than three similar colonies was considered insignificant growth and not included in overall data anaylsis. Hsu-shotts cultures were examined for growth characteristics and appearance similar to those of *F. columnare* as described by Griffin (1992). All agar plates exhibiting no growth or insignificant growth were incubated for an additional 24 h to ensure that no slow growing bacteria were over looked. All BHI isolates were initially characterized by gram stain and cytochrome oxidase test.

Gram negative isolates on BHI were identified using a series of biochemical and phenotypic characteristics including a cytochrome oxidase test (Brenner et al. 2001; Buller 2004), glucose metabolism (Furuwatari et al. 1994; Brenner et al. 2001), sensitivity to vibriostat (Holt et al. 1994; Brenner et al. 2001; Buller 2004), growth in 0% NaCl (Furuwatari et al. 1994; Austin and Ausitn 1999; Brenner et al. 2001), and sensitivity to novobiocin (AFS-FHS 2005). Growth of colonies characteristic of *F. columnare* was confirmed by fatty acid methyl ester analysis (FAME) for samples isolated during the spring/summer of 2006 (Shoemaker et al. 2005). This was done to confirm the accuracy of *F. columnare* identification based on colony morphology.

Aging

Age estimation was performed by an examination of the saggital otolith from each freshwater drum (Goeman et al. 1984; Pereira et al. 1995; Campana and Thorrold 2001). Saggital otoliths were extracted at the end of the necropsy procedure. Otoliths were burnt on a hot plate to enhance the contrast of the annuli and were then cross-sectioned using a wet stone grinder (Rypel et al. 2006). Otoliths were then sanded with fine sand paper and mounted in black putty for reading. Otoliths were viewed under a dissecting scope with oil immersion and fiber optic light. The opaque bands of the otolith were counted and recorded (Clayton and Maceina 1999). Two independent readers examined and recorded age estimates for each otolith. A third independent reader was used to settle any discrepancies in age estimates between the first two independent readers (Rypel et al. 2006).

Statistics

All statistical analyses were performed using SPSS (Version 14.0 for Windows, SPSS, Chicago, Illinois) and SAS (Version 8.2, SAS Institute, Cary, North Carolina). A Mann-Whitney rank-sum test was used to detect significant differences in length of fish collected for each species by impinged and reference sampling locations for all seasons (Navidi 2006). Length-weight relationships were analyzed using simple regression analyses. The variables were natural log-transformed to ensure that the statistical assumptions of normality and homogeneous variance for the residuals were satisfied (Anderson and Neumann 1996; Rodgers 1995). The selection of final models were based on a series of stepwise and enter-and-remove-variable regression analyses using variables known to satisfy relationships between a fish's length and weight (Anderson and Neumann 1996). Additional selection criteria included adjusted R^2 and the existence of randomly distributed residuals (Navidi 2006). Potential interaction or confounding effects between length and treatment (reference fish collected from designated areas) were also tested. When an interaction effect was detected , an analysis of covariance was used to test at which lengths the differences in weight were significant (Kleinbaum et al. 1998). These analyses were conducted separately for each season. Differences in predicted body weight, between impinged and reference fish at equivalent lengths were considered to be significant for all species if the treatment coefficients were significant at $p \leq 0.05$. Percent differences in predicted weights was calculated by divinding the difference in predicted reference weight and predicted impinged weight by predicted treatment weight and multiply by 100.

Chi-square tests were used to compare, by species, the frequency of external necrotic lesions, external parasites, bacteria, age, and disease prevalence in impinged versus reference fish. When sample sizes were not large enough so that expected cell counts were a minimum of five, frequencies were compared using Fisher's exact test (Devore and Peck 1993; Dowdy et al. 2004). Differences in prevalence between treatments were considered significant at $p \leq 0.05$.

V. RESULTS

Temperature - Plant Gorgas

Average daily water temperature data was recorded by a gauge located within the intake canal. Spring/summer sampling season average daily temperatures ranged from 28 to 34°C with a sampling season average of 30°C (Figure 3). Fall sampling season average daily temperatures ranged from 16 to 31°C with a sampling season average of 23°C (Figure 4). Winter/spring sampling season average daily temperatures ranged from 21 to 29°C with a sampling season average of 26°C (Figure 5).

Seasonal Length – Total Weight Relationships - Plant Gorgas

Total length and weight were obtained for all fish that were necropsied as well as additional lengths and weights for fish collected but not necropsied (Tables 1, 2, and 3). Length frequencies of impinged fish were used to determine length of the control fish collected whenever possible. Differences in sample sizes for total length and weight occurred because total length was not available for fish with severe caudal erosion. Therefore, only paired data were used for the length-weight analysis.

Predicted weights of impinged gizzard shad were determined to be significantly less ($p<0.0001$) than the river reference fish of equivalent lengths during all three sampling seasons (Figures 6, 7, and 8). The percent difference in predicted weight at equivalent length between impinged and river reference gizzard shad collected during the spring/summer, fall, and winter/spring were 27.2%, 31.9%, and 27.0%, respectively (Tables 1, 2, and 3). In addition to the comparison of impinged gizzard shad to river reference gizzard shad, regression analyses were also run to make comparisons between impinged gizzard shad and those collected from the intake hole and between impinged gizzard shad and those collected from the intake canal. Predicted weights of impinged gizzard shad ($p<0.0001$) were determined to be significantly less than the intake hole reference fish of equivalent lengths during all three sampling seasons (Figures 9, 10, and 11). The percent difference in predicted weight at equivalent lengths between impinged and intake hole reference gizzard shad collected during the spring/summer, fall, and winter/spring where 16.0%, 20.0%, and 21.6%, respectively (Tables 1, 2, and 3). It was also determined that the predicted weights of the impinged gizzard shad were significantly less ($p<0.0001$) than the intake canal fish of equivalent lengths during all three sampling seasons (Figures 12, 13, and 14). The percent difference in predicted weight at equivalent lengths between impinged and intake canal reference gizzard shad collected during the spring/summer, fall, and winter/spring where 29.2%, 32.0%, and 37.7%, respectively (Tables 1, 2, and 3).

Threadfin shad were not examined for the spring/summer of 2006 due to insufficient number of fish collected (Table 1). No significant differences in predicted weight at length between impinged versus river and impinged versus intake hole threadfin shad was observed during the fall of 2006 or the winter/spring of 2007 (Figures 15, 16, 17, and 18). However, significant differences in predicted weight of impinged threadfin shad compared to threadfin shad collected from the intake canal was observed in both the fall of 2006 and winter/spring of 2007 sample seasons (Figures 19 and 20). The percent difference in predicted weights at equivalent lengths between the impinged and intake hole threadfin shad collected during the fall and winter/spring were 21.6% and 11.6%, respectively (Tables 2 and 3).

Due to lack of sample size of freshwater drum collected from the intake hole and the intake canal, analysis was only conducted for impinged compared to river reference freshwater drum. Predicted weights of impinged freshwater drum were determined to be significantly less (p<0.0001) than the river reference fish of equivalent lengths during all three sampling seasons (Figures 21, 22 and 23). The percent difference in predicted weight at equivalent lengths between impinged and river reference freshwater drum collected during the spring/summer, fall and winter/spring 25.9%, 22.9%, and 22.7%, respectively (Tables 1, 2, and 3).

Seasonal Lesion Prevalence - Plant Gorgas

Total number of fish that were used to calculate the prevalence rates of necrotic lesions, parasites, and bacteria is presented by species and season in Table 4. Necrotic lesions observed were distinguished from mechanical lesions and included ulcerative lesions, skin depigmentation, eroded fins, and hemorrhagic areas. External necrotic lesions, suspect of being bacterial or parasitic in nature, were significantly more prevalent in the impinged gizzard shad and freshwater drum compared to the reference fish during the spring/summer sample. During the winter/spring sampling period, only impinged threadfin shad were observed to have significantly higher lesion prevalence than the reference fish (Table 5).

Seasonal Parasite Prevalence - Plant Gorgas

During the three sampling periods of this study over eight genera of protozoan parasites, including *Apiosoma* spp., *Henneguya* spp., *Ichthyobodo necator, Ambiphyra* spp., *Trichodina* spp., *Trichophyra* spp., plus Monogenea and Digenea were observed in wet mount preparations of the gills. *Ichthyobodo necator* was observed to be significantly more common on the gills of impinged fish compared to the gills of the reference fish for gizzard shad during the fall sampling period (p=0.028), and freshwater drum during the spring/summer and winter/spring sampling periods (p=0.005 and p<0.0001, respectively); (Table 6). *Ichthyobodo necator* was the only external parasite observed to be significantly more common on the impinged fish compare to the reference fish.

Seasonal Bacterial Prevalence - Plant Gorgas

Throughout the duration of this study over seven different known bacterial fish pathogens were isolated from internal organs and lesions of both the impinged and reference fish at Plant Gorgas (Tables 7). It was determined that *A. hydrophila,* and *A. veronii biotype sobria* showed a difference in prevalence rate between impinged and reference fish (Tables 8 and 9). It was determined that fish with the presence of *F. columnare* on the gills without the presence of lesions, systemic infections and histological evaluations of the gills for microscopic lesions could not be conclusively classified as health-compromised fish. Therefore, only fish from which *F. columnare* was isolated from internal organs and/or lesions were considered diseased fish, and used in the statistical analyses (Table 10).

Impinged gizzard shad had a significantly higher prevalence of *A. hydrophila* throughout all seasons when compared to the reference fish (spring/summer *p*=0.019, fall *p*=0.007, winter/spring *p*=0.003; Table 8). A higher percent of impinged versus reference threadfin shad were also determined to be infected with *A. hydrophila* during the winter/spring sample season (*p*=0.002). During the fall sample season impinged gizzard shad were observed with higher rates of *A. veronii biotype sobria* compared to the reference fish (*p*=0.028; Table 9). Internal and lesion associated *F. columnare* was determined to be present in significantly higher rates in impinged gizzard shad throughout all seasons (spring/summer $p \leq 0.0001$, fall $p \leq 0.0001$, winter/spring $p \leq 0.0001$) and threadfin shad in the winter/spring ($p \leq 0.0001$) compared to reference fish (Table 10).

Prevalence of Disease - Plant Gorgas

Overall disease prevalence at Plant Gorgas was determined by using the previously discussed significant fish pathogens and the presence of necrotic lesions as disease indicators. These disease indicators include the presence of *Ichthyobodo necator*, *A. hydrophila*, *A. veronii biotype sobria*, internal and lesion associated *F. columnare,* and necrotic lesions. Disease prevalence at Plant Gorgas is illustrated in Table 11. Significant disease prevalence is seen in all three species. Impinged gizzard shad have a higher prevalence of disease compared to the reference fish in all three seasons (spring/summer 50.0%, *p*=0.019; fall 32.0%, *p*=0.007; winter/spring 87.5%, *p*=0.003). Impinged threadfin shad show a higher prevalence of disease during the winter/spring sampling (40.0%, *p*=0.002), while impinged freshwater drum show a higher prevalence of disease in both the winter/spring and spring/summer (100.0%, $p \leq 0.0001$ and 83.3%, $p = 0.005$, respectively).

Age Evaluation - Plant Gorgas

Freshwater drum otoliths collected from both impinged and reference fish for all three sample season showed no significant difference in age. This was true when seasons were grouped as well as considered individually (Table 12).

Prevalence of Disease Greene County

Impinged and reference threadfin shad and freshwater drum were collected from Plant Greene County during the fall of 2006 (Table 13). Gizzard shad were omitted from the analysis due to lack of sample size. Significant difference in length-weight analysis was determined for freshwater drum. The percent difference in predicted weights at equivalent lengths between the impinged and river freshwater drum collected was 3.9% (Figure 24). Length-weight analysis for threadfin shad was not significant (Figure 25). Several different bacterial and parasite pathogens were isolated from fish collected at Plant Greene County (Table 14). No parasitic pathogens were determined to be significant. The only bacterial pathogen determined to be significant was internal and lesion associated *F. columnare* between impinged and reference freshwater drum with a prevalence rate of 36.2% observed in the impinged compared to 0.0% observed in the reference fish ($p = 0.040$). No other bacterial pathogens were observed to be significant.

Prevalence of Disease – Plant EC Gaston

A total of 11 impinged and reference fish were collected from EC Gaston Steam Plant during the fall of 2006 (Table 14). Due to lack of sample size no significant correlations could be determined.

VI. DISCUSSION

Environmental factors such as temperature (Griffith and Tomljanovich 1975; Mclean et al. 1985) and dissolved oxygen (Bodensteiner and Lewis 1992) have previously been correlated with impingement. These factors may account for much of the variability reported in observed impingement rates. However, it has also been suggested that disease may also be a contributing factor to impingement rates (Sprengel and Luchtenberg 1991; Rodgers 1995; Rohlwing et al 1998; Baker 2007). The severity of a given disease is dependent on the interaction of numerous variables associated with the host, the pathogen, and the environment. Biotic factors such as, lack of food availability or starvation (Shoemaker et al. 2003) and stress (Fujihara and Olson 1962; Haley et al. 1967; Lom and Dykova 1992; Walters and Plumb 1980) have been shown to have adverse affects on the health of fish. Changes in abiotic factors such as, water temperature and dissolved oxygen (Pacha and Ordal 1970; Becker and Fujihara 1978; Austin and Austin 1999; Roberts 2001; Hurst 2007) may create stressful environments that can adversely affect fish health. Unfavorable stressful conditions can increase the susceptibility of a fish to infectious agents. To understand diseases and their impacts on fish populations and the relationship to impinged fish, it is important for this relationship to be examined (Hedrick 1998).

In this study, impinged fish collected at Plant Gorgas throughout three sampling seasons, were observed to have indications of a decrease in general health condition compared to the collected reference fish. Gizzard shad, threadfin shad, and freshwater drum exhibit a decrease in weight at equivalent lengths in the impinged population as compared to the reference populations for all three seasons. An increase in external lesions and bacterial pathogen prevalence in impinged fish was observed for all species sampled, as well as an increase in external parasite in impinged gizzard shad and freshwater drum.

The relationship between the length and weight of fish is one method that can be used to evaluate the general health status of fishes (Anderson and Neumann 1996). Length frequencies of impinged fishes were used to ensure reference fish of similar length were collected whenever possible. Variables evaluated in the length-weight regression models for impinged and reference fish included length and treatment. No significant difference in length frequencies was determined by species per treatment for any season. Length-weight regression models were observed independently for each sampling season due to seasonal differences such as reproductive status, metabolic demand, food availability, and age class of fish (LaJeone and Monzingo 2000). Predicted weights at equivalent lengths were significantly less (16.0% to 37.7%, depending the species, treatment, and season) for impinged gizzard shad and freshwater drum for all three sampling seasons at Plant Gorgas (Figures 6 - 14, and 21 - 23). Predicted weights at equivalent lengths were significantly less (fall = 21.6% and winter/spring = 11.6%) for impinged threadfin shad compared to intake hole reference threadfin shad at Plant Gorgas (Figures 19 and 20).

Similar observations of decreased weight at equivalent lengths in impinged fish have been made for freshwater drum (LaJeone and Monzingo 2000; Baker 2007), and alewives (Rodgers 1995).

The additional regression analysis between impinged gizzard shad and gizzard shad collected from the intake hole, as well as impinged gizzard shad and gizzard shad collected from the intake canal also showed significantly different predicted weights at equivalent lengths (Figures 12, 13, 14, 15, 16, and 17). The highest percent differences occurring between the impinged gizzard shad and gizzard shad collected from the intake canal during all sampling seasons (Figures 15, 16, and 17). These results could indicate that the man-made intake canal at Plant Gorgas is a highly dynamic part of the CWIS. Additional data, such as habitat and food availability, reproduction, and other factors are needed to further assess how these areas may affect impingement rates.

The significant decreases in weight at equivalent lengths of the impinged fish compared to the reference fish may also contribute to the increased likelihood of these smaller length class fish to become impinged. Correlations between the length of fish and their ability to maintain position in the water column at different water velocities have previously been made (Rulifson 1977). Bodensteiner and Lewis (1992) and Saalfeld (2007) also identified fish length as a factor affecting impingement rates. The trend of decreased weight at equivalent lengths of impinged versus reference fish in this study as well as Baker (2007) supports this hypothesis.

Gross necrotic lesions were observed in many of the impinged fish and all three species had a significantly higher prevalence compared to reference fish in at least one of the sampling seasons at Plant Gorgas (Table 5). Lesions of a mechanical nature were distinguished from gross necrotic lesions typical of bacterial infections (Pacha and Ordal 1970; Austin and Austin 1999). Observed gross necrotic lesions included eroded fins and fin bases, and erosion of entire caudal fin, to skin depigmentation, necrotic gill filaments, ulcerative areas, as well as hemorrhagic areas. These lesions, especially the erosion of fins, would affect the swimming ability of the fish, that could increase the likelihood of impingement. Most of the observed mechanical lesions would be presumed to be a result of contact with the intake screen and collection basket. These mechanical lesions leave open portals of entry for bacterial pathogens if and when they are returned to the river through a fish return system. However, it is very unlikely for the observed gross necrotic lesions to have developed in the short period of contact with the intake screen and collection basket. Due to the severity of necrosis of lesions and subsequent isolation of bacterial pathogens, it is most likely that the lesions were present before, and in turn could have contributed, to impingement. These findings are supported by Rohlwing et al. (1998) in that fish made contact with the intake screen due to parasitic infection rather than being infected by contact with the intake screen.

In this study, only one protozoan parasite, *Ichthyobodo necator*, was determined to be of significantly higher prevalence for impinged gizzard shad and freshwater drum relative to reference fish, depending on season at Plant Gorgas. This could indicate that the intake is selectively impinging infected, weaker fish, comparable to selective predation. *Ichthyobodo necator* is known to cause injury to the epithelial cells of the fish (Lom and Dykova 1992). Detection of *I. necator* was associated with the gills and skin of freshwater drum in the spring/summer and winter/spring and in gizzard shad in the fall (Table 6). These findings support similar results observed by Baker (2007). Other parasitic fish pathogens such as, *Ichthyophonus* sp., have been shown to significantly impair the swimming ability of infected fish (Tierney and Farrell 2004). Therefore, it is very likely that the gizzard shad and freshwater drum infected with *I. necator* would have compromised swimming ability and would be more likely to succumb to the intake velocity thus becoming impinged.

Flavobacterium columnare, *A. hydrophila*, and *A. veronii* biotype *sobria* were detected in significantly higher prevalence rates for impinged fish compared to reference fish, depending on season and species at Plant Gorgas (Tables 9, 10, and 11). Prevalence of *F. columnare* isolated from gross necrotic lesions typical of columnaris disease (Austin and Austin 1999; Pacha and Ordal 1970; Roberts 2001) and internal organs was significantly higher for gizzard shad during all sampling seasons and for threadfin shad during the spring sampling season at Plant Gorgas. The prevalence rate of *F. columnare* detected for impinged and reference fish in this study is similar to prevalence rates previously reported in wild fish populations (Fujihara et al. 1964; Becker and Fujihara 1978; Bowser 1973; Pacha and Ordal 1970). There is a slight discrepancy between the previous methodology and the methodology of the current study. Previous studies used a different agar media that could have resulted in failure to culture the bacteria even when present, where as, the current study used a Hsu-shott’s media supplemented with Tobramycin that enhanced the ability to isolate *F. columnare* (Bullock et al. 1986). Gill samples were routinely inoculated on selective media and routinely increased prevalence rates of *F. columnare* in all treatments. Finding in this study are similar to those of Fijan (1969).

Pathology of external columnaris disease primarily involves gill lesions, which include congestion of blood vessels, dissociation of the lamellar epithelium, and scattered hemorrhage (Hawke and Thune 1992). Prevalence of *F. columnare* was determined to be highly significant in impinged gizzard shad compared to the three reference treatments with the highest prevalence always being associated with impinged fish. The highest prevalence being detected in gizzard shad during the winter/spring sampling season and the second highest prevalence rate being associated with reference fish collected from the intake hole (Table 10). This could indicate that gizzard shad inhabiting the intake hole were more likely to be infected by *F. columnare* and in turn, more likely to become impinged when the severity of infection began to disrupt their normal behavior such as listlessness, lack of feeding, and decreased ability to uptake oxygen through the gills. Baker (2007) observed similar trends in prevalence rates in impinged compared to reference fish at Plant Barry. However, prevalence rates in this study may be higher due to the attempt to isolate *F. columnare* from more organs on a more routine basis.

Further histopathological investigation of the gills would be needed to evaluate typical primary pathology associated with *F. columnare* was present on the gill filaments without gross necrosis or isolation from internal organs to conclude that the pathogen was causing harm that could alter behavior and subsequently increase impingement rates. However, the isolation of *F. columnare* from gross necrotic lesions typical of columnaris disease (Austin and Austin 1999; Pacha and Ordal 1970; Roberts 2001) and internal organs are signs of a much more severe infection compared to isolation from the gills without any clinical signs. Severe infections resulting in columnaris disease, like those used to calculate prevalence rate of *F. columnare* in the current research, could alter behavior and subsequently increase impingement rates. Previous research has shown that delta smelt infected with *Mycobacterium* spp. swam significantly slower than uninfected fish (Swanson et al. 2002). A decrease in swimming speed due to a bacterial infection could result in higher impingement rates of infected, unhealthy fish.

Aeromonas hydrophila, the causative agent of motile aeromonas septicemia (MAS), has long been recognized as a pathogen in amphibians, reptiles, and fish. Studies have suggested that densities of *A. hyrophila* in natural water bodies may be an important contributing factor in fish (Hazen et al. 1978). *Aeromonas hydrophila* prevalence was significantly higher for gizzard shad during all three sampling seasons and threadfin shad during the spring compared with reference fish (Table 9). Hazen (1979) determined that *A. hydrophila* occurred in greatest densities in the water column during March and June with a second peak in October and that the mean monthly densities were positively correlated with water temperature and the incidence of infection in largemouth bass. The peaks in *A. hydrophila* densities described by Hazen (1979) similarly overlaps with the current sampling seasons and observed water temperatures in the present study (Figures 3, 4, and 5). Hazen (1979) concluded that the thermal effluent from a single nuclear production reactor significantly affected the ecology of *A. hydrophila* and the epizootiology of red-sore disease within the reservoir. It is unknown if the thermal discharge at Plant Gorgas has an affect on the ecology of *A. hydrophila* present in the water column.

Aeromonas veronii biotype *sobria* was the only other bacterial fish pathogen determined to be of significant prevalence rate in the impinged fish compared to reference fish at Plant Gorgas. Prevalence rates were found to be significant in impinged gizzard shad during the fall sampling season. Many of the impinged fish sampled in this study had both *Aeromonas* spp. infections coupled with external lesions and clinical signs of disease. *Aeromonas* spp. have previously been reported to be involved in gizzard shad (Toranzo et al. 1989) and threadfin shad mortality (Haley et al. 1967). It is highly likely that the fish exhibiting clinical signs of disease, bacterial or parasitic, become moribund and are unable to maintain their position in the water column (Swanson et al. 2002). However, most *Aeromonas* spp. can cause low level infections in fish with no clinical signs of disease until the fish encounters significant stress which will lead to a full blown infection with clinical signs of disease.

Moribund fish are a part of natural fish community. Diseased fish that are unable to maintain swimming ability will end up down stream of their point of origin according to the flow and velocity of the water until they die or are consumed by a predator. Within the CWIS of generating power plants, especially Plant Gorgas, the moribund or sick fish follow the flow of the intake canal downstream into the intake hole, and subsequently may become selectively impinged. Currently, it has not been determined if fish that end up in the intake hole can make passage back to the intake canal and subsequently the river. Future studies should investigate the movement of healthy, diseased, dead, and dying fish in the vicinity of a CWIS to determine if diseased fish are becoming selectively impinged due to lack of available passage back to the river.

In this study, a disease prevalence rate was created, based on length-weight analysis, presence of necrotic lesions, and prevalence of significant parasitic and bacterial pathogens comparable to the Bodensteiner and Lewis (1992). Disease prevalence rates were higher for impinged fish compared to the reference fish at Plant Gorgas for all seasons (Table 11). These observations indicate that the CWIS at least at Plant Gorgas is selectively impinging moribund, diseased and dying fish. Additional sample seasons with larger sample sizes need to be collected from the additional two plants in this study, Plant EC Gaston and Plant Greene County, to determine if this in a common trend among other generating power plants in Alabama. Similar tends may have become more evident if larger sample sizes were analyzed, especially since some similar tends were observed such as significantly higher prevalence of *F. columnare* observed in freshwater drum, as well as significant difference observed in the length-weight analysis at Plant Green County. It is also possible that due to methodological and field restraints of the current study certain pathogens, such as virus and internal parasites went undetected. Similar to the findings of Baker (2007), this study indicates that the rate of impingement at a CWIS may be a predictor of the general health of the local fish community. The intake screens may be selecting for and concentrating sick, dying, and dead fish that populate or are taken into the HZI. Continued impingement monitoring, as well as health evaluations of fish, is necessary to establish if this trend breaks geographic barriers within and outside the southeastern United States.

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Table 1. Total length and weight (mean ± sd; ranges in parentheses) of fish collected at and around Plant Gorgas during the spring/summer of 2006. Data include fish where only metrics were recorded. No significant difference ($p \leq 0.05$) in mean length between treatments was determined per species. Percent weight difference (Dif.) between the impinged fish and other treatments is reflected in the last column. If no number is present then no significant percent difference was observed.

Species Treatment	Total (N)	Total Length (mm)	Total (N)	Weight (g)	Dif. (%)
Gizzard Shad					
Impinged	130	308 ± 39 (134-400)	132	210 ± 82 (19-500)	
Intake Hole	98	296 ± 53 (117-421)	98	231 ± 103 (13-670)	16.0
River	100	259 ± 38 (155-336)	100	176 ± 65 (37-337)	27.2
Intake canal	50	315 ± 28 (253-375)	50	309 ± 98 (144-686)	29.2
Threadfin Shad					
Impinged	18	120 ± 20 (95-154)	19	15 ± 7 (7-25)	
Freshwater Drum					
Impinged	6	281 ± 126 (180-479)	6	325 ± 416 (44 - 1058)	
Intake Hole	2	296 ± 87 (234-357)	2	315 ± 269 (125 - 505)	
River	14	237 ± 45 (192-336)	14	176 ± 109 (72 - 427)	25.9
Intake canal	5	330 ± 88 (226-470)	5	575 ± 509 (147 -1456)	

Table 2. Total length and weight (mean ± sd; ranges in parentheses) of fish collected at and around Plant Gorgas during the fall of 2006. Data include fish where only metrics were recorded. No significant difference ($p \leq 0.05$) in mean length between treatments was determined per species. Percent weight difference (Dif.) between the impinged fish and other treatments is reflected in the last column. If no number is present then no significant percent difference was observed.

Species Treatment	Total (N)	Total Length (mm)	Total (N)	Weight (g)	Dif. (%)
Gizzard Shad					
Impinged	81	287 ± 58 (99-415)	82	173 ± 92 (7-412)	
Intake Hole	50	279 ± 45 (162-369)	50	196 ± 95 (36-463)	20.0
River	50	247 ± 46 (105-379)	50	162 ± 80 (11-505)	31.9
Intake canal	49	266 ± 57 (145-363)	50	215 ± 123 (26-582)	32.0
Threadfin Shad					
Impinged	141	95 ± 26 (59-171)	141	9 ± 8 (2-40)	
Intake Hole	22	146 ± 9 (130-165)	22	28 ± 4 (19-37)	21.6
River	43	82 ± 13 (55-110)	43	5 ± 2 (1-11)	
Intake canal	50	96 ± 19 (61-136)	50	8 ± 6 (1-24)	
Freshwater Drum					
Impinged	41	253 ± 64 (161-473)	41	186 ± 251 (28-1457)	
Intake Hole	2	456 ± 6 (451-460)	2	1202 ± 252 (1024-1381)	
River	8	276 ± 91 (173-421)	8	357 ± 413 (52-1175)	22.9

Table 3. Total length and weight (mean ± sd; ranges in parentheses) of fish collected at and around Plant Gorgas during the winter/spring of 2007. Data include fish where only metrics were recorded. No significant difference ($p \leq 0.05$) in mean length between treatments was determined per species. Percent weight difference (Dif.) between the impinged fish and other treatments is reflected in the last column. If no number is present then no significant percent difference was observed.

Species Treatment	Total (N)	Total Length (mm)	Total (N)	Weight (g)	Dif. (%)
Gizzard Shad					
Impinged	24	264 ± 86 (159-411)	24	171 ± 155 (28-675)	
Intake Hole	40	291 ± 49 (187-387)	40	248 ± 131 (54-576)	21.6
River	102	253 ± 48 (159-375)	102	174 ± 105 (35-548)	27.0
Intake canal	50	273 ± 64 (80-376)	50	271 ± 57 (4-657)	37.7
Threadfin Shad					
Impinged	38	118 ± 21 (79-153)	38	15 ± 7 (3-29)	
Intake Hole	50	134 ± 7 (118-150)	50	23 ± 4 (15-33)	11.6
River	50	131 ± 8 (119-152)	50	20 ± 4 (15-32)	
Intake canal	50	119 ± 19 (77-145)	50	16 ± 8 (3-27)	
Freshwater Drum					
Impinged	37	254 ± 77 (167-460)	37	193 ± 223 (39-1062)	
River	11	309 ± 82 (218-510)	11	290 ± 169 (114 - 628)	22.7

Table 4. Sample size (N) of impinged and reference fish collected and necropsied at and near Plant Gorgas.

Species	Season		
Treatment	Spring/Summer	Fall	Winter/Spring
Gizzard Shad			
Impinged	50	50	24
Intake Hole	50	50	40
River	50	50	50
Intake Canal	50	50	50
Threadfin Shad			
Impinged	18	50	30
Intake Hole	-	14	50
River	-	21	50
Intake Canal	-	50	50
Freshwater Drum			
Impinged	6	36	37
Intake Hole	2	2	-
River	14	8	11
Intake Canal	5	-	-

Table 5. Percentage of fish collected at and around Plant Gorgas that had necrotic lesions. Asterisk (*) next to the impinged value indicates a significantly ($p \leq 0.05$) higher prevalence compared to other treatments.

Species	Treatment			
Season	Impinged (%)	Intake Hole (%)	River (%)	Intake Canal (%)
Gizzard Shad				
Spring/Summer	18.0*	2.0	0.0	6.0
Fall	6.0	0.0	0.0	6.0
Winter/Spring	8.3	2.5	6.0	4.0
Threadfin Shad				
Spring/Summer	16.7	-	-	-
Fall	4.0	0.0	0.0	0.0
Winter/Spring	13.3*	0.0	0.0	2.2
Freshwater Drum				
Spring/Summer	33.3*	0.0	0.0	0.0
Fall	8.3	0.0	0.0	-
Winter/Spring	2.7	-	0.0	-

Table 6. Percentage of fish collected at and around Plant Gorgas that were infected with *Ichthyobodo necator*. Asterisk (*) next to the impinged value indicates a significantly ($p \leq 0.05$) higher prevalence of infection compared to other treatments.

Species	Treatment			
Season	Impinged (%)	Intake Hole (%)	River (%)	Intake Canal (%)
Gizzard Shad				
Spring/Summer	4.0	4.0	0.0	0.0
Fall	6.0*	0.0	0.0	0.0
Winter/Spring	25.0*	7.5	0.0	0.0
Threadfin Shad				
Spring/Summer	0.0	-	-	-
Fall	0.0	0.0	0.0	0.0
Winter/Spring	16.7	4.0	8.0	16.0
Freshwater Drum				
Spring/Summer	83.3*	0.0	0.0	0.0
Fall	36.1	0.0	0.0	-
Winter/Spring	97.3*	-	9.1	-

Table 7. Presence and absence of all detected bacterial fish pathogens across all seasons and treatments per species at Plant Gorgas. (Im = impinged, Ho = intake hole, Ca= intake canal, Ri = river)

Bacteria	Gizzard Shad				Threadfin Shad				Freshwater Drum			
Season	Im	Ho	Ca	Ri	Im	Ho	Ca	Ri	Im	Ho	Ca	Ri
F. columnare												
Spring/Summer	+	+	+	+	+				+	+	-	-
Fall	+	+	+	+	+	-	+	-	+	-		-
Winter/Spring	+	+	+	+	+	+	-	+	+			+
P. shigeloides												
Spring/Summer	-	-	+	-	-				-	-	-	-
Fall	-	-	-	-	+	-	-	-	-	-		-
Winter/Spring	-	-	-	-	+	-	-	-	-			-
Aeromonas spp.												
Spring/Summer	-	-	-	-	-				-	-	-	-
Fall	-	-	-	-	+	-	-	-	+	-		-
Winter/Spring	+	+	-	+	-	+	+	-	+			-
A. hydrophila												
Spring/Summer	+	-	+	-	-				-	-	-	-
Fall	+	-	-	-	+	-	-	-	+	-		-
Winter/Spring	+	+	-	+	+	+	-	-	-			-
A. ver. bt. sobria												
Spring/Summer	+	-	+	-	-				-	-	-	-
Fall	+	-	-	-	-	-	-	-	-	-		-
Winter/Spring	+	+	-	-	+	+	-	-	-			-
A. schubertii												
Spring/Summer	-	-	-	-	-				-	-	-	-
Fall	-	-	-	-	-	-	-	-	-	-		-
Winter/Spring	-	-	-	-	+	-	+	+	-			-
A. trotta												
Spring/Summer	-	-	-	-	-				-	-	-	-
Fall	-	-	-	-	+	-	-	-	-	-		-
Winter/Spring	-	+	-	+	+	-	-	-	-			-

Table 8. Percentage of fish collected at and around Plant Gorgas with *Aeromonas hydrophila* infections detected. Asterisk (*) next to the impinged value indicates a significantly ($p \leq 0.05$) higher prevalence of infection compared to other treatments.

Species	Treatment			
Season	Impinged (%)	Intake Hole (%)	River (%)	Intake Canal (%)
Gizzard Shad				
Spring/Summer	10.0*	0.0	0.0	4.0
Fall	8.0*	0.0	0.0	0.0
Winter/Spring	16.7*	2.5	2.0	0.0
Threadfin Shad				
Spring/Summer	0.0	-	-	-
Fall	2.0	0.0	0.0	0.0
Winter/Spring	10.0*	0.0	0.0	0.0
Freshwater Drum				
Spring/Summer	0.0	0.0	0.0	0.0
Fall	2.8	0.0	0.0	-
Winter/Spring	0.0	-	0.0	-

Table 9. Percentage of fish collected at and around Plant Gorgas with associated *Aeromonas veronii* biotype *sobria* infections. Asterisk (*) next to the impinged value indicates a significantly ($p \leq 0.05$) higher prevalence of infection compared to other treatments.

Species	Treatment			
Season	Impinged (%)	Intake Hole (%)	River (%)	Intake Canal (%)
Gizzard Shad				
Spring/Summer	2.2	0.0	0.0	4.0
Fall	6.0*	0.0	0.0	0.0
Winter/Spring	4.2	5.0	2.0	0.0
Threadfin Shad				
Spring/Summer	0.0	-	-	-
Fall	0.0	0.0	0.0	0.0
Winter/Spring	0.0	0.0	0.0	0.0
Freshwater Drum				
Spring/Summer	0.0	0.0	0.0	0.0
Fall	0.0	0.0	0.0	-
Winter/Spring	0.0	-	0.0	-

Table 10. Percentage of fish collected at and around Plant Gorgas with internal and necrotic lesion associated *Flavobacterium columare* infections. Asterisk (*) next to the impinged value indicates a significantly ($p \leq 0.05$) higher prevalence of infection compared to other treatments.

Species	Treatment			
Season	Impinged (%)	Intake Hole (%)	River (%)	Intake Canal (%)
Gizzard Shad				
Spring/Summer	44.0*	0.0	0.0	2.0
Fall	28.0*	0.0	2.0	2.0
Winter/Spring	87.5*	15.0	4.0	0.0
Threadfin Shad				
Spring/Summer	5.6	-	-	-
Fall	6.0	0.0	0.0	0.0
Winter/Spring	20.0*	0.0	2.0	0.0
Freshwater Drum				
Spring/Summer	16.7	0.0	0.0	0.0
Fall	2.8	0.0	0.0	-
Winter/Spring	18.9	-	0.0	-

Table 11. Percentage of disease prevalence in fish collected at and around Plant Gorgas. Asterisk (*) next to the impinged value indicates a significantly ($p \leq 0.05$) higher prevalence compared to other treatments.

Species	Treatment			
Season	Impinged (%)	Intake Hole (%)	River (%)	Intake Canal (%)
Gizzard Shad				
Spring/Summer	50.0*	4.0	0.0	10.0
Fall	32.0*	0.0	2.0	2.0
Winter/Spring	87.5*	25.0	8.0	0.0
Threadfin Shad				
Spring/Summer	5.6	-	-	-
Fall	8.0	0.0	0.0	0.0
Winter/Spring	40.0*	8.0	10.0	16.0
Freshwater Drum				
Spring/Summer	83.3*	0.0	0.0	0.0
Fall	38.9	0.0	0.0	-
Winter/Spring	100.0*	-	9.1	-

Table 12. Age (mean ± sd; ranges in parentheses) of freshwater drum collected at and around Plant Gorgas during the spring/summer of 2006, fall of 2006, and winter/spring of 2007. Data includes fish where otoliths were removed. No significant difference ($p \leq 0.05$) in mean length between treatments was determined per season by treatment.

	Impinged		River	
Season	Total (N)	Age	Total (N)	Age
Spring/Summer	6	4.8 ± 3.6 (1-10)	14	3.3 ± 2.5 (2 - 7)
Fall	36	5.5 ± 3.5 (1-17)	8	6.7 ± 5.4 (2-16)
Winter/Spring	37	4.9 ± 3.0 (1-11)	11	7.0 ± 4.7 (2-18)

Table 13. Total length and weight (mean ± sd; ranges in parentheses) of fish collected at and around Plant Greene County during the fall of 2006. Data include fish where only metrics were recorded. No significant difference (*p*≤0.05) in mean length between treatments was determined per species. Percent weight difference (Dif.) between the impinged fish and other treatments is reflected in the last column. If no number is present then no significant percent difference was observed.

Species Treatment	Total (N)	Total Length (mm)	Total (N)	Weight (g)	Dif. (%)
Threadfin Shad					
Impinged	80	73 ± 16 (39-113)	80	4 ± 3 (1 - 11)	
River	50	101 ± 13 (80-125)	50	10 ± 4 (4 - 17)	
Freshwater Drum					
Impinged	66	116 ± 52 (52-290)	58	26 ± 44 (2 - 196)	
River	8	202 ± 113 (92-472)	8	243 ± 565 (7 - 1641)	3.9

Table 14. Presence and absence of all detected bacterial fish pathogens across both treatments per species at Plant Greene County for the fall of 2006.

	Threadfin Shad		Freshwater Drum	
Bacteria	Impinged	River	Impinged	River
F. columnare	+	-	+*	-
Aeromonas sp.	-	-	+	-
A. hydrophila	-	-	+	-
A. ver. bt. sobria	+	-	-	-
A. jandei	-	-	+	-
A. trotta	-	-	+	-
Unidentified	-	-	+	-
Parasites				
Henneguya sp.	+	+	+	-
Trichodina sp.	-	-	+	-
Ichthyobodo necator	-	-	+	-
Digene sp.	-	-	+	-

*The only bacterial pathogen observed to be significant (*p*=0.040) between treatments at Greene County was *Flavobacterium columnare* with a prevalence of 36.2% detected in impinged freshwater drum compared to 0.0% detected in river freshwater drum.

Figure 1. Plant Gorgas intake structures and associated intake canal perpindicular to the Black Warrior River with sample collection areas indicated.

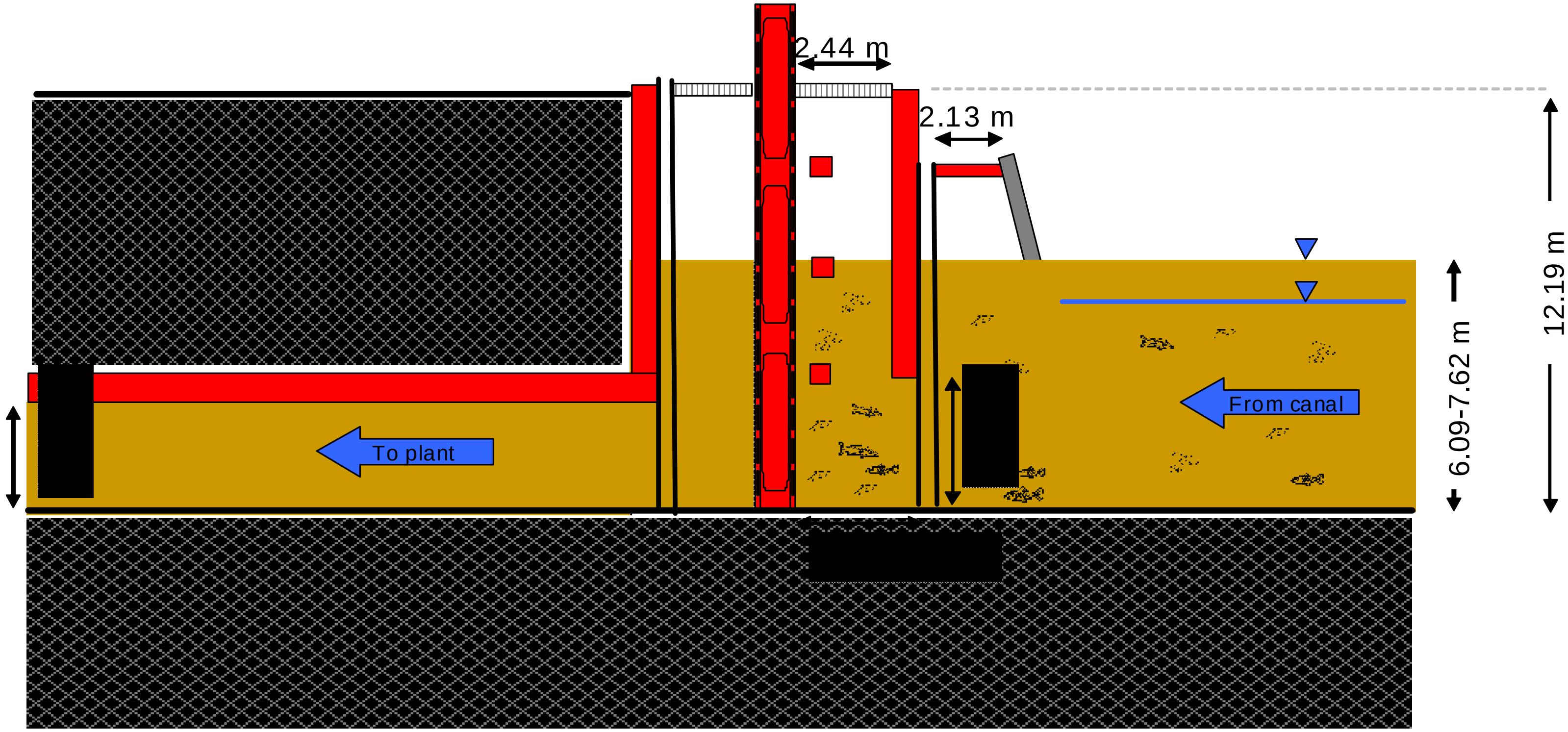

Figure 2. Cooling water intake system (CWIS) located at Plant Gorgas intakes with relevant dimensions presented.

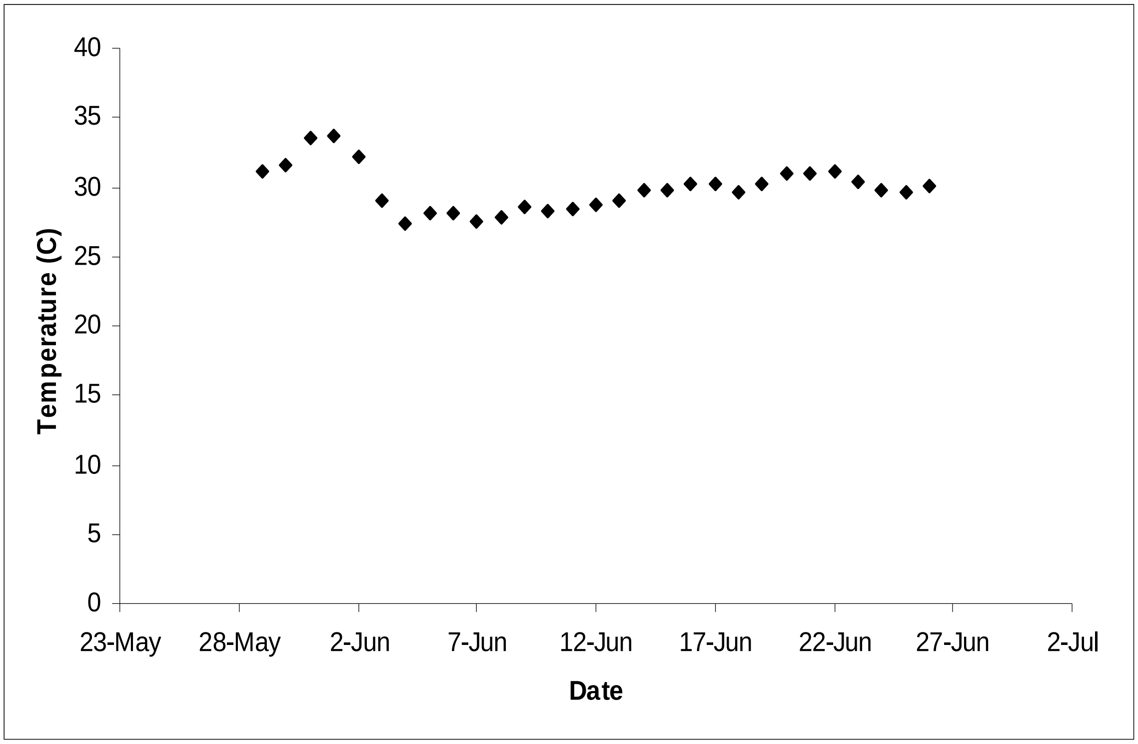

Figure 3. Average daily temperature in the intake canal at Plant Gorgas during the spring/summer 2006 sampling season.

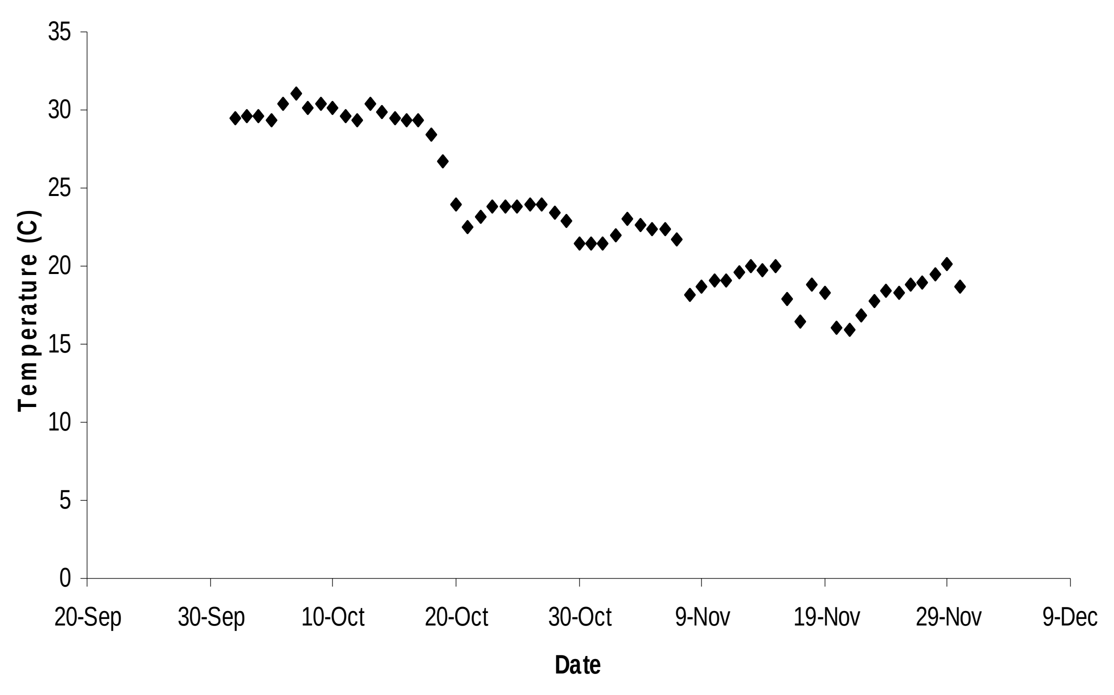

Figure 4. Average daily temperature in the intake canal at Plant Gorgas during the fall 2006 sampling season.

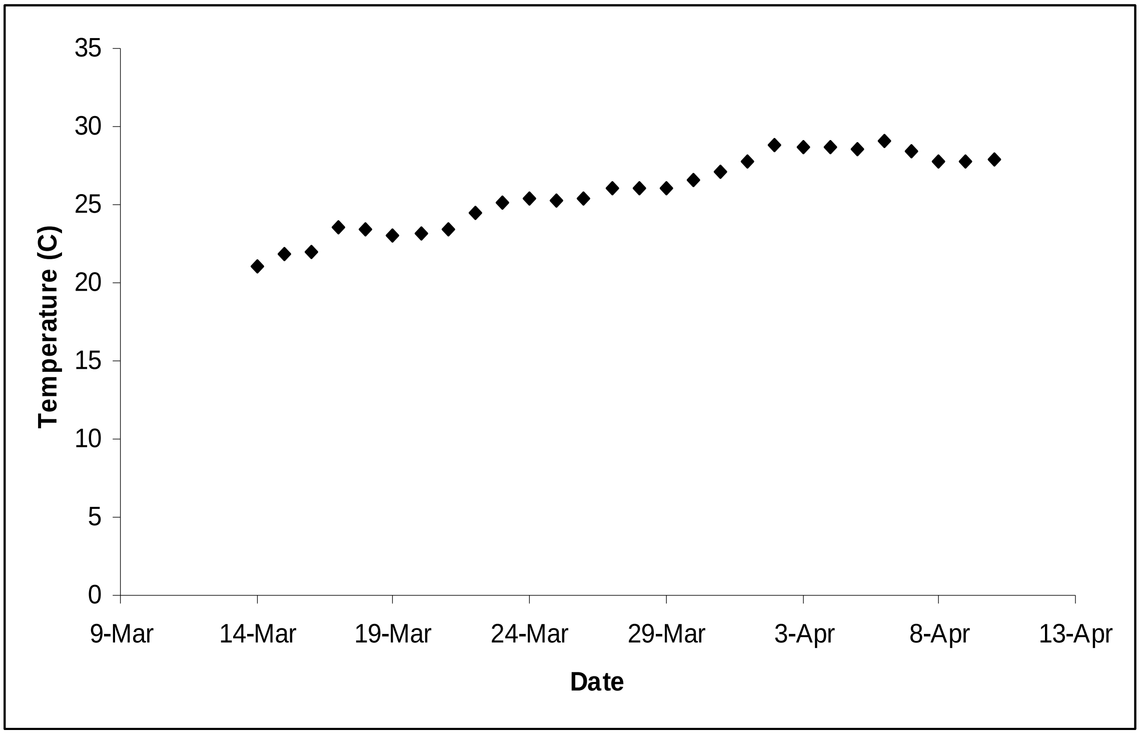

Figure 5. Average daily temperature in the intake canal at Plant Gorgas during the winter/spring 2007 sampling season.

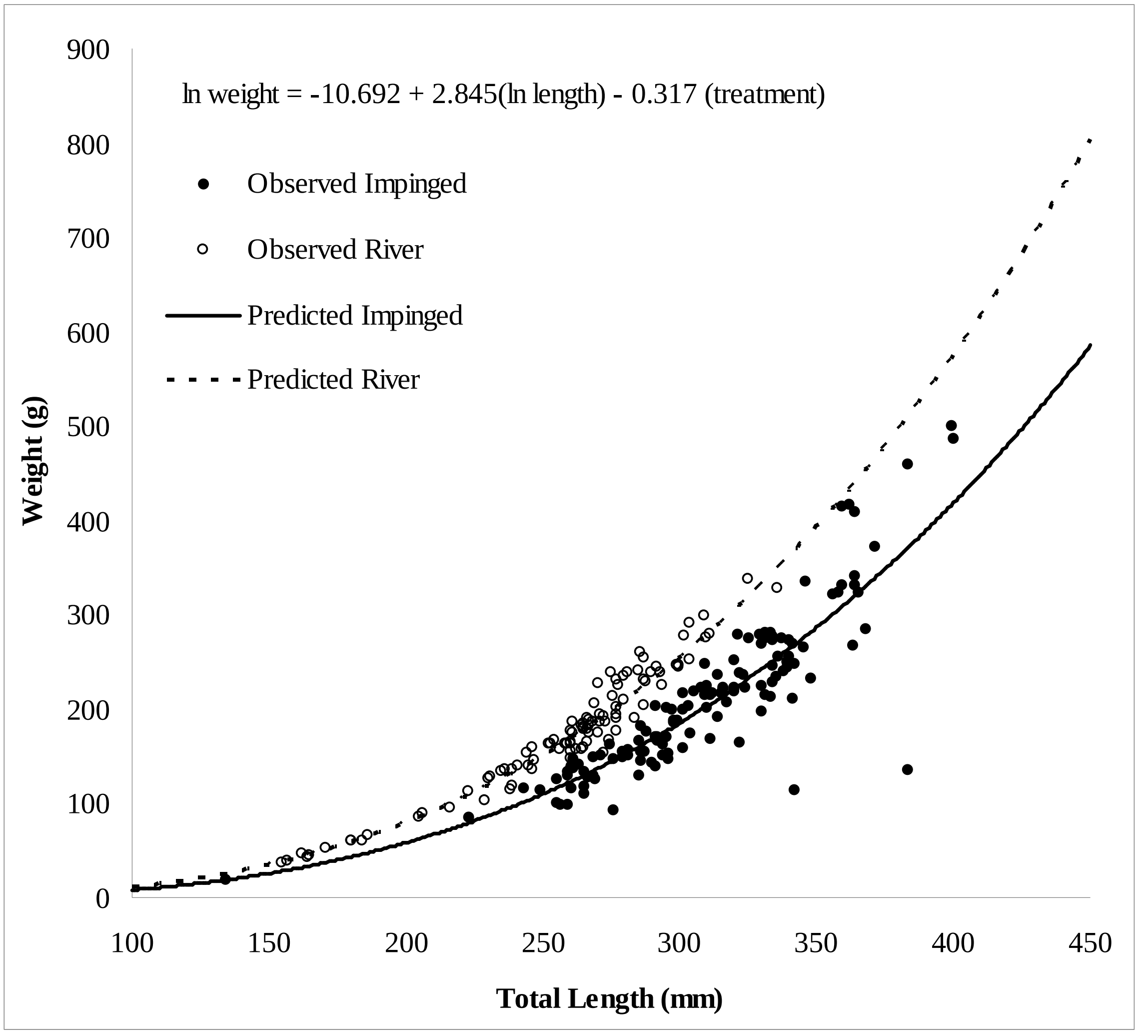

Figure 6. Predicted and observed weights for impinged and river reference gizzard shad collected at Plant Gorgas during the spring/summer of 2006. Predicted weights of impinged fish were significantly different from that of reference fish ($p \leq 0.0001$).

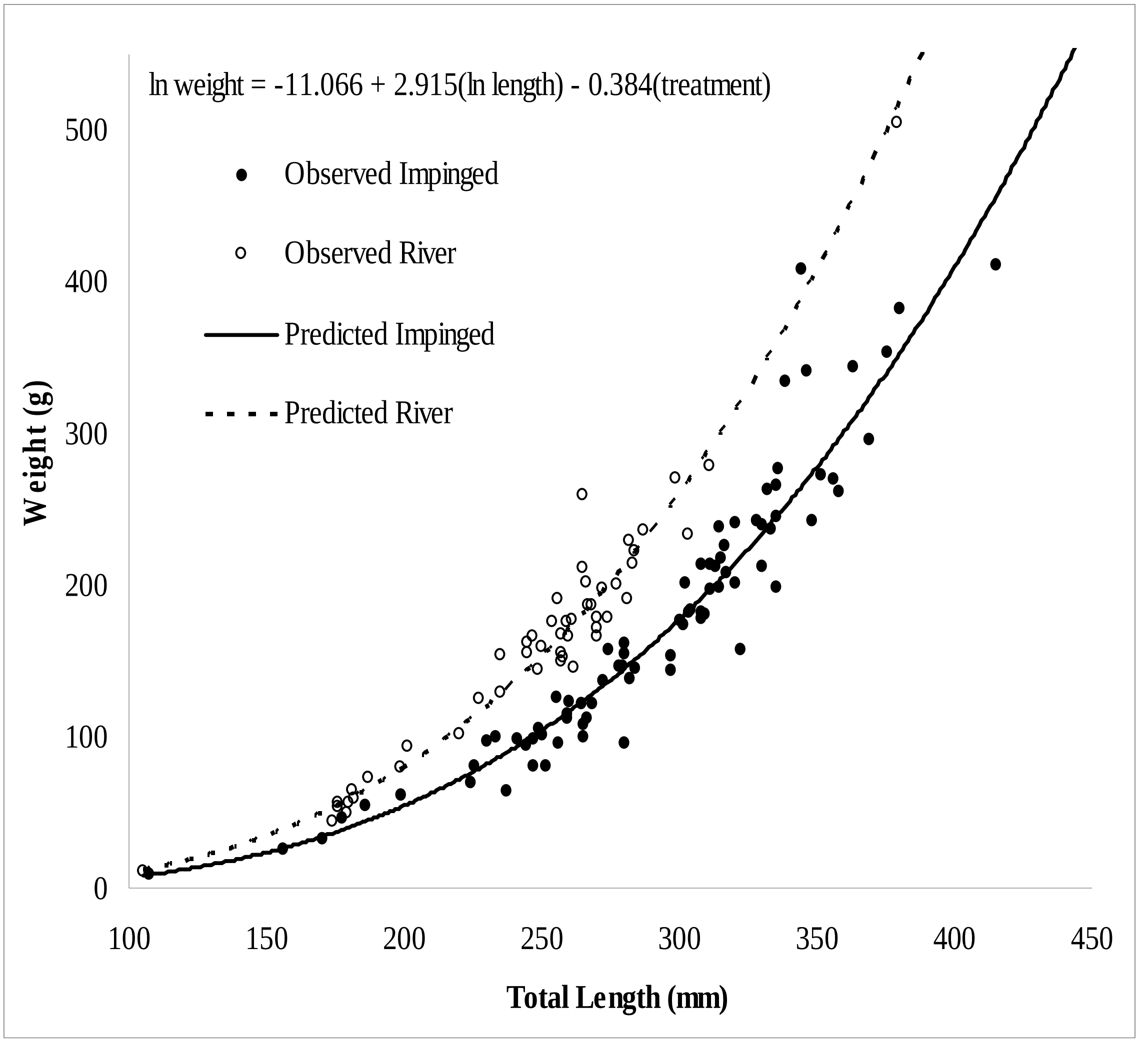

Figure 7. Predicted and observed weights for impinged and river reference gizzard shad collected at Plant Gorgas during the fall of 2006. Predicted weights of impinged fish were significantly different from that of reference fish (*p*≤0.0001).

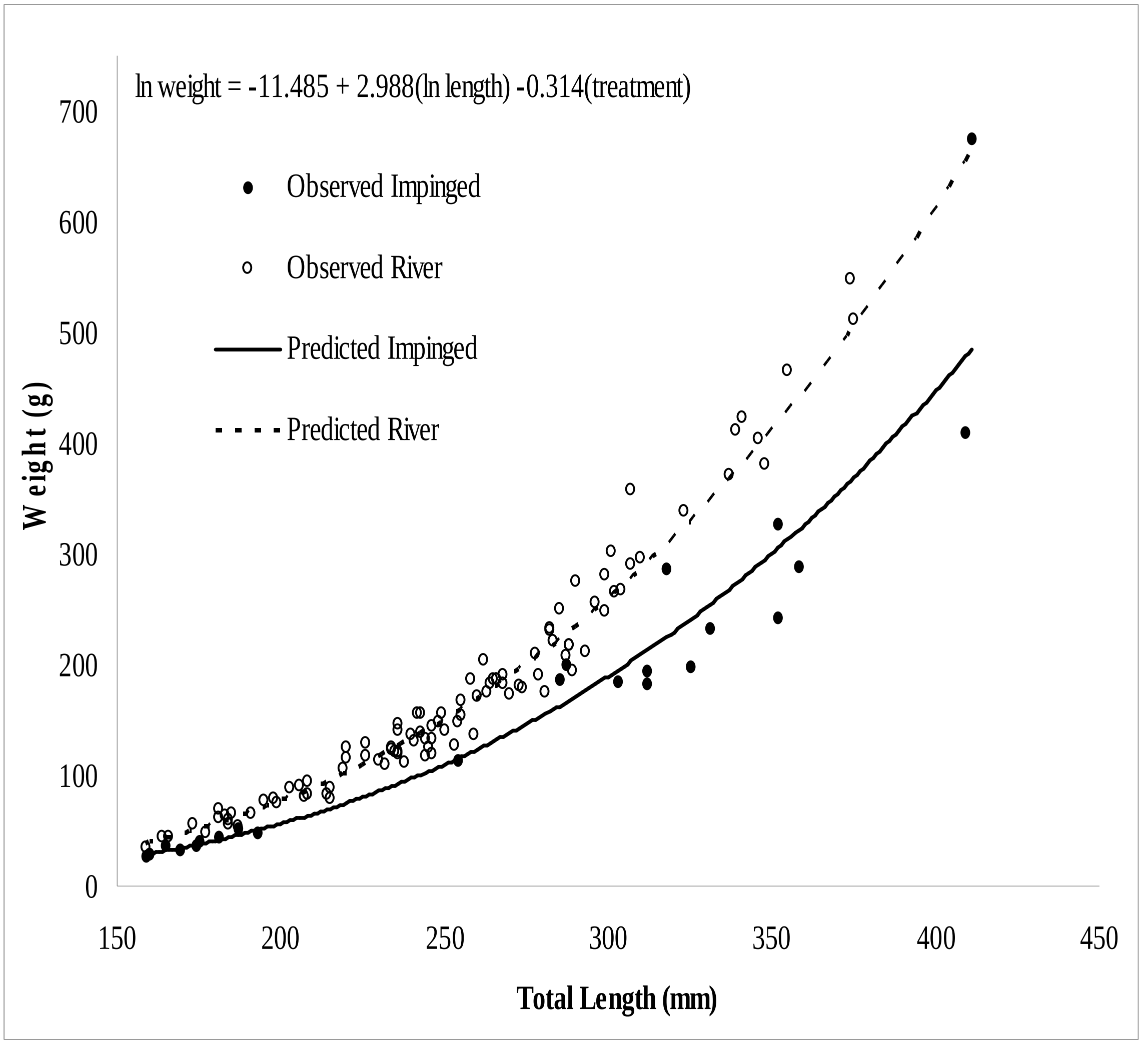

Figure 8. Predicted and observed weights for impinged and river reference gizzard shad collected at Plant Gorgas during the winter/spring of 2007. Predicted weights of impinged fish were significantly different from that of reference fish ($p \leq 0.0001$).

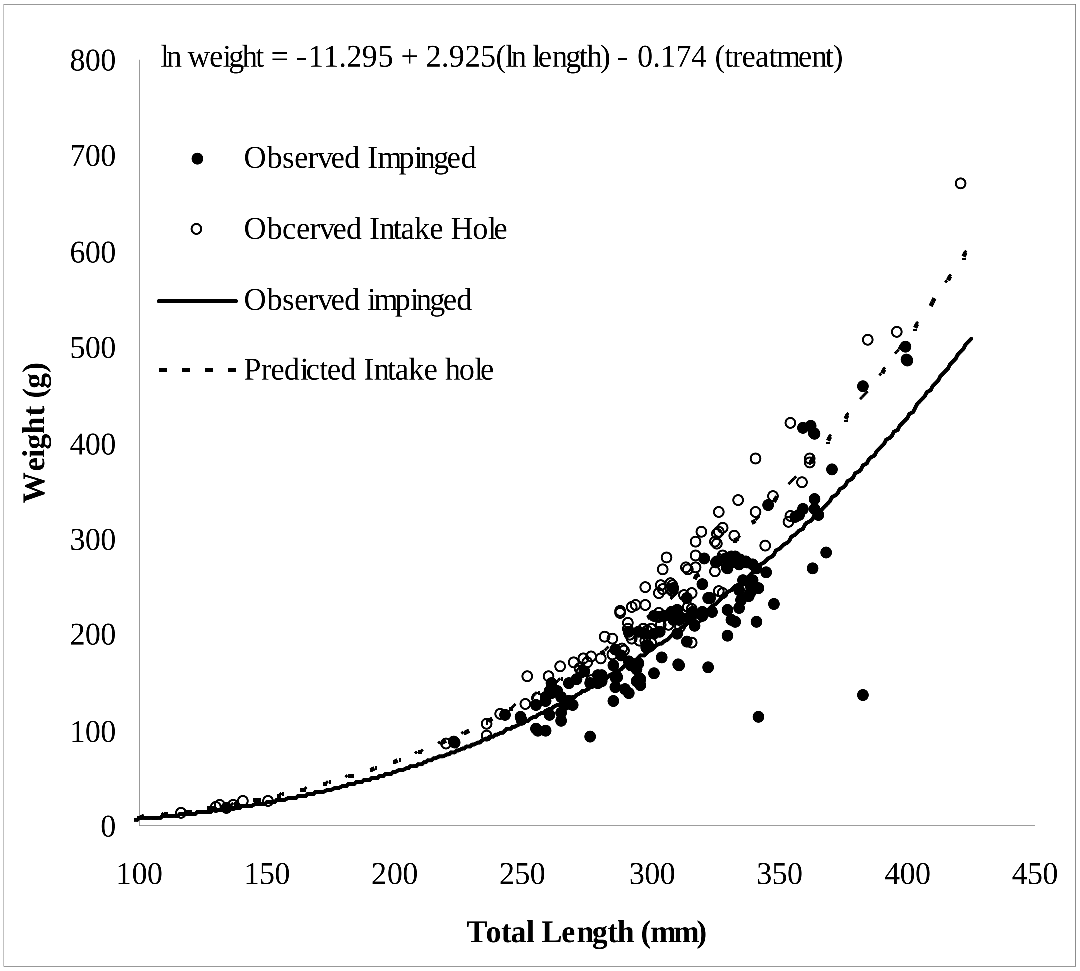

Figure 9. Predicted and observed weights for impinged and intake hole reference gizzard shad collected at Plant Gorgas during the spring/summer of 2006. Predicted weights of impinged fish were significantly different from that of reference fish ($p \leq 0.0001$).

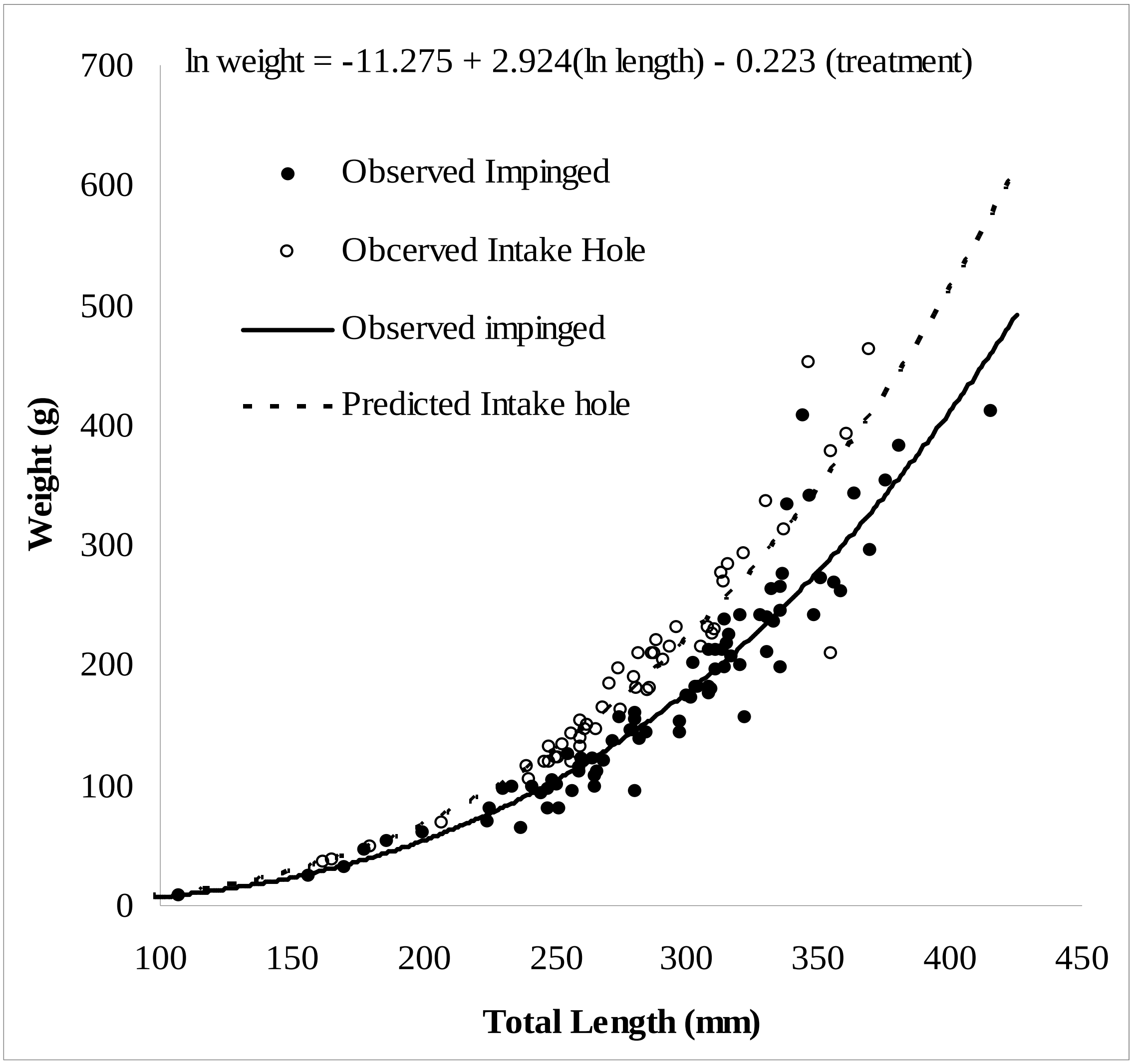

Figure 10. Predicted and observed weights for impinged and intake hole reference gizzard shad collected at Plant Gorgas during the fall of 2006. Predicted weights of impinged fish were significantly different from that of reference fish ($p \leq 0.0001$).

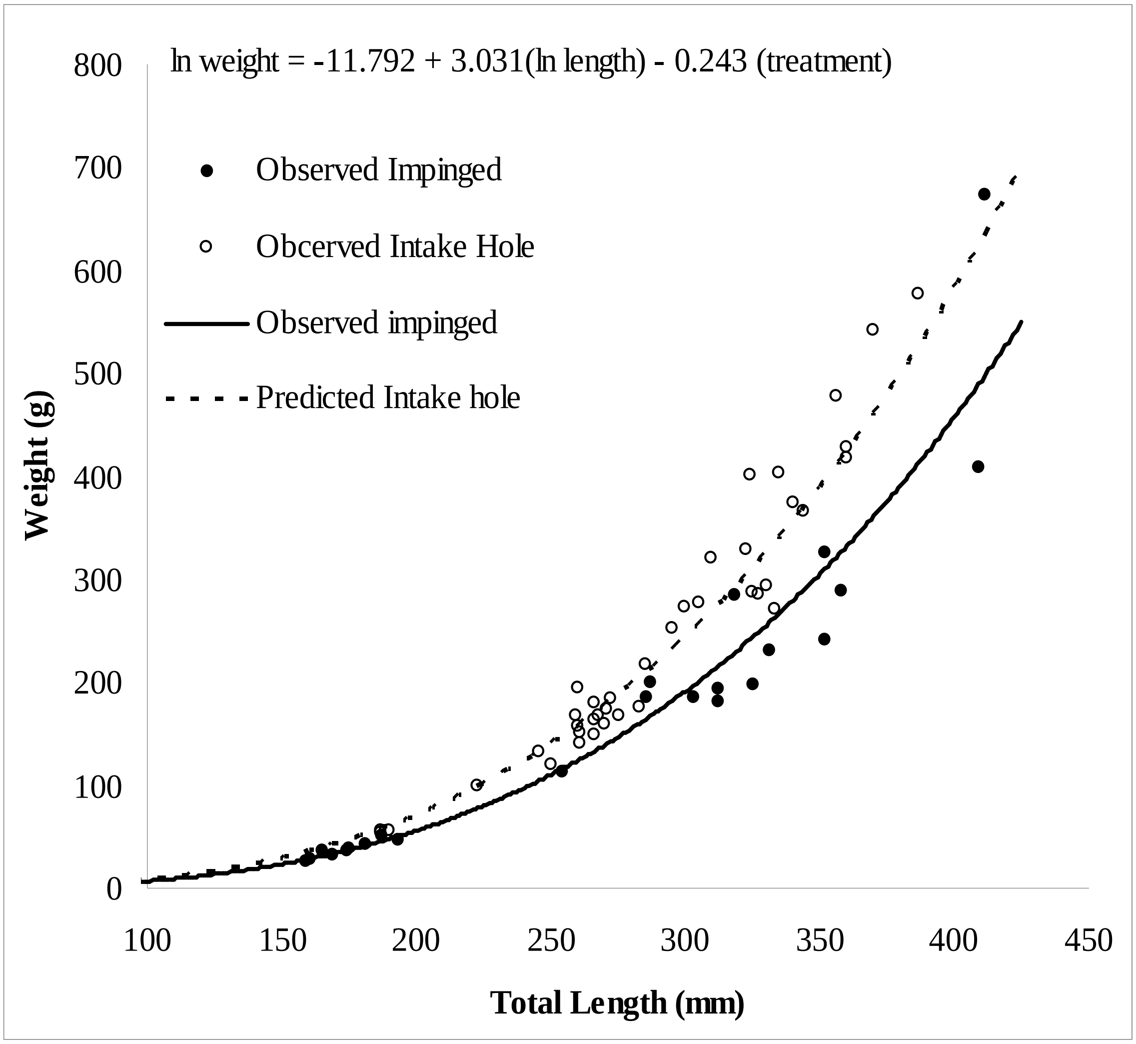

Figure 11. Predicted and observed weights for intake impinged and intake hole reference gizzard shad collected at Plant Gorgas during the winter/spring of 2007. Predicted weights of impinged fish were significantly different from that of reference fish ($p \leq 0.0001$).

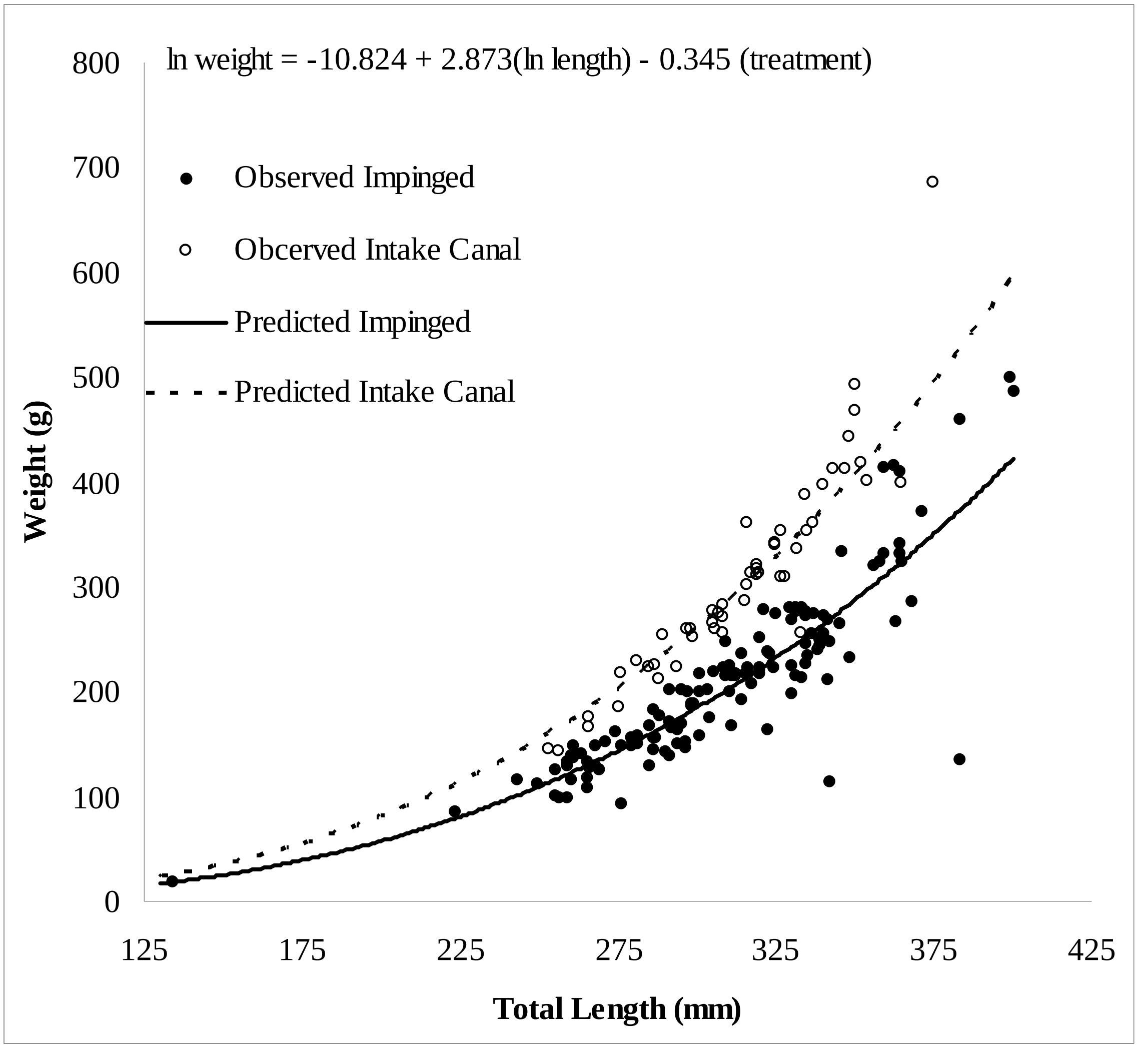

Figure 12. Predicted and observed weights for impinged and intake canal reference gizzard shad collected at Plant Gorgas during the spring/summer of 2006. Predicted weights of impinged fish were significantly different from that of reference fish ($p \leq 0.0001$).

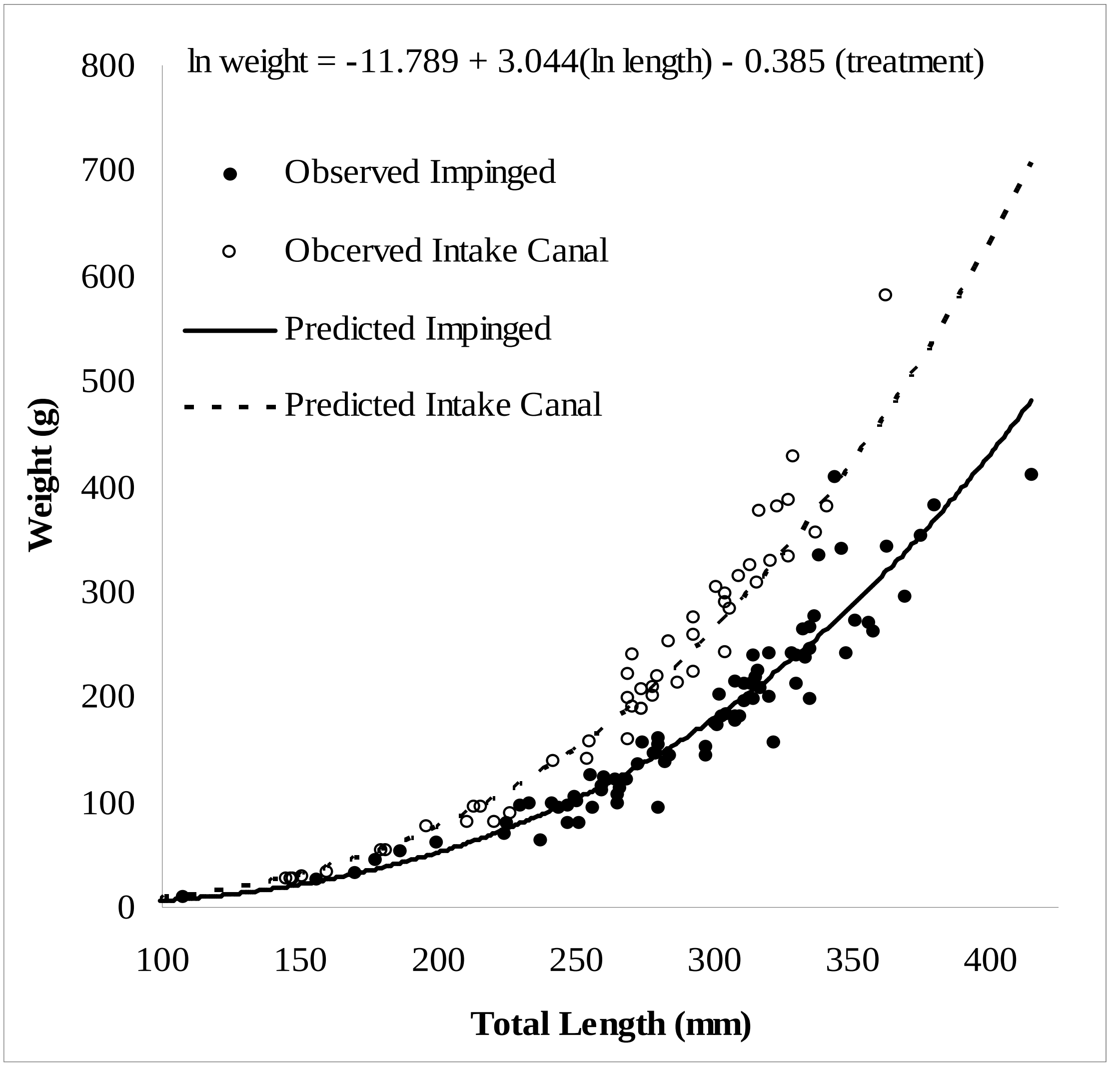

Figure 13. Predicted and observed weights for impinged and intake canal reference gizzard shad collected at Plant Gorgas during the fall of 2006. Predicted weights of impinged fish were significantly different from that of reference fish (*p*≤0.0001).

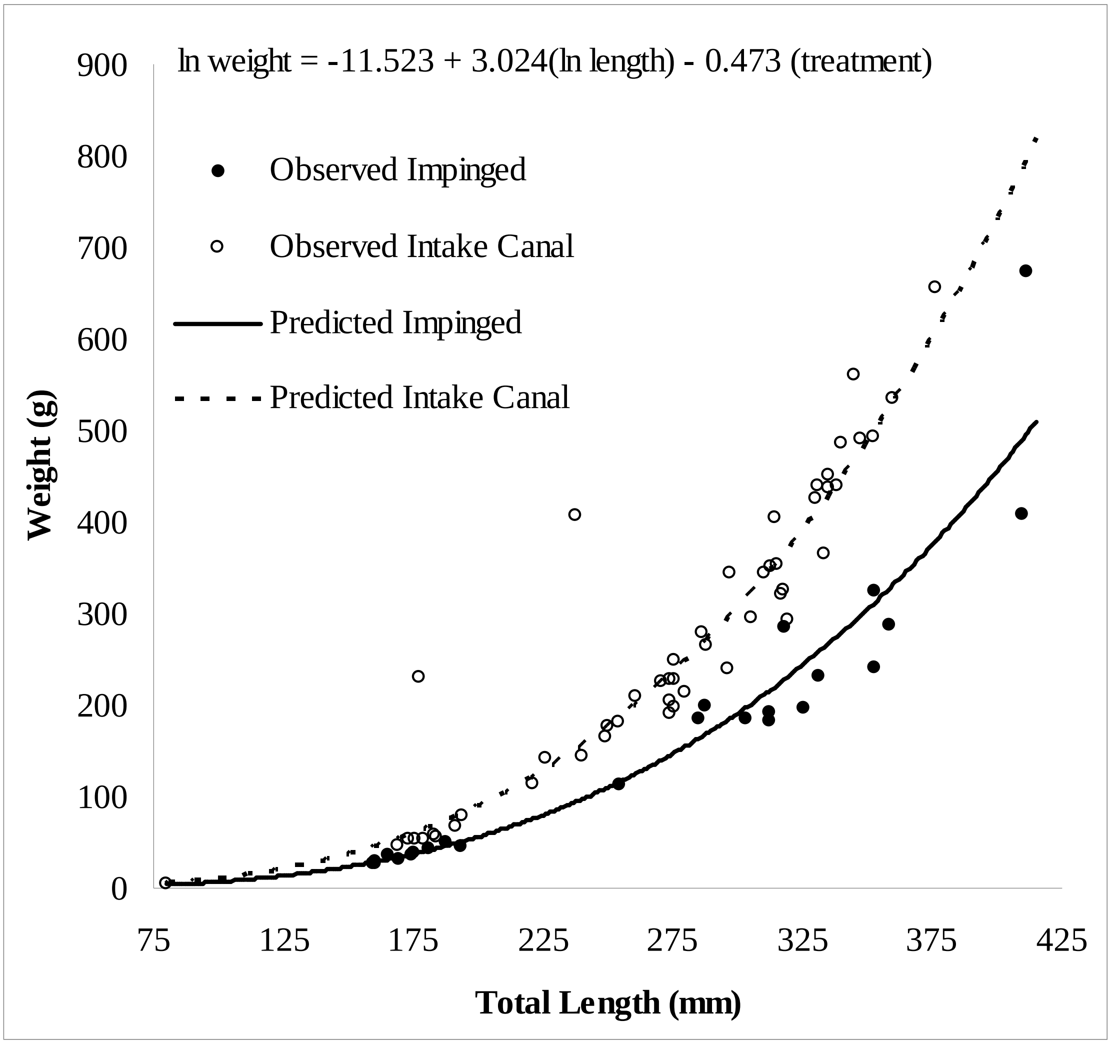

Figure 14. Predicted and observed weights for impinged and intake canal reference gizzard shad collected at Plant Gorgas during the winter/spring of 2007. Predicted weights of impinged fish were significantly different from that of reference fish ($p \leq 0.0001$).

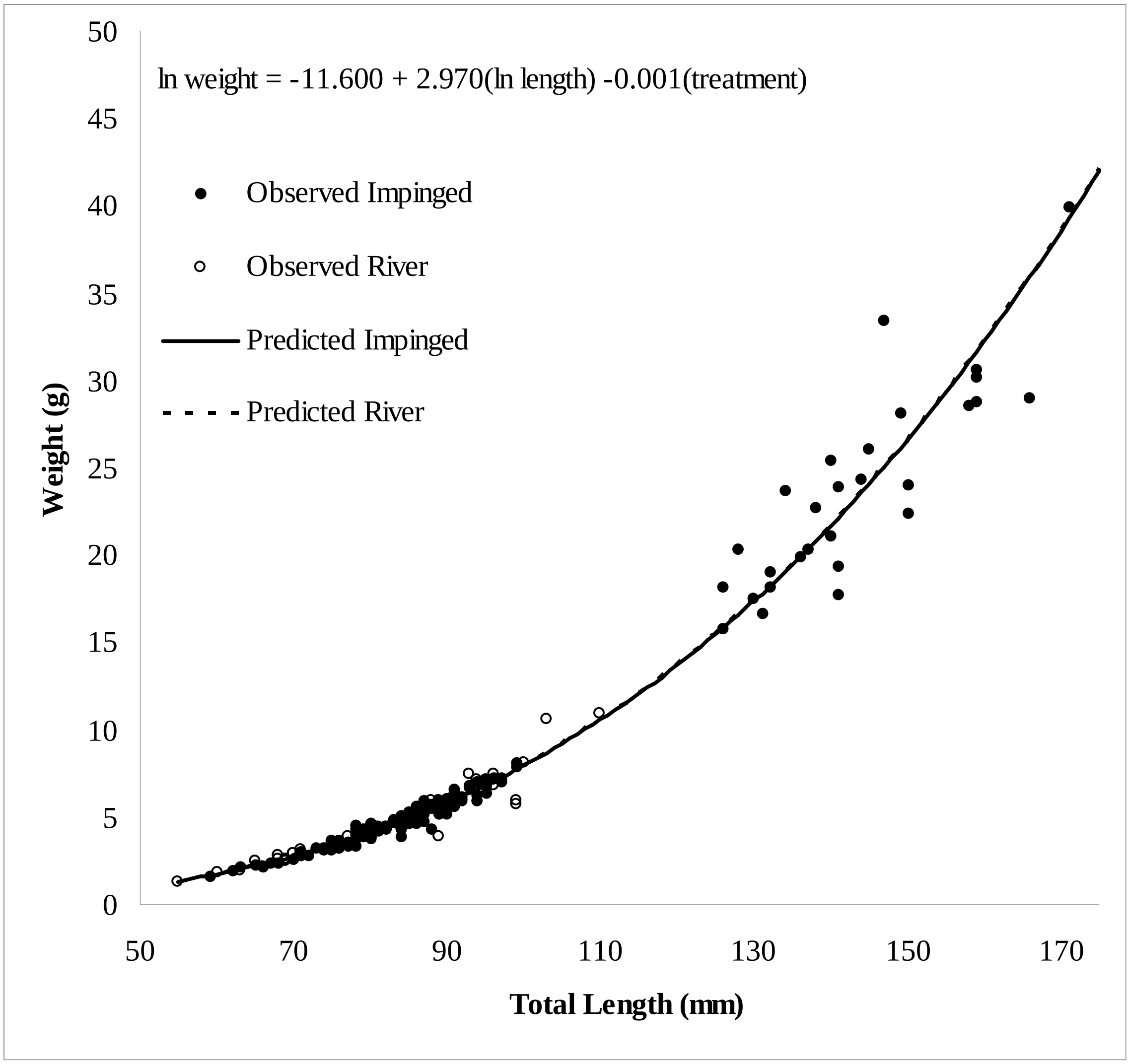

Figure 15. Predicted and observed weights for impinged and river reference threadfin shad collected at Plant Gorgas during the fall of 2006. Predicted weights of impinged fish were not significantly different from that of reference fish.

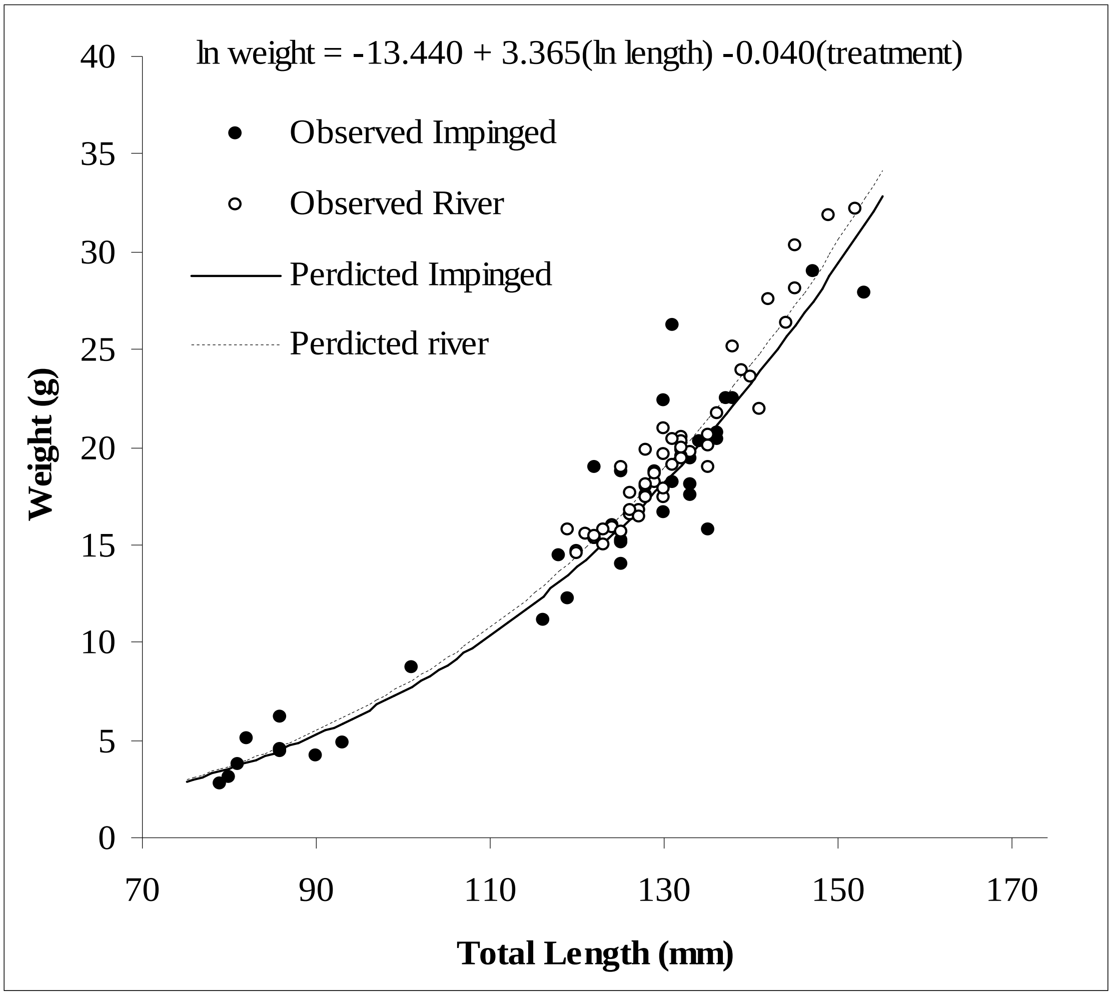

Figure 16. Predicted and observed weights for impinged and river reference threadfin shad collected at Plant Gorgas during the winter/spring of 2007. Predicted weights of impinged fish were not significantly different from that of reference fish.

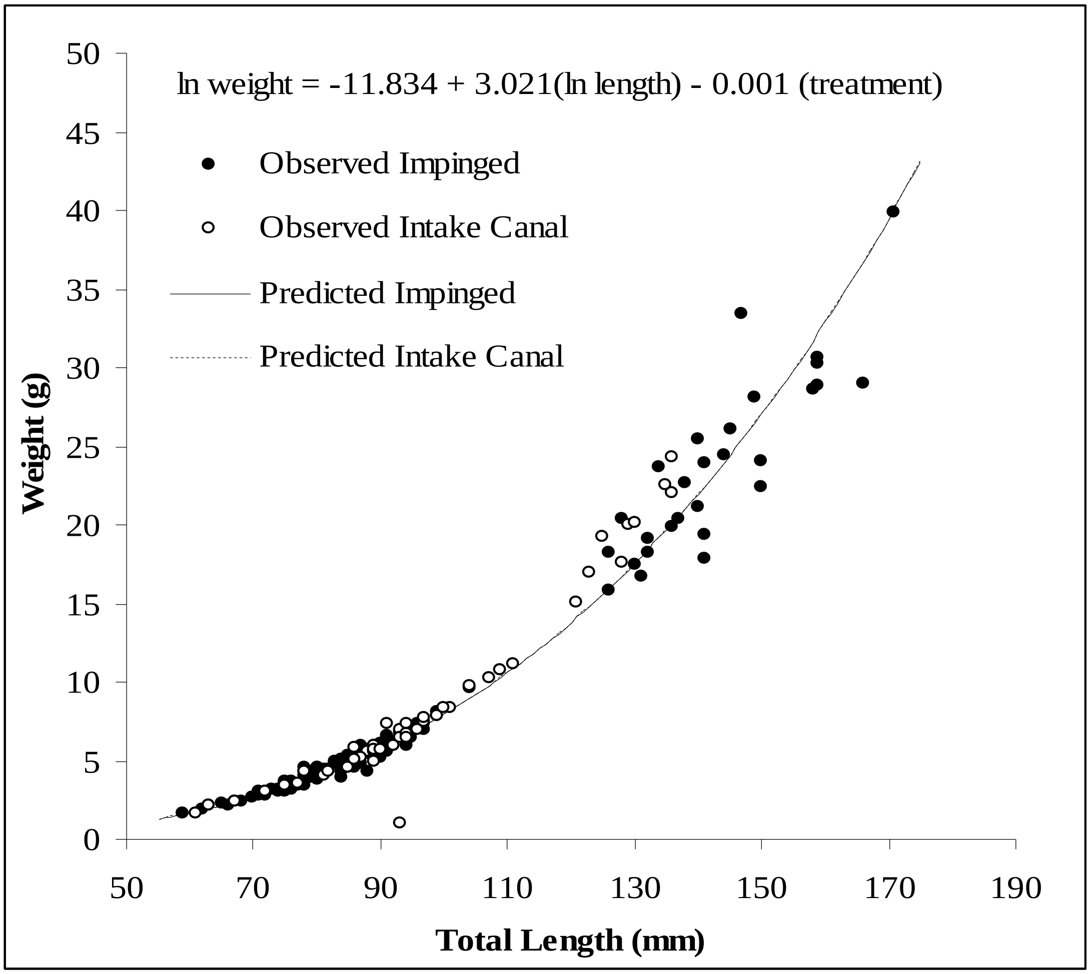

Figure 17. Predicted and observed weights for impinged and intake canal reference threadfin shad collected at Plant Gorgas during the fall of 2006. Predicted weights of impinged fish were not significantly different from that of reference fish.

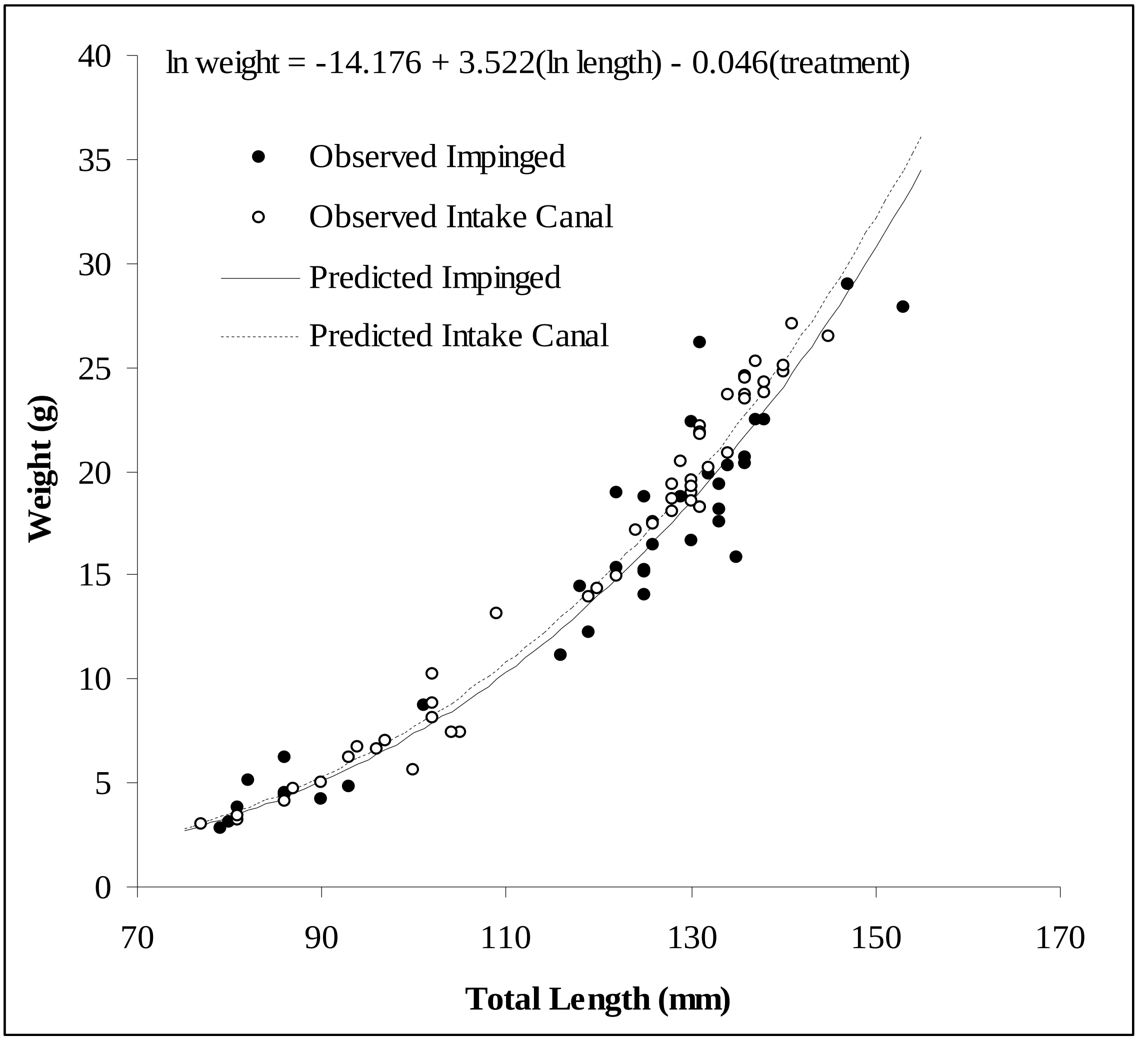

Figure 18. Predicted and observed weights for impinged and intake canal reference threadfin shad collected at Plant Gorgas during the winter/spring of 2007. Predicted weights of impinged fish were not significantly different from that of reference fish.

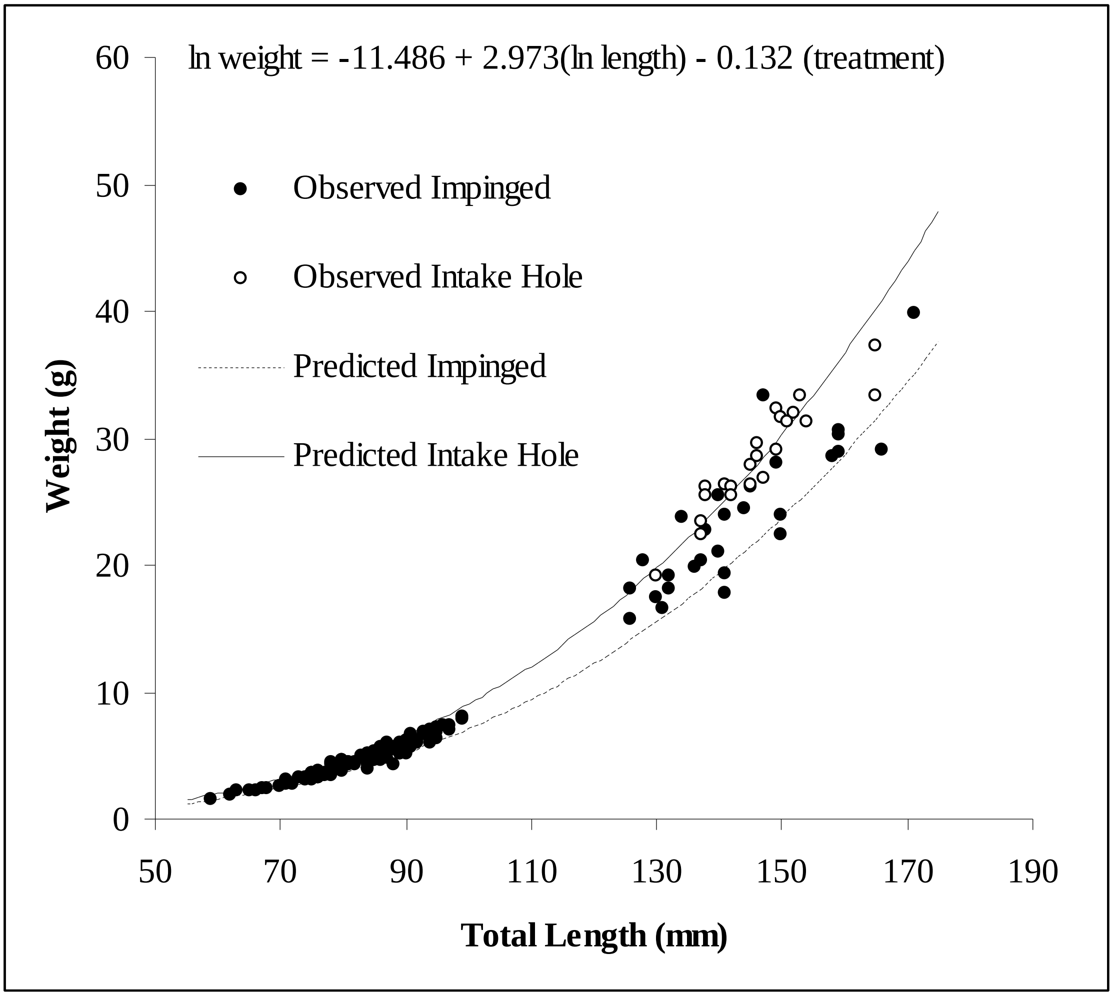

Figure 19. Predicted and observed weights for impinged and intake hole reference threadfin shad collected at Plant Gorgas during the fall of 2006. Predicted weights of impinged fish were significantly different from that of reference fish (*p*≤0.0001).

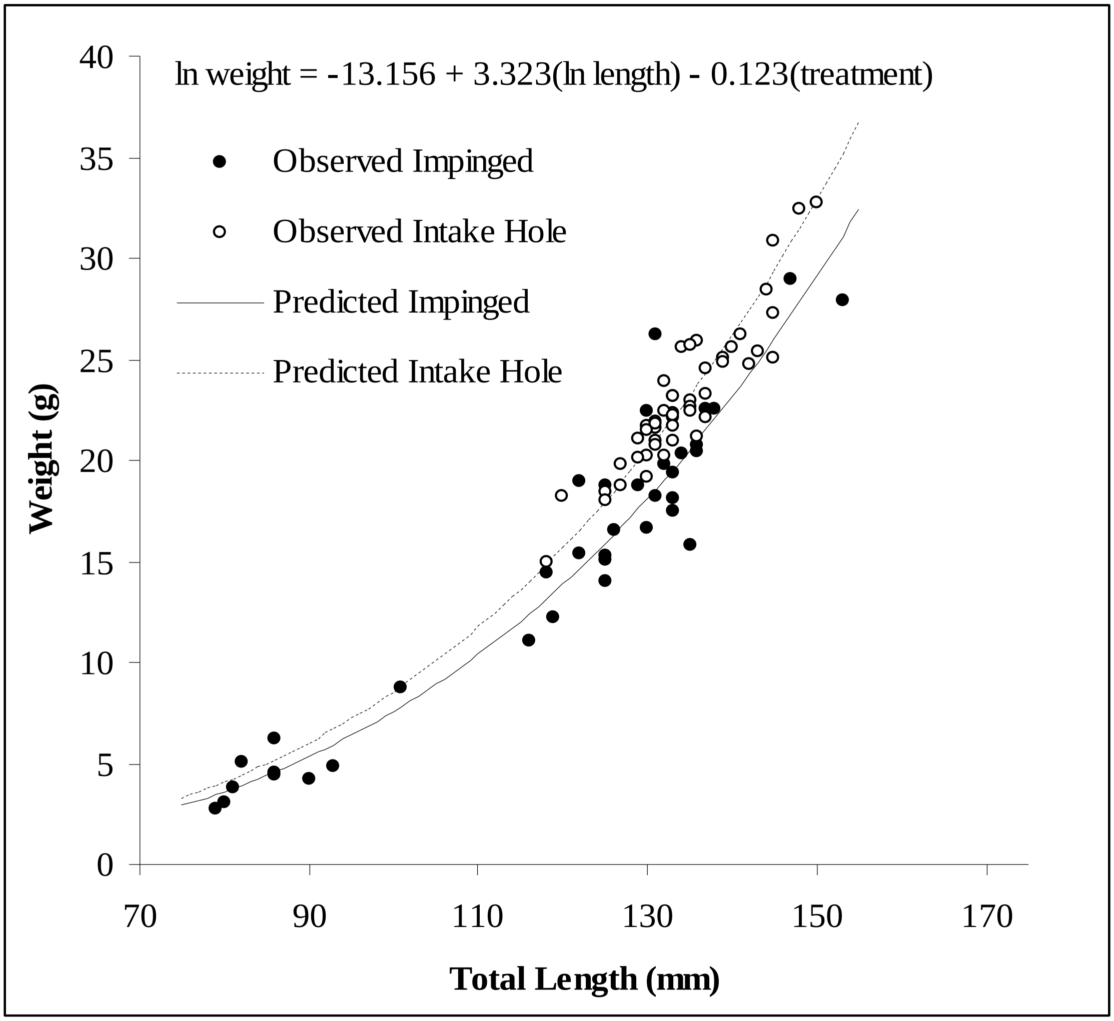

Figure 20. Predicted and observed weights for impinged and intake hole reference threadfin shad collected at Plant Gorgas during the winter/spring of 2007. Predicted weights of impinged fish were significantly different from that of reference fish ($p \leq 0.0001$).

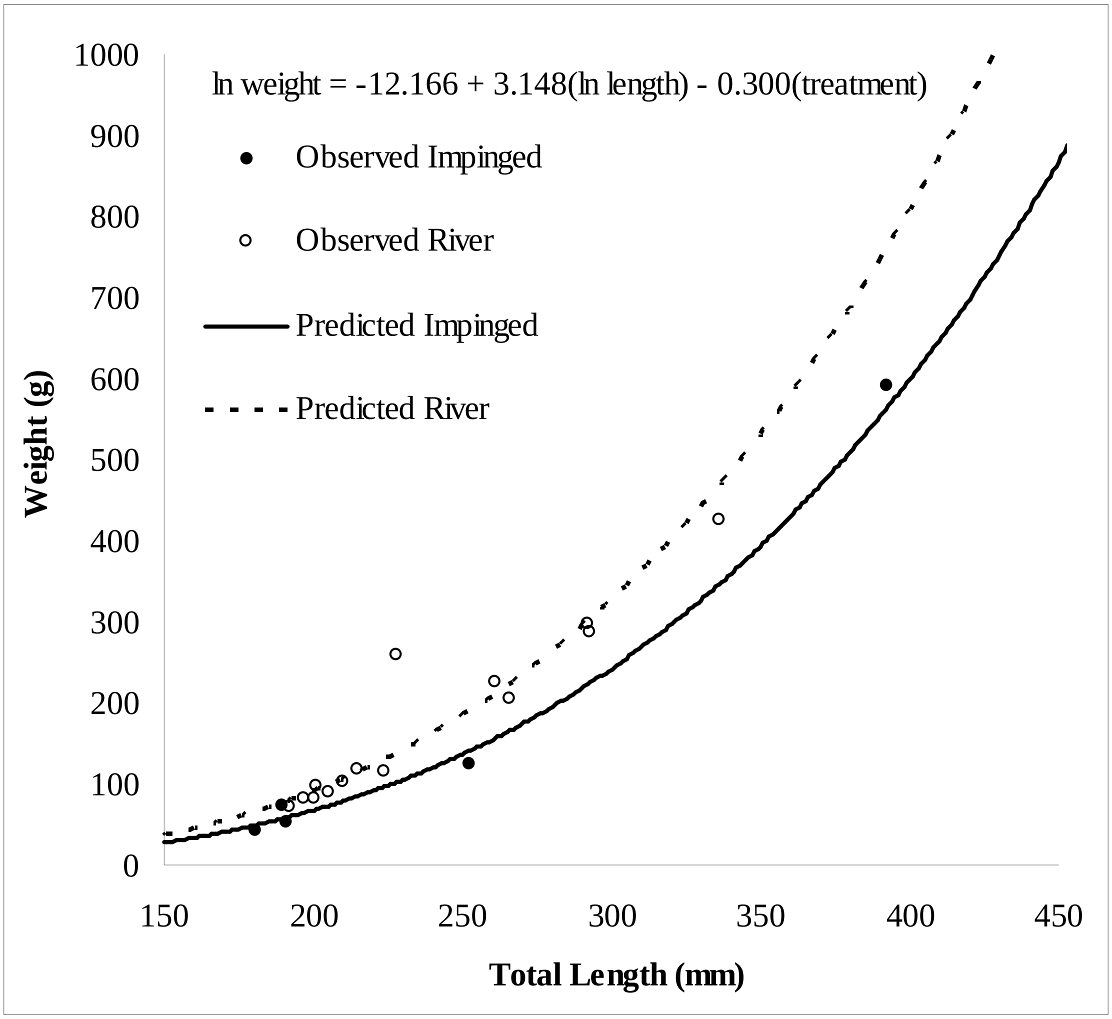

Figure 21. Predicted and observed weights for impinged and river reference freshwater drum collected at Plant Gorgas during the spring/summer of 2006. Predicted weights of impinged fish were significantly different from that of reference fish (*p*≤0.0001).

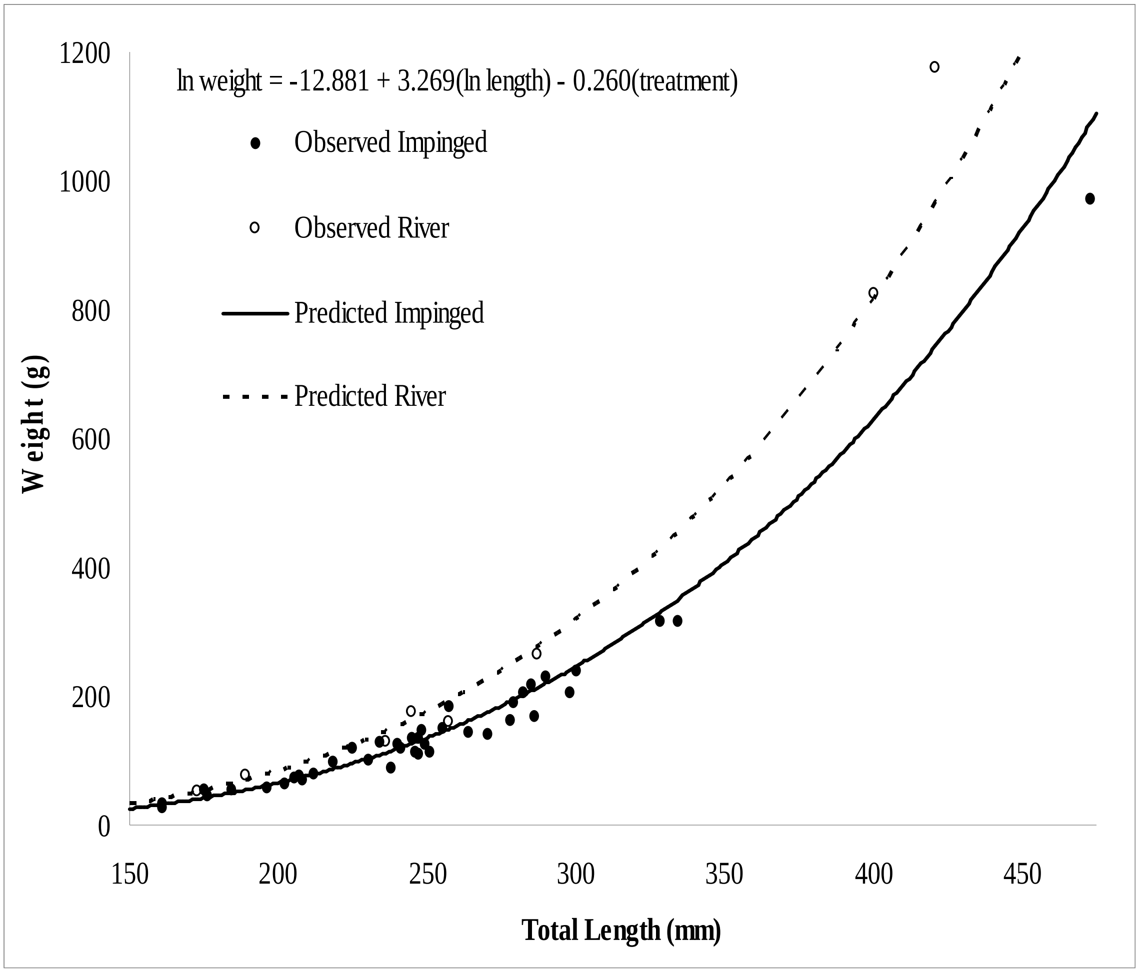

Figure 22. Predicted and observed weights for impinged and river reference freshwater drum collected at Plant Gorgas during the fall of 2006. Predicted weights of impinged fish were significantly different from that of reference fish ($p \leq 0.0001$).

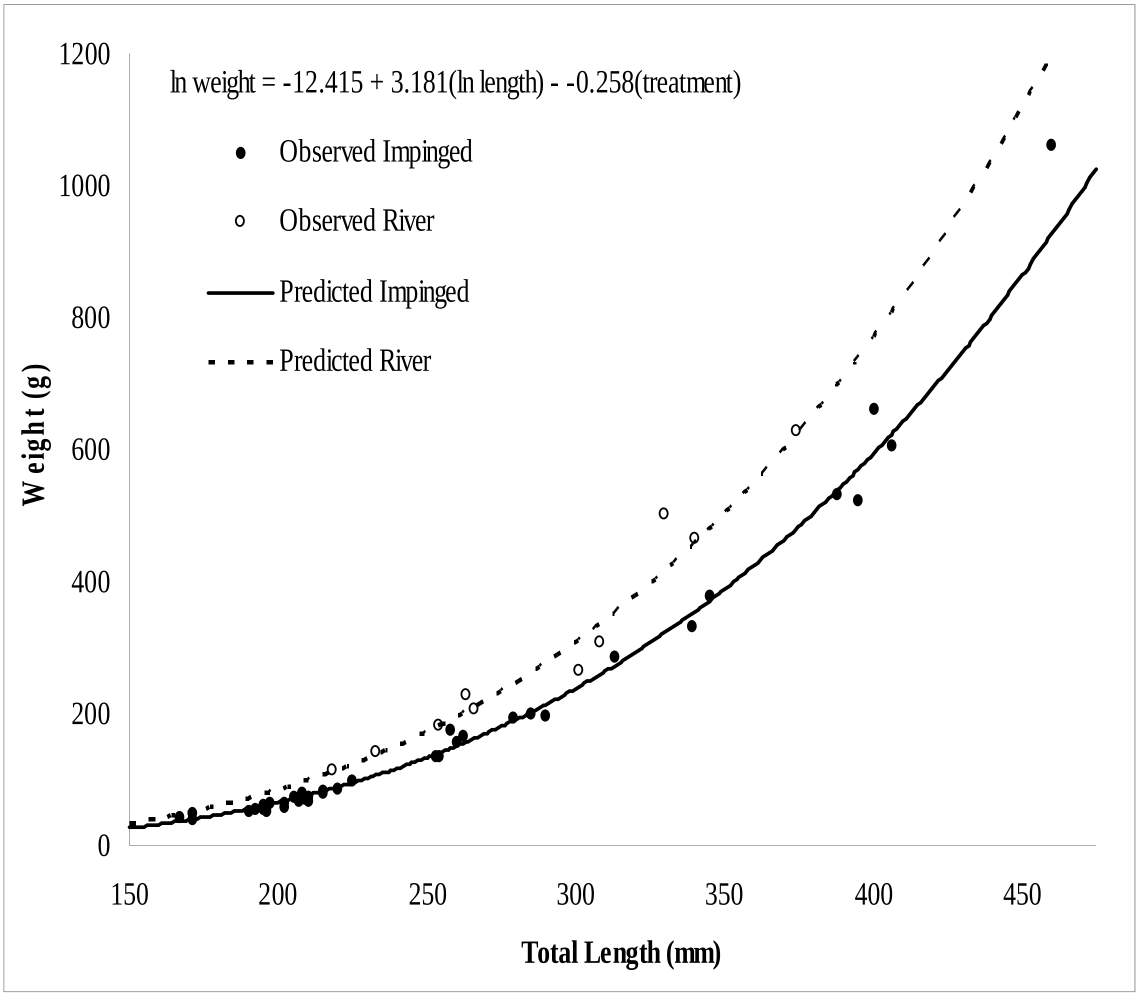

Figure 23. Predicted and observed weights for impinged and river reference freshwater drum collected at Plant Gorgas during the winter/spring of 2007. Predicted weights of impinged fish were significantly different from that of reference fish ($p \leq 0.0001$).

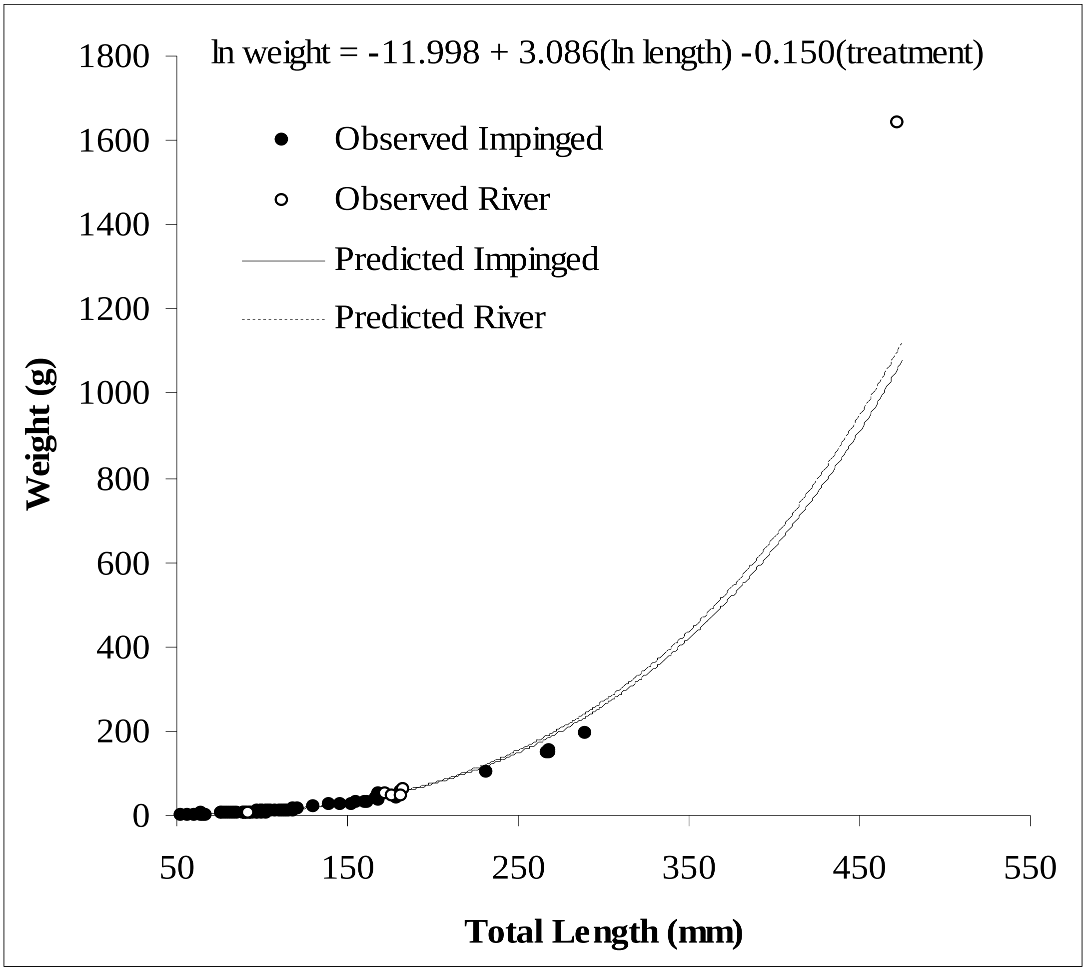

Figure 24. Predicted and observed weights for impinged and river reference freshwater drum collected at Plant Greene County during the fall of 2006. Predicted weights of impinged fish were significantly different from that of reference fish ($p \leq 0.0001$).

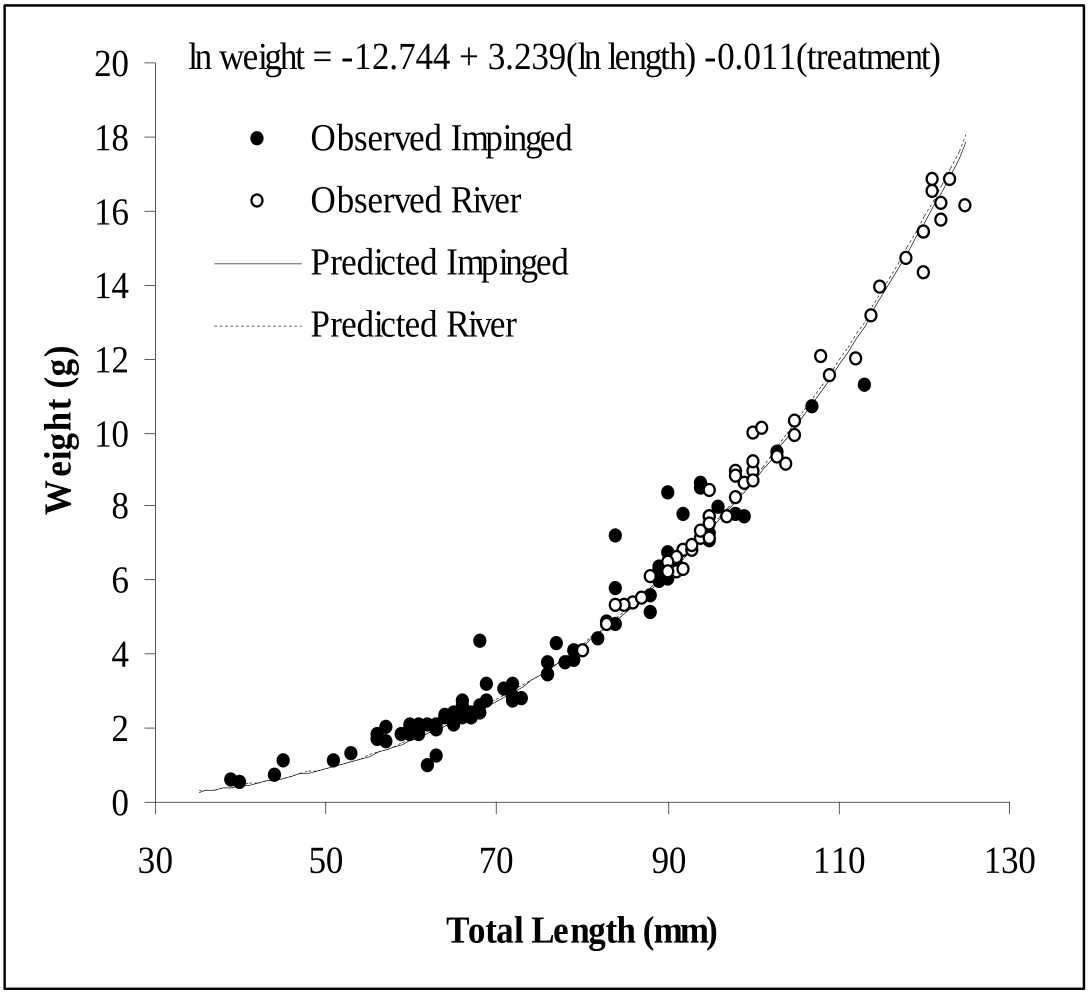

Figure 25. Predicted and observed weights for impinged and river reference threadfin shad collected at Plant Greene County during the fall of 2006. Predicted weights of impinged fish were not significantly different from that of reference fish.

APPENDIX

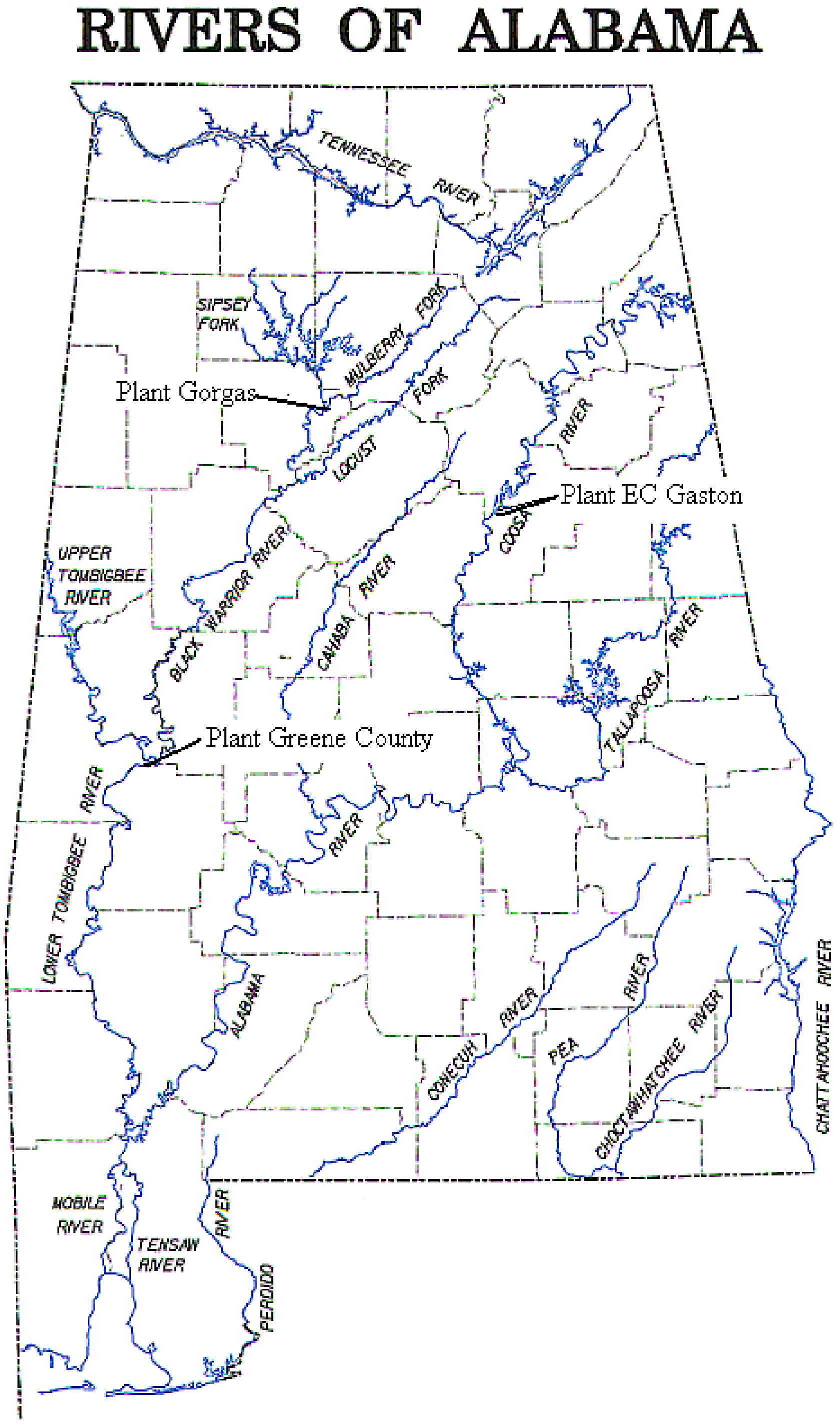

Figure A.1. Map of the rivers of Alabama with study sites indicated.