HEALTH OF FISH IMPINGED ON COOLING-WATER INTAKE SCREENS

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HEALTH OF FISH IMPINGED ON COOLING-WATER INTAKE SCREENS

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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
May 10, 2007

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THESIS ABSTRACT

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Master of Science, May 10, 2007
(B.S., University of Alabama at Birmingham, December 2003)

111 Typed Pages

Directed by J. S. Terhune

Fish health evaluations were made between impinged and reference fish collected at and in the vicinity of Plant Barry (Mobile River, Mobile County, Alabama) from April 19, 2005, until May 19, 2005, and again from September 26, 2005, until November 2, 2005. The four species of fish investigated were threadfin shad (*Dorosoma petenense*), channel catfish (*Ictalurus punctatus*), blue catfish (*Ictalurus furcatus*), and freshwater drum (*Aplodinotus grunniens*). Evaluations were based on body weight-length and liver weight-body weight indices, gross external lesions, and pathogens. Impinged threadfin shad, blue catfish, and freshwater drum weighed significantly less (7-30%) during the spring, and impinged threadfin shad and freshwater drum weighed significantly less (12-32%) during the fall when compared with reference fish of equivalent lengths. Additionally, lesions were significantly more common for all species of impinged fish than for reference fish during the spring (14-73%) and for species except threadfin shad during the fall (25-64%). Lesions observed included necrotic gill filaments, eroded fins and fin bases, ulcers, skin depigmentation, and hemorrhaging. Liver, trunk kidney, and the periphery of lesions on gills and skin were sampled for bacteria. *Flavobacterium columnare* was detected in a significantly greater percentage of impinged fish than in reference fish during the spring (13-61%) and fall (16-74%). The protozoan parasite, *Ichthyobodo necator*, was also observed at a significantly higher prevalence during the spring (50%) and fall (30%) in impinged freshwater drum.

The weight of blue catfish (10.1% and 18.4%) and channel catfish (12.6% and 9.5%) collected from October 24, 2005, until October 27, 2005, from the intake pit at Plant Barry was significantly greater at equivalent lengths compared with fish of the same species collected from both the river and impinged population during the fall. Threadfin shad collected from the intake pit weighed 12.9% less than impinged threadfin shad and 11.5 to 35.4% less than river threadfin shad at equivalent lengths. External lesions were significantly higher in impinged blue catfish and channel catfish collected during the fall (39-64%) compared with similar species sampled from the intake pit. *Aeromonas* spp. were significantly higher in impinged blue catfish (18%) compared with intake pit fish. The prevalence of *F. columnare* was significantly higher in impinged blue catfish, channel catfish, and threadfin shad (33-74%), collected during the fall, compared with fish from the intake. *Aeromonas* spp. prevalence was significantly higher in river blue catfish and channel catfish (12.3-12.7%) compared with fish from the intake. However, threadfin shad from the intake had a higher prevalence of *I. necator* (10%) compared with threadfin shad from the river.

ACKNOWLEDGMENTS

I would like to thank Alabama Power for funding this research. I am grateful to my advisory committee for their support and advice. Justin Mitchell, Larry Craft, John Sieweke, David Griffin, Kevin Chalk, and Jon Ponstein of Alabama Power assisted in the collection of samples. Completion of the research would not have been possible without the assistance of Chris Sayles, Abel Carrias, Josh McNelly, and Casey Knight. Karl Hayden (Auburn University Fish Disease Lab) was consulted throughout the project for advice concerning technique. I also thank my fiancée, Jennifer Brown, for her constant support and encouragement.

Style manual or journal used: Journal of Aquatic Animal Health

Computer software used: Microsoft Word

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I. INTRODUCTION

Almost half of the utility-owned steam generating power plants in the United States use a once-through cooling water process. Once-through cooling involves the removal of water from a water body, such as a river, to be used for cooling purposes within the plant before an eventual return of the water to the same or nearby water body (Veil 2000). According to a study conducted in 1996, 44% of the utility-owned steam-electric generating plants in the United States employ this type of system (EEI 1996). Depending upon water use demands, varying intake velocities are generated (Tatham et al. 1978). Rotating intake screens are placed at the intake structures to prevent debris from entering the plant via cooling water and are generally constructed with a wire opening of approximately 1 cm (Kelso and Milburn 1979).

Impingement and entrainment of objects occur because of the cooling water intake flow generated by power plants. Entrainment takes place when small organisms such as plankton and small fish pass through the intake screens as cooling water is pumped into the power plant (Kelso and Milburn 1979; USEPA 2002). Most studies on entrainment have concentrated on fish larvae and eggs (Marcy 1975). Impingement, however, involves the entrapment of larger organisms against the screens located at the intake structures (USEPA 2002). Ecological concerns associated with impingement deal primarily with fish, with the species at risk being site-specific.

The Clean Water Act (CWA, Section 316(b)) requires that the best technology available be employed to limit any potential adverse environmental impact of these cooling water intake structures (CWIS) on the body of water where the power plant is located (Veil 2000; Dey 2002; USEPA 2002). Regulations defined by CWA 316(b) have been divided into phases by the United States Environmental Protection Agency (EPA). Power plants are categorized into one of three phases depending upon requirements established by the EPA. Phase I applies to new plants while Phase II applies to existing power plants that withdraw approximately 189 million liters of water a day or plants that use 25% or more of the water withdrawn for cooling, and Phase III includes both small power plants and other industrial facilities (USEPA 2002). Hydroelectric facilities that lack CWIS are not affected by the 316(b) ruling.

Plants affected by the Phase II ruling must reduce impingement mortality by 80 to 95% under the ruling. This reduction is based upon and measured against a baseline calculation defined as the amount of fish mortality that would occur at a plant with a shoreline intake absent of any impingement and entrainment controls (USEPA 2002). Monitoring at the power plant must, therefore, be conducted to estimate the rate of fish impingement, identify the species composition, and establish temporal variability. A temporally stratified sampling program must be developed to concentrate sampling during documented times of high impingement if a good sampling program is to be developed (Kumar and Griffith 1978). It has been recommended that initial sampling be performed in order to establish temporal variations in total numbers of fish being impinged. These patterns can be the result of various physical factors such as intake water temperature, pond elevation, and daily river flow (Mathur et al. 1977). Other factors that may be correlated with rates of impingement include diel and seasonal movements (Lifton and Storr 1978), turbidity (Turnpenny 1983), dissolved oxygen (DO) (Bodensteiner and Lewis 1992), temperature (LaJeone and Monzingo 2000), intake velocity (Fletcher 1994), and health of the organisms (Foster and Wheaton 1981). For potential negative impacts on fish populations to be mitigated successfully, the possible correlation between these factors to rates of impingement need to be understood.

A potential correlation between disease and rates of impingement has been previously investigated (Foster and Wheaton 1981; Bamber et al. 1983; Sprengel and Luchtenberg 1991; Rodgers 1995; Rohlwing et al. 1998). However, in previous studies, comparisons of disease occurrence between impinged fish and reference fish have been limited (Rodgers 1995). Fish health factors, as variables affecting impingement, may account for much of the variability in observed rates of impingement and possibly contribute to the baseline calculation from which impingement must be reduced in order to meet compliance standards. The overall goal of this research was to investigate whether or not unhealthy fish may be selectively impinged at the Barry Steam Plant (Mobile River, Mobile, Alabama), which is currently affected by Phase II of the CWA 316(b) ruling.

II. REVIEW OF THE LITERATURE

Geographical Studies

Threadfin shad (*Dorosoma petenense*) and freshwater drum (*Aplodinotus grunniens*) are the predominant fish species impinged in the southeastern United States. In a study of 27 fossil fuel and 5 nuclear plants in nine southeastern states, threadfin shad accounted for 90% of the total number of fish impinged while total impingement rates were reported to vary from as high as 100,000/day to as low as 10/day (Loar et al. 1978). McLean et al. (1985) conducted an impingement study from November of 1976 until April, 1978, on the Clinch River at Kingston Steam Plant (Knoxville, Tennessee) and reported that threadfin shad accounted for 97% of the fish impinged with impingement rates ranging from a peak of 42,000/day to only 500 for the entire period following January until April 1977. Kumar and Griffith (1978) conducted studies at both Browns Ferry Nuclear Plant in Alabama and at a power plant in Arkansas and determined that threadfin shad was the most commonly impinged fish species. Impingement at Quad Cities Nuclear Station (Cordova, Illinois) on the Mississippi River was studied from 1984 to 1985 and demonstrated similar fluctuations in the number of fish impinged on a temporal scale. Fluctuations in the impingement of freshwater drum were correlated with an increase of incapacitated and dead fish observed in the water flow (Bodensteiner and Lewis 1992). Impingement collections conducted at this site until 1992 indicated that the mean annual impingement rate for freshwater drum was 135,000/yr with the highest rates occurring from February to April. Most of the impinged freshwater drum (87%) ranged from 80-150 mm total length and were less than 2 years old.

Various studies on impingement have been conducted in the northeastern United States and in Canada around the Great Lakes region. Species impinged in the northeastern U.S. and Great Lakes region were highly variable and included bay anchovies (*Anchoa mitchilli*), alewives (*Alosa pseudoharengus*), gizzard shad (*Dorosoma cepedianum*), rainbow smelt (*Osmerus mordax*), channel catfish (*Ictalurus punctatus*), white crappie (*Pomoxis annularis*), and bluegill (*Lepomis macrochirus*) (Lifton and Storr 1977; Mathur et al. 1977; Tatham et al. 1977; Kelso and Milburn 1979). Tatham et al. (1978) studied the condition of fish impinged at two generating plants in New Jersey from September 1975 through August 1977. Condition, determined immediately after impingement, was divided into three categories representing live and undamaged, damaged but not dead, and dead. Peak rates of impingement were determined to occur in spring and fall. The most numerous species impinged was bay anchovy and accounted for 72% of all impinged fish. The condition of most fish was reported as either dead (78%) or damaged (15%). Another study, investigating impingement on the Great Lakes, noted the presence of 89 thermal electric generating plants that used once-through cooling (Kelso and Milburn 1979). A summary of reports submitted by individual power plants, from among the plants noted to occur on the Great Lakes, suggested that the most numerous species impinged were alewives, gizzard shad, and rainbow smelt, while commercial and sport species combined to represent only about 15% of fish impinged (Kelso and Milburn 1979). Mathur et al. (1977) reported, in a study conducted at the

Peach Bottom Atomic Power Plant Station (York County, Pennsylvania) from November 1973 to December 1975, that the most numerous species impinged were channel catfish, bluegill, and white crappie. Mean number of fish impinged, out of 174 samples, ranged from 0.1 to 40.8 per 12 h sample period, varying by species, for the entire sampling effort.

Turnpenny (1983) noted temporal variations in the number of fish impinged at Fawley Power Station in Hampshire, England. Over a 26-week period beginning August 3, 1978, the total number of fish impinged per week ranged from 29 to 7,773 fish. The author reported that sprat (*Spratuis sprattus*) was impinged in numbers sufficient to cause interruptions in plant operations seven times from 1969 to 1980. Fish collection data at the plant were recorded along with measurement of multiple variables such as plant unit operation, water temperature, fish movement, water flow rate, and tidal height in an attempt to create a model to predict influxes in the impingement of fish (Turnpenny 1983). A majority of the variability was correlated with cooling water flow, water temperature, tide height, and wind speed.

Seasonal and Diel Movements

One factor contributing to variability in impingement has been reported to be diel movements. Impingement studies at the C.R. Huntley Power Plant (Buffalo, New York) demonstrated seasonal rates, with impingement increasing during fall (Lifton and Storr 1978). A change in the number of fish impinged was also observed to occur on an hourly or daily basis (Lifton and Storr 1978). These researchers suspected that the difference in observed impingement rates may be attributed to fish location due to diel or seasonal movements. The variation, in regards to location of fish, can be attributed to spawning behavior, diurnal movement or migration cycles, sky cover, and changes in other environmental factors (Lifton and Storr 1978). The diurnal cycles for some species results in more active feeding at night, and this has been directly correlated with increased impingement (Lifton and Storr 1978). Tatham et al. (1978) noted that 86.4% of impingement occurred at night over a 3-d sampling period at Oyster Creek Generating Station in Ocean County, New Jersey where bay anchovy comprised 72% of the total impingement.

An inverse relationship between sky cover and rates of impingement for fish that actively feed in daylight was observed (Lifton and Storr 1978). The increase in sky cover was associated with decreased impingement in sampling conducted at C.R. Huntley Power Plant (Buffalo, New York) located on the Niagara River. Lifton and Storr (1998) suggested that this was due to fish normally active in the daytime becoming less active with increased sky cover.

It has been suggested that much of the observed variability in numbers of fish impinged may also be related to seasonal movements or migrations. Seasonal movements are often triggered by factors such as DO and temperature (Lifton and Storr 1978). Spawning behavior can bring certain species into the general intake area or hydraulic zone of influence (HZI), causing fish to become more susceptible to impingement (Lifton and Storr 1978).

Turbidity

Turbidity and ambient light levels could have an effect on impingement rates and therefore account for some of the observed variability in numbers of fish impinged. Fish use visual or tactile cues to correctly orient spatially, and turbidity may have an effect on the ability of a fish to orient, therefore contributing to impingement (Rulifson 1977). This relationship has also been suggested by Lifton and Storr (1978), who noted a positive correlation between numbers of fish impinged and turbidity that was associated with wind direction. Kumar and Griffith (1978) and Turnpenny (1983) suggested that future attempts to create models forecasting impingement rates should include water turbidity as a variable.

DO and Water Temperature

DO has been suspected of contributing indirectly to an increase in the number of fish impinged (Bodensteiner and Lewis 1992). The authors investigated the role of DO and water temperature on the survival of freshwater drum in the Mississippi River along the border of Iowa and Illinois. Laboratory studies conducted by Bodensteiner and Lewis (1992) demonstrated that juvenile drum became disoriented, incapacitated, and experienced an increased mortality when exposed to water temperatures at and below 1ºC. As water temperatures during winter decreased in the main river channel, freshwater drum aggregated in warmer backwaters where water temperatures were above 1ºC. Other species that have been observed to use thermal refuges include threadfin shad (McLean et al. 1985), smallmouth bass (*Micropterus dolomieu*) (Munther 1970), and the eastern blacknose dace (*Rhinichthys atratulus*) (Cunjak and Power 1986). Low DO concentrations are usually experienced in conjunction with higher water temperature and slower river flow rates. When DO levels declined in the backwater areas, the freshwater drum migrated into the main channels and were exposed to water temperatures as low as 0ºC. This resulted in an increase in the number of moribund freshwater drum observed floating with the direction of the river flow and subsequently at the intake screens. Analysis of these fish collected at the intake screens indicated that 90% of impinged drum were dead or moribund (Bodensteiner and Lewis 1992).

Temperature seems to be the most commonly cited factor affecting rates of impingement. In the southeastern United States, impingement rates of threadfin shad increased as water temperatures decreased (McLean et al. 1985). The authors observed an estimated 240,000 threadfin shad impinged at the Kingston Steam Plant (Knoxville, Tennessee) from 1976-1977. The rate of impingement was correlated with a decrease in water temperatures. Impingement rates increased as water temperatures approached 7ºC and peaked in December when water temperatures were measured at 4ºC. Griffith and Tomlijanovich (1975) demonstrated that threadfin shad exhibited changes in swimming ability when exposed to water temperatures below 12ºC. Threadfin shad died at 4ºC in laboratory experiments (Griffith 1978). Additionally, increases in impingement of gizzard shad has been attributed to stress related with low temperatures (LaJeone and Monzingo 2000). Additional species that exhibit size dependent low-temperature mortality include smallmouth bass, largemouth bass (*Micropterus salmoides*) and bluegill (Oliver et al. 1979; Toneys and Coble 1979). However, low temperature associated mortalities have not been correlated with impingement in these species.

Mathur et al. (1977) observed rates of impingement from November 1973 through December 1975 at Peach Bottom Atomic Power Station (York County, Pennsylvania) to be highest for channel catfish, white crappie, and bluegill. Impingement rates were positively correlated with an increase in river flow, decrease in water temperature, and a decrease in water elevation. Swimming performance of channel catfish in laboratory experiments performed by Hocutt (1973) was positively correlated to water temperature. Temperature was also positively correlated with swimming speeds of striped mullet (*Mugil cephalus*), spot (*Leiostomus xanthurus*), and pinfish (*Lagodon rhomboids*) in a laboratory experiment conducted by Rulifson (1977) and was concluded to be significant in regards to impingement.

Intake Velocity

Experiments have been performed to investigate the effects of intake water velocity on fish exhaustion and impingement. Temperature was positively correlated with time to impingement (TTI) for striped mullet, spot, and pinfish, and water velocity was negatively correlated with TTI for all species (Rulifson 1977). The TTI was also dependent upon fish size, with smaller fish becoming impinged in a shorter amount of time. Time to impingement also increased as the size of fish increased. However, abnormally heavy mullet and spot were less able to maintain swimming position (Rulifson 1977).

Fletcher (1994) investigated the effects of two different flow rates on experimental impingement of juvenile striped bass (*Morone saxatilis*) and golden shiners (*Notemigonus crysoleucas*). The experiment was performed to simulate the flow patterns present at dual-flow intake screens employed at some power plants and investigated the influence of velocity on rates of impingement. Nominal speeds of 30 cm/s and 45 cm/s were compared. Fluid speeds increased at the corner of the entry portal to a range of 50-100 cm/s for the 30 cm/s setting and to 80-150 cm/s for the 45 cm/s setting. The species responded differently to the current, with the stripped bass being able to maintain position against the current for longer periods of time. Only 10% of the striped bass were able to maintain position at the 45 cm/s flow rate while none of the golden shiners were able to do so. All experimentally impinged fish at both flow settings were killed or injured (Fletcher 1994).

Fish Health

Factors previously discussed, such as DO and water temperature, have an effect on the health of fish. Low DO concentration is an environmental factor that can lead to increased susceptibility of fish to opportunistic pathogens (Hayley et al. 1967; Pacha and Ordal 1970; Walters and Plumb 1980). Historically, water temperature has been correlated with epizootic events and various pathogens have been associated with fish die-offs during specific temperature intervals (Austin and Austin 1999; Becker and Fujihara 1978; Pacha and Ordal 1970; Roberts 2001). A sick or weakened fish, if present in the HZI, could be more susceptible to flow rates present at intake structures.

In an experiment unrelated to impingement, Tierney and Farrell (2004) investigated the swimming ability of adult sockeye salmon (*Oncorhynchus nerka*). Critical swimming speed tests were performed on fish with injury or abnormalities and results were compared to previous test performed with apparently healthy fish. They discovered that fish infected with *Ichthyophonus* spp. and *Saprolegnia* spp. were not able to achieve critical swimming speeds equivalent to those of healthy fish. Cutaneous lesions were considered serious if > 1 cm^2 in size and if < 1 cm^2 then they were considered 'moderate' and fish were classified as sick (lesion > 1 cm^2) or moderately sick (lesion < 1 cm^2). Inability to recover from initial critical swimming speed test and slower swimming speeds were observed in fish classified as sick (Tierney and Farrell 2004). The authors also concluded that disease impairment could cause a fish to be more vulnerable to human induced impacts on their environment. Additionally, delta smelt (*Hypomesus transpacificus*) infected with *Mycobacterium* sp. had a significant reduction in swimming speed compared with uninfected smelt (Swanson et al. 2002).

While the methods used to investigate the health status of impinged and reference fish has varied in past studies, the general hypothesis investigated was that a decreased health status or infection increases the likelihood that a fish is impinged. Bamber et al. (1983) suggested that fish with diplostomiasis, commonly referred to as black spot disease, would be more susceptible to impingement because of decreased ability to escape intake flows. The authors compared the prevalence of diplostomiasis, in impinged and reference sand smelt (*Atherina presbyter*) at Fawley Power Station (Hampshire, England). Diplostomiasis had been previously observed in sand smelt in the vicinity of Fawley Power Station and was therefore chosen as the disease for evaluation (Bamber et al. 1983). For basis of comparison, fish were separated into size groups greater and less than 100 mm total length and divided into impinged and reference fish. There were no statistical differences detected in occurrence of *Neodiplostomum* sp. in the four fish populations (Bamber et al. 1983). The authors concluded that black spot disease did not lead to selective impingement of sand melt, which could be due to the fact that black spot disease is not debilitating to most fish (Post 1983). Bamber et al. (1983) also noted that a high prevalence of *Sacculina* and *Lernaeocera* sp. had been observed previously on pout (*Trisoptrerus luscus*) impinged at Fawley Power Station, although no direct comparison was made between the infection rates of impinged and reference fish.

Bodensteiner and Lewis (1992) investigated health factors affecting impingement and incapacitation of freshwater drum from 1984 until 1985 at Quad Cities Nuclear Station (Cordova, Illinois). The factors evaluated to establish heath status of the fish were fluid in the body cavity, gall bladder distension, bile color, muscle texture, liver color, liver weight, and presence of material in the gut. A Fulton-type condition factor was calculated for the freshwater drum. These indices were compared between drum collected on a barrier net at the water intake with fish collected by seine nets from nearby sloughs. Mean body condition of the two populations was not significantly different. It was concluded that starvation was not significantly correlated with impingement. The authors, however, noted that 33% of recently impinged fish had small oomycete growths on their pectoral or pelvic fins but an infection rate of open water fish was not reported. *Philometra* spp. were also found on impinged fish; however, there was no difference in the prevalence between impinged and reference fish. The size distribution of fish impinged throughout the sample period led the authors to conclude that freshwater drum in the first and second year of life were more susceptible to incapacitation and subsequent impingement. Factors contributing to incapacitation were concluded to be low temperature and DO. The authors also observed that during the sample collection for 1985, that 98% of impinged freshwater drum were either live or recently dead. This was in contrast to the previous winter when it was noted that up to 90% of impinged freshwater drum was dead (Bodensteiner and Lewis 1992).

Rodgers (1995) performed a histopathological assessment of alewives from both an impinged and a reference population in June 1986 at Pickering Nuclear Generating Station (northwest shore of Lake Ontario). Organs examined were spleen, liver, kidney, intestine, gonad, heart, muscle, gill, brain, eye, and skin. A total of 25 fish from both the impinged and reference population were investigated in this manner. Rodgers (1995) observed a significantly higher prevalence of nephrocalcinosis in impinged fish. Nephrocalcinosis is known to be caused by an imbalance in dietary cation intake and high levels of carbon dioxide; however, the author noted that it was unlikely that the fish encountered these conditions within the intake channel and that the natural occurrence of the lesion has been noted in wild fish (Rodgers 1995). Based upon the prevalence of lesions other than nephrocalcinosis, Rodgers (1995) suggested that impingement did not select unhealthy fish. However, the impinged fish had lower body weights and liver weights, compared with reference fish of the same size. The body weight of an impinged alewife was 10% lower than the weight of a reference alewife of the same length. A possible explanation, given by the author, for the lower liver and body weight was depletion of energy reserves due to exhaustion from maintained swimming velocities required to avoid impingement. Rodgers (1995) concluded, however, that it is unlikely that an otherwise healthy fish would be unable to leave the intake prior to impingement.

Sprengel and Luchtenberg (1991) investigated whether infection by endoparasites would reduce maximum swimming speed in European smelt (*Osmerus eperlanus*) and European eel (*Anguilla anguilla*) thus increasing susceptibility to impingement. The authors performed their study at a nuclear power plant located on the Elbe River (Brunsbuttel, Germany). European smelt were chosen as a target species because they were the most frequently impinged species and European eel because they were the most commercially valuable species impinged. In European smelt, *Pseudoterranova decipiens* (Nematoda) and *Pleistophora ladogensis* (Microspora) infections were evaluated to test for possible effects on swimming ability of the fish. *Pseudoterranova decipiens* and *P. ladogensis* infest muscle (Sprengel and Luchtenberg 1991). The effect of *Anguillicola crassus* (Nematoda) infection in European eel was also investigated. A total of 249 European smelt and 161 European eel were collected from the intake screens of the plant. The fish were then held in aquaria for observation prior to tests designed to measure swimming performance. Reduction in the swimming speed of European smelt, depending on the number of *P. decipiens* found infecting the fish, ranged from 18.6-32.2% with an average reduction in swimming speed of 19.3%. European smelt infected with both *P. decipiens* and *P. ladogensis* experience a 29.9% reduction in swimming speed. Reduction in the swimming speed of European eel ranged from 2.9-18.6% depending upon level of infection of *A. crassus*. The authors concluded that infected fish could be affected and thus, less likely to escape the cooling-water intake. A comparison between the prevalence of these parasites among fish in the intake area and fish from the reference population, however, was not reported.

Rohlwing et al. (1998) investigated the effect on survival of a *P. decipiens* infection in European smelt impinged at the same power plant studied by Sprengel and Luchtenberg (1991). This power plant has a fish return system that includes a reservoir where fish are held prior to return to the river. The authors hypothesized that infected fish would experience more contact with the intake screens, resulting in more damage and thus, a lower survival. To test this, a total of 354 fish representing year classes one and two (7 to 20 cm) were collected from the holding reservoir. The authors discovered greater damage due to contact with the intake screen and lower survival for infected fish resulting in a lower percentage of living parasitized smelt present in the local population (Rohlwing et al. 1998).

Epidemiological Studies

Columnaris disease, caused by the bacterial pathogen *Flavobacterium columnare* and first described by Davis (1922), is known to affect most species of fish in fresh water (Roberts 2001). Prevalences as high as 60% for black bullheads (*Ameiurus melas*) were reported (Bowser 1973). Fujihara and Olson (1962) compared the prevalence of *F. columnare* on fish from three sample sites in the Columbia River to fish from an artificial spawning channel. Fish collected from 1960 to 1961 were divided into temperature ranges, reflecting sample period, of less than 15.6°C, 15.6 to 20.6°C, and greater than 20.6°C. A total of 577 fish from three collection sites in the Columbia River and 218 from the McNary spawning channel were sampled. Prevalence rates ranged from 1.8% for the lower temperature range to 3.6% for the upper temperature range in fish sampled from the Columbia River. This contrasted with prevalence rates, in fish collected from the artificial spawning channel, of 22% (fish held <15.6° C) and 54% for fish held between 15.6-20.6° C. The authors concluded that high fish densities contributed to the levels of infection.

Fujihara et al. (1964) compared the prevalence of *F. columnare* in 214 fish collected from three sampling sites on the Columbia River to 120 fish collected from the Yakima River during 1963. Detection of *F. columnare* was divided into two categories, "incidence" and number "infected", based upon the number of colonies growing on the culture plate. The growth of only a few colonies from tissue smears were considered to be incidental ("incidence") and growth of a "host of colonies" was considered to indicate "infection" or disease. Fish sampled from the Columbia River included suckers (*Catostomidae*), chiselmouth (*Acrocheilus alutaceus*), common carp (*Cyprinus carpio*), whitefish (*Prosopium* sp.), smallmouth bass, yellow perch (*Perca flavescens*), and pikeminnow (*Ptychocheilus* sp.). Species were grouped together and "incidence" of *F. columnare* was calculated at 3.3% compared to an "infection" rate of <1%. Species sampled from the Yakima River included suckers, chiselmouth, common carp, whitefish, smallmouth bass, yellow perch, pikeminnows, and chinook salmon. Incidence of *F. columnare* for all fish collected from the Yakima River was 93% compared with 15% determined to be infected. The authors further concluded that the mere presence of skin lesions or necrotic gills did not indicate the presence of *F. columnare*, as the organisms were frequently not isolated from these organs.

Pacha and Ordal (1970), summarized data collected during field studies performed on the Columbia River from 1955 to 1959. The authors noted a wide prevalence range of columnaris disease in salmonids throughout the study period. From 1955 until 1957, the disease was not detected among fish sampled at Bonneville Dam on the Lower Columbia River. *Flavobacterium columnare* was isolated in only two of 543 salmonids during 1956 and from only one of 140 fish sampled during 1957. However, columnaris disease ranged from 28-75% in adult Chinook and sockeye salmon sampled from the Snake River during July and August of 1957. In 1955, prevalence of columnaris disease in sockeye salmon ranged from 1.5-6.7% during the early part of the spawning year to 55% near the end of spawning (Pacha and Ordal 1970). A study conducted at Bonneville Dam during the summer of 1957 reported almost no columnaris in the salmonids sampled. However, 51.9% of sockeye salmon sampled at McNary Dam on July 10 and 11, 1957, exhibited lesions typical of columnaris disease. In 1958, an extensive study investigating columnaris disease was conducted in the Columbia River Basin. Water temperatures during this sample effort were the highest recorded since 1939-1949 (Pacha and Ordal 1970) and *F. columnare* was isolated from 1,200 fish sampled during the effort. The authors concluded that temperature and high density of fish was responsible for the number of fish infected. Similar relationships between temperature and columnaris disease have been observed in other studies (Becker and Fujihara 1978).

The epizootiology of *F. columnare* in fish from the Columbia River was summarized for the years 1964 to 1973 by Becker and Fujihara (1978). Methods of isolation of *F. columnare* from fish included plating tissue samples from external lesions, gill, and head kidney onto agar made with 1% nobel agar, 0.4% bactotryptone, and 0.04%

bacto-yeast extract (final pH=7.3), followed by incubation at 25°C for 48 h. They noted a percentage of bacterial isolation from lesions as low as 10% and the successful isolation of the bacteria from gill and head kidney in fish demonstrating no external signs of the disease. The authors further divided detection of *F. columnare* into two categories. Samples yielding 1 to 10 colonies were considered by the authors as “exposed” and those with more than 10 colonies were considered to be “infected”. Sampling occurred at 4 sites in 1965 and from 1969 to 1973 and included 7,000 fish. Species of fish included largescale sucker (*Catostomus macrocheilus*), bridgelip sucker (*Catostomus columbianus*), northern squawfish (*Ptychocheilus oregonensis*), peamouth (*Mylocheilus caurinus*), chiselmouth, white sturgeon (*Acipenser transmontanus*), common carp, channel catfish, smallmouth bass, largemouth bass, black crappie (*Pomoxis nigromaculatus*), white crappie, yellow perch, and mountain whitefish (*Prosopium williamsonii*). This study was performed to investigate the possible effect of operation of the Hanford reactor on the pathogenesis of *F. columnare* (Becker and Fujihara 1978). All fish were grouped by site and year and summarized as percent “exposed” and “infected”. In sampling efforts from May to November 1965, percent “exposed” for sites, on the mainstem Columbia River, at 6.4 km below Bonneville Dam, 9.6 km below McNary Dam, near the town of Hanford, and at Wenatchee just above Rock Island Dam ranged from 4 to 7.5% compared to a percent “infected” range of 1.9 to 4.3%. Percent “exposed” ranged from 1.7 to 34.9% and percent “infected” ranged from 3.3 to 22.7% during sampling efforts from 1969 to 1973.

Epizootic events occurring at water bodies in Alabama have been attributed to various pathogens. In an investigation of fish kills during the spring of 1973 and 1974 at seven impoundments located in Alabama, *Aeromonas hydrophila* was determined to be the etiological agent in 90% of diseased fish (Hawke 1974). However, detection of the pathogen did not necessarily indicate disease in the fish as an examination of apparently healthy fish sampled from 1973 to 1974 by electrofishing at lakes Martin and Logan Martin revealed a carrier rate of *A. hydrophila* from 10% to 25%, respectively (Hawke 1974). The etiological agent of a freshwater drum kill in the Mobile River Delta was determined to *Pseudomonas fluorescens* and an additional fish kill involving crappie and shad at Lake W. F. George was attributed to *F. columnare* (Hawke 1974). A fish kill of gizzard shad and threadfin shad from the Coosa River in Alabama during the winter of 1962 was determined to be caused by *Ichthyophthirius multifiliis*, a ciliated protozoan (Allison and Kelly 1963). Detection of any of these pathogens, among others, in impinged fish may be an indicator of a decreased health status and thus an increased susceptibility to impingement.

Hazen (1979) studied the prevalence of *A. hydrophila* in largemouth bass in Par Pond, a cooling water reservoir for nuclear reactors at the Savannah River Plant (Aiken, South Carolina). A minimum of 200 largemouth bass were sampled monthly from December 1975 until December 1977. During 1976, the percent infection ranged from 10% to 44% with the highest infection rates (33-44%) occurring during the spring months. During 1977, percent infection followed the same general trends with lows of approximately 5% infection in January and peaks of approximately 25% infection in

May. Hazen (1979) concluded that increased water temperatures along with increased densities of *A. hydrophila* in the water column caused the largemouth bass to become more susceptible to infection. Esch and Hazen (1978) noted a correlation between body conditions of largemouth bass to vulnerability of infection with *A. hydrophila*. Hazen et al. (1978) attributed the outbreaks of some epizootics, such as one in North Carolina in 1973 when 37,500 fish (commercial and sport species) died over a 13-day period, to *A. hydrophila.*

In a study conducted from June 2, 1958, to December 3, 1959, 350 fish including brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*), splake (*S. namaycush x S. fontinalis*), rainbow trout, brown trout (*Salmo trutta*), brown bullhead (*Ameiurus nebulosus*), creek chub (*Semotilus atromaculatus*), common sucker (*Catostomus commersonii*), common shiner (*Luxilus cornutus*), and sunfish (*Lepomis* spp.) were sampled from the freshwater streams, lakes, ponds, and hatcheries of Ontario. Bacterial genera found (prevalence in parentheses) were: *Pseudomonas* (29%), *Aeromonas* (24%), *Streptococcus* (1%), *Escherichia* (13%), and *Flavobacterium* (1%) (Evelyn and McDermott 1961). Rabb and McDermott (1962) conducted a follow up bacteriological investigation from June 6, 1959, to November 7, 1960. The presence of *Aeromonas salmonicida* was investigated in fish collected by electroshocking from freshwater streams in Ontario, Canada. With the exception of one mottled sculpin (*Cottus bairdii*), *A. salmonicida* was isolated only from brook trout collected from two of the streams sampled. A total of 2,655 isolates were obtained from 1,044 fish, however, only 41 of the isolates were identified as *A. salmonicida* for a total prevalence of 3.9% for all fish from the Credit River and Beaver River. Varying by location, 34-54% of the fish sampled tested negative for any bacterial growth. The fish that tested positive for *A. salmonicida* were otherwise healthy fish (Rabb and McDermott 1962). Results from wild fish surveys such as these are valuable when applied to a comparison of pathogen prevalence between impinged and reference fish. The chronic effects of these diseases need to be considered in conjunction with many of the previously discussed factors when attempting to explain the variability associated with rates of impingement.

III. STATEMENT OF RESEARCH OBJECTIVES

The purpose of this study was to determine if there was increased pathogen prevalence or a decrease in general health status in four species of fish impinged at Plant Barry on the Mobile River relative to reference fish. The four species of concern were chosen due to either high impingement rates or to high replacement cost in terms of mitigation. The four species of concern are threadfin shad, channel catfish, blue catfish, and freshwater drum. Alabama Power Company has 7 intake structures, located at 5 power plants, presently subject to the 316(b) Phase II regulations. Two of these intake structures are located at Plant Barry and have the highest impingement rates among the 7 CWIS (David Saalfeld, Auburn University, unpublished data). In addition to determining whether impinged fish were unhealthy relative to the river population, fish from the intake bays located within the CWIS were sampled over a three day period and health status determined.

IV. METHODS

Study Site

Plant Barry, a coal-fired steam generating power plant owned by Alabama Power Company (APCO), is located in Mobile County, Alabama, on the Mobile River approximately 49 km from the confluence of the Mobile River and the Gulf of Mexico. Plant Barry has two CWIS located in a man-made canal that is perpendicular to the main river channel and has a maximum depth of 5.18 m (Figure 1). The Mobile River is 198.1 m wide and has a maximum depth of 12.19 m, depending upon river flow, at the junction of the intake canal and river. Rotating screens, 6 at one CWIS (Units 1-3) and 5 (Units 4-5) at the other, are contained within individual intake bays. The intake bays at both intake structures are approximately 3.35 m wide and have a perimeter trash rack for initial filtering of objects from incoming water flow. The height of the 6 rotating screens at the CWIS for Units 1-3 is 13.26 m, while the five screens located at the CWIS for Units 4-5 are 12.8 m high (Figure 2). The rotating screens, with 1-cm mesh wire openings, are placed at the entrance to the intake tunnels of the plant to further filter out objects from the incoming water. The 11 rotating screens take approximately 8 to 10.5 min to make one-half a revolution.

CWIS Velocities and Water Temperatures

At maximum generation capacity, the measured CWIS flow volumes through the trash racks were 20.5 m^3/s and 29.3 m^3/s for Units 1-3 and Units 4-5, respectively. The

CWIS flow velocities were calculated by APCO from data for river stage, volume of water withdrawn from the CWIS, and the surface area of the screen. The through-screen water velocity during the sampling varied from 0.16 to 1.64 m/s for Units 1-3 and averaged 0.47 m/s. The through-screen water velocity mean was calculated to be 0.64 m/s for Units 4-5 with a range of 0.21 to 1.43 m/s for the sampling events. Data temperature loggers (Hobo Water Temp Pros Onset, Bourne, Massachusetts) were placed at the intake structures approximately 1 m beneath the water surface and recorded water temperatures every 30 minutes throughout the spring and fall sampling periods.

Fish Sampling

Impinged fish and reference fish from the riverine population were collected from the Mobile River in the vicinity of the plant and at the two CWIS during the spring and fall of 2005. Impinged fish were collected in baskets that received the backwash water used to clean the screens at the CWIS. The baskets were emptied every 1 to 2 h and fish were separated by species and whether they were dead or alive, sealed in a plastic bag, and placed on ice. Impingement sampling was conducted from 0800 until 1800 hours four days a week throughout the duration of the spring sampling from April 19, 2005, to May 19, 2005. Fall sampling was conducted as above from September 26, 2005, to November 2, 2005.

Reference fish were collected from inside the intake canal and approximately 5 km upstream and downstream of the plant site. Adjacent water bodies connected to the Mobile River that were sampled included Jose Creb Bayou, Matche Bayou, and Zedol Lake (Figure 3). However, approximately 75% of fish sampled came from within and

0.5 km upstream and downstream of the intake canal. Reference fish were collected by electrofishing, gill nets, and traps. The electric current used was 60-Hz in pulsed DC. Habitat was selected to target specific fish species. Gill nets were set and then retrieved approximately every 3-4 hr while box traps were set overnight. All reference fish were placed in sealable bags and stored on ice. Impinged and reference fish were collected concurrently throughout the sampling periods.

General Necropsy Procedures

All fish collected were processed on site within 3-4 h of collection. At the beginning of the necropsy procedure, live fish were euthanized with a solution of Finquel (Argent Chemical Laboratories, Redmond, Washington) buffered with equal parts of sodium bicarbonate. Weight and total length was then recorded for all fish. Impinged fish determined to be dead at the time of collection were examined to determine whether they were suitable for necropsy by evaluating redness of the gill along with skin and eye color. Gill and skin scrape wet mounts were examined microscopically for parasites and bacteria. Any gross lesions including mechanical damage were noted.

The body cavity of the fish was aseptically opened and calibrated 1 μL disposable inoculating loops (Apogent Company, Portsmouth, New Hampshire) were used to streak samples of liver and trunk kidney onto brain heart infusion agar (BHI) and Hsu-Shotts agar (Bullock et al. 1986). Tobramycin was added to Hsu-Shotts agar to a final concentration of 4 μg/ml. The periphery of gill and external lesions not appearing to be mechanically induced were also streaked for bacterial isolation. Livers from all species except threadfin shad were excised and weighed to the nearest tenth of a gram in the spring and from the blue catfish and channel catfish in the fall.

Bacteriology

Bacterial media plates were incubated at 30°C and growth was recorded initially at 24 hours. Any plates demonstrating no growth at this time were incubated an additional 24 hours. Bacterial colonies were counted and described according to shape and color. In the spring sampling when three or more colonies with similar growth characteristics were observed from a primary isolation on BHI, one of the colonies was streaked for isolation. Growth of fewer than three similar colonies was considered to be insignificant growth. For bacteria growing on Hsu-Shotts plate medium, only the colonies having growth characteristics and appearance similar to those of *F. columnare*, as described by Griffin (1992), were streaked for isolation. Isolated colonies restreaked on BHI or Hsu-Shotts were allowed to grow for 24 to 48 hours at 30°C to check for purity. Suspected isolates of *F. columnare* were identified as described by Griffin (1992). All isolates on BHI were characterized by a gram stain and cytochrome oxidase test. Gram positive cocci were presumptively identified as *Staphylococcus* sp. if catalase positive and as *Streptococcus* sp. if catalase negative (Holt et al. 1994). Gram negative isolates cultured on BHI were identified to genus using API 20 E rapid identification strips (Biomerieux Company, Durham, North Carolina) incubated at 30° C (Toranzo et al. 1986; Santos et al. 1993; Austin and Austin 1999).

In the fall sample, growth of colonies occurring on BHI, regardless of number, was streaked for isolation. 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 Austin 1999; Brenner et al. 2001), and sensitivity to novobiocin (AFS-FHS 2005). Growth of colonies characteristic of *F. columnare* was confirmed by polymerase chain reaction (Welker et al. 2005) and fatty acid methyl ester analysis (FAME) (Shoemaker et al. 2005).

Histology

Liver, trunk kidney, spleen, and gill were preserved in Bouin's solution and then stored in 50% isopropyl alcohol within approximately 96 h. Samples selected for confirmation of field observations were then embedded in paraffin, sectioned, and stained with hematoxylin and eosin (Luna 1992).

Intake Pit Study

Fish were sampled from inside the perimeter trash barrier immediately in front of the intake screens (pit area; Figure 4) for evaluation of weight-length relationships, lesion prevalence, and pathogen prevalence. The sampling was performed from October 24-October 27, 2005, by inserting concrete barriers in front of and behind the intake screen to seal the area off from the CWIS and intake canal. Water was removed from the pit area and fish in front of the intake screens were collected by seine and gill net. The four targeted fish species were sampled until 60 fish/species were collected. Fish were weighed and measured as previously described. Wet mounts and necropsy procedures were performed as described earlier except that only trunk kidney streaks were performed on fish from the intake pit. Liver weight measurements were not performed for fish collected from the intake pit.

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 treatment for both seasons (Navidi 2006). Liver somatic index (LSI) was calculated based on Adams and McLean (1985). Weight-length and liver weight-body weight relationships were analyzed using regression analyses. The variables were natural log-transformed to ensure that statistical assumptions of normality and homogeneous variance for the residuals were satisfied (Anderson and Neumann 1996; Rodgers 1995). The selection of the 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 weight and length (Anderson and Neumann 1996). Additional selection criteria included the adjusted R^2 and the existence of randomly distributed residuals (Navidi 2006). In the regression analyses, the impingement treatment term was coded "1" and the river treatment term was coded "0" in comparisons between the impinged and river population. Potential interaction or confounding effects between length and treatment was also tested (Kleinbaum et al. 1998). 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). The analyses were conducted separately for each season. Differences in predicted body and liver weight, between impinged and reference fish at equivalent fish lengths were considered to be significant for all species if the treatment coefficients were significant at $P \leq 0.05$. Weight-length regression analyses were also performed as above to test for significant differences in weight at equivalent lengths, by species, in pair wise comparisons between fish collected from the intake pit and fall impingement samples and reference fish. In these pair wise comparisons, the intake population treatment term was coded "1" while both the river and impinged treatment terms were coded "0" in the respective analyses.

Chi-square tests were used to compare, by species, frequency of external necrotic lesions, external parasites occurring on gill or skin, and bacteria in impinged and reference fish. When sample sizes were not large enough so that expected cell counts were a minimum of 5, 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 $P \leq 0.05$. For comparisons with intake fish, the prevalence of bacteria in impinged fish and river fish collected during the fall was adjusted to represent only bacteria detected in trunk kidney tissues. Bonferroni's inequality method was used and significance was tested to $P \leq 0.017$ to maintain an overall P-value of 0.05 (Milliken and Johnson 1984). This method was applied to all statistical test used during the fall analyses. However, comparisons of impinged freshwater drum with reference freshwater drum collected from the river were tested to $P \leq 0.05$ because freshwater drum from the intake pit were not collected.

V. RESULTS

Comparison of Impinged and River Reference Fish

The daily mean water temperature during the spring collection period ranged from 19.7 to 25.1° C (Figure 5). The daily mean water temperature during the fall collection period ranged from 24.8 to 28.8° C (Figure 6). Data loggers were removed by plant personnel on October 18, 2005, and therefore temperatures were not available after this date.

Weight-Length Relationship

Total length and weight were obtained for all fish that were necropsied plus additional fish that were not necropsied (Tables 1 and 2). During the spring, impinged blue catfish and threadfin shad collected had a significantly ($P \leq 0.003$ and $P \leq 0.021$, respectively) greater mean length compared with blue catfish and threadfin shad collected from the river. However, channel catfish collected from the river had a significantly ($P \leq 0.0001$) greater mean length compared with impinged channel catfish. During the fall, channel catfish, freshwater drum, and threadfin shad ($P \leq 0.0001$) collected from the river had a significantly greater mean length compared with impinged fish of the same species (Table 2). Differences between the sample sizes for total length and weight occurred because total length was not available for fish with severe caudal fin erosion. Only paired data were utilized in regression analyses.

Predicted weights of impinged blue catfish ($P \leq 0.006$), freshwater drum ($P \leq 0.002$), and threadfin shad ($P \leq 0.0001$) were determined to be significantly less than for reference fish of equivalent lengths during the spring (Figures 7-9). No significant difference was observed in channel catfish during the spring (Figure 10). During the fall sampling, only impinged threadfin shad ($P \leq 0.0001$) and freshwater drum ($P \leq 0.013$) had predicted weights less than reference fish at equivalent lengths (Figures 11 and 12). No significant differences were observed in channel catfish or blue catfish during the fall (Figures 13 and 14). The percent difference in predicted weight at equivalent lengths between impinged and reference blue catfish collected during the spring was 9.24% and was constant over different lengths (Figure 7). Impinged threadfin shad predicted weight was 7.4% and 12.0% less during the spring and fall, respectively, compared with reference threadfin shad of equivalent lengths for all size classes sampled (Figures 8 and 11). Significant interaction between length and treatment was not observed for blue catfish in the spring nor for the threadfin shad during the spring or fall. For both seasons, impinged freshwater drum weighed significantly less at equivalent lengths than reference fish and had significant interaction between total length and treatment. Predicted weight of freshwater drum impinged during the spring was 9.2-29.6% less for fish 120-360 mm in total length compared with reference freshwater drum of equivalent length (Figure 9). During the fall, predicted weight of impinged freshwater drum ranged from 6.8 to 30.8% less for fish 90 to 274 mm in total length compared with reference freshwater drum of equivalent length (Figure 12).

Liver Weight Analysis

Mean LSI for the blue catfish during the spring were 1.8% for the reference samples and 1.9% for impinged fish and 2.5% and 2.6% for reference and impinged fish, respectively, during the fall. In channel catfish, mean LSI were 1.8% for reference fish and 2.3% for impinged fish in the spring and 1.5% and 2.0% for reference and impinged fish, respectively, in the fall. During the spring the mean LSI of river reference freshwater drum was 1.5% compared with 1.7% in impinged freshwater drum. No significant differences were observed in the regression models between impinged and reference fish in any of the species sampled in either season (Tables 3 and 4).

Lesion Prevalence

Total number of fish that were necropised and used to calculate the prevalence rates of lesions, parasites and bacteria is presented by species and season in Table 5. Lesions observed included ulcerative lesions, skin depigmentation, eroded fins, and hemorrhagic areas. External lesions, suspected of being bacterial or parasitic in origin, were significantly more prevalent in all four species of impinged fish than in reference fish of the same species during the spring sample. Similar results were observed for all species except threadfin shad during the fall sample (Table 6). The percentage of fish having lesions that also had bacteria or parasites associated with the lesion ranged from 62 to 100% in the spring and from 83 to 100% in the fall (Table 7).

External Parasite Prevalence

In the spring sampling, eight genera of protozoans plus Monogenea were observed in wet mount preparations of gill and skin (Table 8) and six genera of protozoan and Monogenea were observed in the fall (Table 9). Compared with reference fish, *Ichthyobodo necator* was significantly more common on impinged freshwater drum ($P \leq 0.0001$) during both the spring and fall sample and on impinged threadfin shad ($P \leq 0.031$) during the spring. *Chilodonella* sp. prevalence was also significantly higher on impinged threadfin shad than on reference fish during the fall sample period ($P \leq 0.0001$). Prevalence of other external parasites was not significantly different for impinged and reference fish for either season.

Bacterial Prevalence

Because of the use of API 20 E identification strips during the spring sample period, bacteria isolated in the spring sample were reported at the genus level due to variability in biochemical results associated with the use of the API 20 E identification strips (Austin and Austin 1999; Overman et al. 1985; Santos et al. 1993; Toranzo et al. 1986). During the spring sampling period, 20 different profiles, with 1-8 different isolates giving the same profile number, were identified as an *Aeromonas* sp. This was evident in other genera as well with three different profiles being identified as *Plesiomonas* sp. and five different profiles as *Pseudomonas* sp. (Appendix 1). Additionally, two isolates generated profiles that were indexed as *Aeromonas* spp. even though they were both oxidase negative. These two isolates were not included when calculating prevalence rates because bacteria from that genus are oxidase positive (Brenner et al. 2001). During the fall, *Aeromonas* spp. were characterized as being gram negative, oxidase positive, fermenting glucose, and vibriostat resistant. *Plesiomonas* and *Vibrio* were characterized as being gram negative, oxidase positive, and fermenting glucose but were distinguished from *Aeromonas* spp. by being sensitive to vibriostat (Brenner et al. 2001; Buller 2004). *Plesiomonas* and *Vibrio* spp. were separated by testing sensitivity to novobiocin with *Plesiomonas* considered resistant and *Vibrio* spp. sensitive to this antibiotic (AFS-FHS 2005). Isolates presumptively identified as *Vibrio* spp. were considered to be within this genus even though they grew in 0% NaCl because certain species such as *Vibrio mimcus* do not require salt for growth (Austin and Austin 1999).

The percentage of fish that had at least one potential bacterial pathogen identified (total bacteria prevalence) was significantly higher for impinged fish than for reference fish of all species during both seasons (Tables 10 and 11). In the spring sampling, prevalence ranged from 0.0 to12.1% in reference fish and from 30.3 to 75.6% in the impinged fish. In the fall sample, total bacterial prevalence in reference fish ranged from 0.0 to 33.3% and from 23.9 to 80.0% in impinged fish.

Aeromonas spp. prevalence rates during the spring ranged from 0.0 to 5.9% for reference fish and from 7.7 to 43.9% for impinged fish. During the fall, prevalence ranged from 0.0 to 14.5% for reference fish and from 4.5 to 18.0% for impinged fish. The percentage of fish testing positive for *Aeromonas* spp. were found at a significantly higher prevalence ($P \leq 0.0001$) for impinged blue catfish during the spring sample and for impinged threadfin shad during the spring ($P \leq 0.0001$) and fall ($P \leq 0.0001$; Tables 10-11) as compared with the respective reference species. *Plesiomonas* spp. were found at a significantly higher prevalence ($P \leq 0.02$) for impinged threadfin shad than for reference threadfin shad during the fall. However, the prevalence rates of *Vibrio* spp. were not significantly different for impinged and reference fish (Table 11).

F. columnare prevalence was significantly higher for impinged fish of all species compared with reference fish during both the spring and fall (Tables 10 and 11). Prevalence ranged from 13.0 to 60.9% for impinged fish during the spring and from 16.4 to 74.0% for impinged fish during the fall. All reference fish tested negative for *F. columnare* during both seasons. No significant difference was observed for impinged and reference fish for any other bacterial genus isolated. During both sample periods, additional bacteria that could not be identified to at least the genus level were also isolated but were not considered in any analyses.

Intake Pit Study

A total of 186 fish were collected for necropsy from the intake pit area in the CWIS and compared with both impinged and river reference fish necropsied during the fall (Tables 12 and 13). With the exception of freshwater drum (N = 6), a total of 60 fish of each target species were collected. Due to the low number of freshwater drum, data collected from this species were not used in any analyses. Blue and channel catfish collected from both the river and impinged population during the fall had a significantly greater mean length compared with blue and channel catfish collected from the intake population. Intake pit threadfin shad collected during the fall had a significantly greater mean length when compared with impinged threadfin shad and had a significantly smaller mean length when compared with threadfin shad collected from the river (Tables 12 and 13).

The predicted weights of both blue catfish ($P \leq 0.002$) and channel catfish ($P \leq 0.0001$) from the intake pit were significantly higher at equivalent lengths compared with impinged fish collected during the fall (Figures 15 and 16). The predicted weight of intake pit blue catfish was 18.4% higher compared with impinged blue catfish of equivalent lengths (Figure 15) and intake pit channel catfish weighed 9.5% more at equivalent lengths compared with impinged channel catfish (Figure 16). Treatment was also significant ($P \leq 0.0001$) in the threadfin shad model; however, the predicted weight of intake pit threadfin shad was 12.9% less compared with impinged threadfin shad of equivalent lengths (Figure 17). No interaction between length and treatment was observed in any model for any species; therefore, difference in weight between intake pit fish and impinged fish collected during the fall sample period was consistent across all total lengths.

Blue catfish ($P \leq 0.0001$) and channel catfish ($P \leq 0.0001$) from the intake pit weighed more at equivalent lengths when compared with reference blue catfish and channel catfish collected from the river (Figures 18 and 19). The predicted weight of intake pit blue catfish was 10.1% higher compared with river blue catfish of equivalent lengths (Figure 18) and intake pit channel catfish weighed 12.6% more at equivalent lengths compared with river channel catfish (Figure 19). Significant differences were observed between length, treatment, and interaction between length and treatment ($P \leq 0.0001$) in threadfin shad (Figure 20). However, threadfin shad collected from the intake pit weighed 35.4 to 11.5% less at equivalent length, with the differences inversely related to length, compared with threadfin shad collected from the river (Figure 20). This difference was significant for all lengths collected.

Lesions, as previously defined, were significantly higher for impinged blue catfish ($P \leq 0.0001$) and impinged channel catfish ($P \leq 0.0001$) from the fall compared with the respective fish species collected from the intake pit (Table 14). No significant difference in the prevalence of lesions was observed in reference fish from the riverine population compared with intake pit fish for any species (Table 14). Analysis of parasite prevalence indicated that monogene prevalence was higher for intake blue catfish compared with both impinged and river blue catfish ($P \leq 0.015$ and $P \leq 0.004$, respectively). *Chilodonella* sp. prevalence was significantly ($P \leq 0.002$) higher for impinged threadfin shad compared with threadfin shad from the intake pit (Table 15). However, *I. necator* was significantly more prevalent ($P \leq 0.003$) for threadfin shad from the intake pit compared with threadfin shad from the river (Table 16).

Analysis of bacterial prevalence in intake pit fish compared with impinged fish indicated that percentage of fish with at least one bacterial pathogen (total bacteria) and *F. columnare* was significantly ($P \leq 0.0001$) higher for the three impinged fish species compared with respective fish species from the intake pit (Table 17). *Aeromonas* spp. prevalence was significantly ($P \leq 0.001$) higher for impinged blue catfish compared with blue catfish from the intake pit. Comparison of bacteria prevalence in intake pit fish with respective fish species from the river indicated that the prevalence of at least one bacterial pathogen (total bacteria) was significantly higher for blue catfish ($P \leq 0.002$) and channel catfish ($P \leq 0.015$) from the river compared with fish from the intake pit. *Aeromonas* spp. prevalence was significantly ($P \leq 0.005$) higher for both blue catfish and channel catfish from the river compared with those sampled from the intake pit. Whereas, *Plesiomonas* spp. were significantly ($P \leq 0.009$) higher only for blue catfish from the river compared with blue catfish from the intake pit (Table 18).

VI. DISCUSSION

Environmental factors such as temperature (Griffith and Tomljanovich 1975; McLean et al. 1985), dissolved oxygen (Bodensteiner and Lewis 1992), and turbidity (Lifton and Storr 1978), as well as seasonal and diel movements of fish (Lifton and Storr 1978) have been previously correlated with impingement and may account for much of the variability in observed impingement rates. Disease has also been previously suggested as a factor contributing to impingement (Sprengel and Luchtenberg 1991; Rohlwing et al. 1998) and may also account for some of this variability. Food deprivation (Shoemaker et al. 2003), stress (Fujihara and Olson 1962; Haley et al. 1967; Lom and Dykova 1992; Walters and Plumb 1980), and other environmental characteristics such as water temperature (Pacha and Ordal 1970; Becker and Fujihara1978; Austin and Austin 1999; Roberts 2001) can have an affect on the susceptibility of fish to infectious disease and virulence of pathogens. Therefore, it is important to establish whether fish arrive at the intake structure in a disease state. If this question is addressed, then a portion of the fish collected during impingement may be viewed as representing natural mortality.

In this study, impinged fish collected during both spring and fall 2005 tended to have characteristics indicative of a decrease in general health condition compared with reference fish. These characteristics included a decrease in weight at equivalent length for all species except blue catfish during the fall and channel catfish during both seasons and an increase in external lesions, external parasites, and bacteria. Blue catfish and channel catfish collected from the intake pit had a lower prevalence of bacteria and weighed more at equivalent lengths compared with fish from the river and impinged population (Tables 14-18 and Figures 15-16, 18-19). However, threadfin shad from the intake pit weighed less at equivalent lengths compared with both impinged and river fish and had a significantly higher prevalence of *I. necator* compared with fish from the river.

Weight-length relationship is one method used as an indicator of general health condition in fish (Anderson and Neumann 1996). Although an attempt was made in this study to collect fish with similar lengths between treatments, average lengths between treatments were significantly different. Because of these significant differences, the analyses of weight differences between treatments were adjusted for length using multiple regression techniques. Variables evaluated in the weight-length regression models for impinged and reference fish included length, treatment, and the interaction between length and treatment in an attempt to account for more of the variability in the response of the dependent variable and to adjust for differences in the length of fish collected between treatments. Seasonal differences such as food availability and metabolic demand may have an effect on the weight of a fish (LaJeone and Monzingo 2000) and, therefore, the weight-length models were developed independently for each season.

Predicted weight at equivalent lengths was significantly less (7% to 31%, depending upon species) for impinged freshwater drum, threadfin shad, and blue catfish during the spring and for threadfin shad and freshwater drum during the fall. Significant interaction between length and treatment was detected in the freshwater drum model comparing reference fish from the river with impinged fish for both seasons and in the threadfin shad model comparing fish from the intake pit with river fish. A decrease in weight at equivalent lengths in impinged fish had been previously reported for alewives (Rodgers 1995), Atlantic tomcod (*Microgadus tomcod*) (Texas Instruments Ecological Services 1980), and in freshwater drum (Lajeone and Monzingo 2000). It is possible that sustained swimming required to avoid impingement resulted in decreased weights, at equivalent lengths, for impinged fish (Texas Instruments Inc. 1980). However, blue catfish and channel catfish collected from the intake pit at Plant Barry actually weighed more at equivalent lengths compared with the same species collected from the impinged and river population (Figures 15-16, 18-19). Severely emaciated blue catfish have been previously observed by Alabama Power and Auburn University research staff while conducting impingement sampling during the winter months (unpublished data). Similar observations of thin, emaciated fish have been made by LaJeone and Monzingo (2000) in freshwater drum impinged at Quad Cities Station (Cordova, Illinois).

Fish length has also been correlated with ability to maintain position at different water velocities (Rulifson 1977) and has also been observed as a factor affecting impingement (Bodensteiner and Lewis 1992). Many of the fish impinged at 5 of the 6 Alabama Power coal-fired steam plants were smaller length classes with 94% of threadfin shad less than 100 mm in total length, 64% of freshwater drum less than 140 mm in total length, and 68% of blue catfish less than 100 mm in total length (D. Saalfeld, Auburn University, AL, unpublished data). It is possible that decreased weight at equivalent length may be increasing the likelihood of these smaller length class fish becoming impinged.

No significant treatment effect in liver weight analyses was observed for species sampled in this study. However, Rodgers (1995) observed that impinged alewives had lower liver weights at equivalent lengths when compared with reference alewives. He stated that his evidence was inconclusive as to whether this was a result of exhaustion due to maintained swimming in an attempt to avoid impingement or if the fish arrived at the intake in the observed condition. Further research needs to be conducted to determine whether nutritional status plays a role in impingement at specific size intervals, time of the year, or whether LSI is an indicator of a health condition that may have a bearing on the susceptibility to impingement.

Lesions were observed in many of the impinged fish (14.5% to 73.2% depending upon species and season) and had a significantly higher prevalence compared with reference fish (Table 8). These lesions were typical of those caused by some bacterial pathogens (Pacha and Ordal 1970; Austin and Austin 1999) and did not appear to be mechanical in nature. Lesions observed included eroded fins and fin bases, and erosion of the entire caudal fin. This type of lesion would have an effect on the ability of a fish to swim and avoid impingement. Other lesions observed included skin depigmentation, necrotic gill filaments, ulcerative areas, and apparent hemorrhaging. While it is possible that contact with the intake screen could cause a fish to be more vulnerable to infectious disease, it is likely that a fish unable to maintain swimming position relative to the intake flow would become quickly impinged and would not have time to develop necrotic lesions following bacterial infection. Additionally, a low prevalence of lesions among fish collected from the intake pit supports the conclusion that lesions on impinged fish were not mechanically induced (Table 14). Further support for this conclusion was provided by the prevalence of pathogens in association with observed lesions on impinged fish as well as the significantly higher number of lesions observed for impinged fish compared with fish from the intake pit. Similarly, Rohlwing et al. (1998) concluded that fish were making contact with the intake screen due to infection by parasites as opposed to becoming infected due to contact with the screen.

The prevalence of two protozoan parasites in this study, *Ichthyobodo necator* and *Chilodonella* spp., was significantly higher for impinged freshwater drum and threadfin shad relative to reference fish indicating a selective impingement of infected fish. *Ichthyobodo necator*, known to injure epithelial cells of fish (Lom and Dykova 1992), was found in many of the cutaneous and gill lesions of freshwater drum (Tables 6 and 7). Although the data are not presented, 21% of freshwater drum during the spring and 18% during the fall had moderate to heavy infestation of *I. necator*. When histological sections of gill were evaluated to verify infection levels, evident epithelial hyperplasia was observed in fish with moderate and heavy infections of the parasite. Parasitic infestation of *Ichthyophonus* sp. has been shown to significantly impair the swimming ability of fish (Tierney and Farrell 2004). It is possible that the infestations of *I. necator* reported in our study with freshwater drum and threadfin shad may have had an affect on swimming ability and increased the risk of impingement.

Flavobacterium columnare, *Aeromonas* spp., and *Plesiomonas* sp. were detected at a significantly higher prevalence for impinged fish (13 to 74%) compared with reference fish and intake pit fish. Prevalence of *F. columnare* was significantly higher for all impinged fish collected during both sampling periods and the advanced external necrotic lesions found on many of the impinged fish were typical of columnaris disease (Austin and Austin 1999; Pacha and Ordal 1970; Roberts 2001). Prevalence rates of *F. columnare* for wild fish populations similar to those detected for impinged and for reference fish in this study have been previously reported with ranges from as low as 0-1.5% to as high as 75-93% (Fujihara et al. 1964; Becker and Fujihara 1978; Bowser 1973; Pacha and Ordal 1970). However, the type of media used in the above studies could contribute to a failure to culture the bacterium. Becker and Fujihara (1978) reported using plate agar made with 1% nobel agar, 0.4% bactotryptone, and 0.04% bacto-yeast extract at a final pH of 7.3. Whereas, isolation of *F. columnare* in the research presented here was enhanced by the use of a selective medium (Bullock et al. 1986). Additionally, gills were not routinely sampled in this study when attempting to culture bacteria unless necrotic lesion were observed in the tissue. If gills had been sampled from all fish necropsied in this study it is possible that more *F. columnare* could have been isolated from both treatment groups.

Aeromonas spp. prevalence was significantly higher for impinged blue catfish during the spring and in threadfin shad during both the spring and fall compared with reference fish. Prevalence rates as high as 35% were detected for blue catfish collected from the river during the fall. Hawke (1974) reported prevalences of *Aeromonas* spp.

infections for samples comprised of 15 species, collected from 1973 to 1974 at Logan Martin Lake, Alabama, as high as 25% for apparently healthy fish. It is possible that infected fish collected from the Mobile River, that appeared healthy in this study, were not adversely affected. Additional investigation, such as histological analysis, would need to be performed to make that determination. Many of the impinged fish sampled in this study had both an *Aeromonas* infection and external lesions indicating disease. An *Aeromonas* sp., has been previously reported with mortalities of gizzard shad (Toranzo et al. 1989), and in an epizootic involving threadfin shad (Haley et al. 1967). *Plesiomonas* sp. was also found at a significantly higher rate (7%) for impinged threadfin shad during the fall. This rate was similar to reference channel catfish collected during the spring and was lower than the prevalence in both river blue catfish and river channel catfish collected during the fall (Tables 10 and 11). Its potential role in the selective impingement of threadfin shad, however, is unknown.

The intake pit fish population represents one that needs to be further investigated in an extended concurrent sampling effort along with impinged and river fish. Weight-length regression analyses of channel catfish and blue catfish indicated that, at equivalent lengths, impinged fish weighed less than fish collected from the intake pit (Figures 15-16). Blue catfish and channel catfish collected from the intake pit had a significantly ($P \leq 0.017$) lower prevalence of pathogens (except for mongenes in blue catfish) compared with the same species collected from the river and impinged population, but threadfin shad from the intake pit had a significantly ($P \leq 0.003$) higher prevalence of *I. necator* compared with threadfin shad from the river (Tables 15-18) and weighed significantly less at equivalent lengths compared with both river and impinged threadfin shad (Figures 17 and 20). Threadfin shad may not have followed the trend exhibited by both catfish species due to species specific differences such as feeding characteristics. It is possible that only threadfin shad which are sick are pulled into the intake environment and are not represented there as a stable population. However, both blue catfish and channel catfish intake populations may represent a stable population benefiting from feeding opportunities provided by the intake environment. Because fish from the intake pit were only collected during a 3-d period, results may not indicate health condition through out the year. Additionally, research needs to be performed to investigate whether fish can freely move into and out of the intake structures and to establish whether debilitated fish become trapped in the current caused by the intake and become impinged. The ability of fish to leave the intake has been documented in the case of alewives. Hutcheson (1981) introduced marked alewives and rainbow trout in the intake area of Pickering Nuclear Generating Station (Lake Ontario, Canada) and was able to recover only a small percentage of the fish over a two-week time frame. It is likely that the fish left the area prior to becoming impinged (Rogers 1995). It is also possible that the numbers of fish living in the intake are in a high density situation and that the intake screens are selecting for diseased individuals within that population.

Future studies should be conducted at other power plants to investigate disease prevalence in impinged fish. Also, studies should be repeated at Plant Barry to see if this is a reoccurring phenomenon in other seasons and years and to determine if diseases change from season to season or year to year. The effect of seasonal factors such as water temperature on rates of infectious disease has been well documented (Pacha and Ordal 1970; Bowser 1973; Roberts 2001). It is possible that as water temperatures change, the distribution or prevalence of pathogens that may be contributing to impingement of fish may also change. It is also possible, as water temperatures change with season, that nutritional status may become more important than infectious diseases as a variable affecting impingement, thus, resulting in a more pronounced difference in weight at equivalent lengths between impinged and reference fish.

Disease rates in this study were higher for impinged fish than for reference fish and indicated a selective impingement of unhealthy fish. Additionally, it is possible that additional pathogens were in the impinged fish but were not detected because of the methods used. For example, fish were not examined for viral infections and internal parasites. This study indicated that the rate of impingement at a CWIS may be a continuous predictor of the general health of the local fish community because the intake screens may be selecting for and concentrating the dead and sick fish that populate the HZI. However, future research should investigate both the condition of fish living in the intake pit area as well as establishing whether fish are able to leave the intake prior to impingement. Sampling should also be performed to investigate whether diseased fish are caught in the intake drift or HZI to determine whether impingement selects for unhealthy fish from the natural environment.

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Table 1. Total length and weight (mean ± sd; ranges in parentheses) of fish collected at and near Plant Barry during the spring 2005. Data include fish where only metrics were recorded. Different letters for the same species within columns indicate significant ($P \leq 0.05$) differences in mean length between treatments.

Species *Treatment*	*Total* N	*Total length (mm)*	*Total* N	*Weight (g)*
Blue catfish				
Reference	156	127±32.7 (77-223) y	156	18.2±16.2 (3.3-81.1)
Impinged	38	152±47.4 (73-281) z	41	34.6±37.1 (2.7-184.3)
Channel catfish				
Reference	166	130±34.7 (71-233) y	166	20.5±18.3 (2.3-94.3)
Impinged	62	95±33.7 (57-231) z	65	9.3±15.3 (1.4-86.6)
Freshwater drum				
Reference	25	214±101.8 (91-359) y	25	228.9±232.5 (7.7-648.8)
Impinged	26	167±83.5 (80-307) y	26	81.1±94.4 (3.7-259.1)
Threadfin shad				
Reference	240	102±11.9 (86-148) y	240	9.3±4.6 (5.2-35.9)
Impinged	70	106±11.3 (85-142) z	76	9.9±5.0 (4.7-39.4)

Table 2. Total length and weight (mean ± sd; ranges in parentheses) of fish collected at and near Plant Barry during the fall 2005. Data include fish where only metrics were recorded. Different letters for the same species within columns indicate significant ($P \leq 0.05$) differences in mean length between treatments.

Species *Treatment*	*Total* N	*Total length* *(mm)*	*Total* N	*Weight* *(g)*
Blue catfish				
Reference	55	114±21.2 (73-190) y	55	10.4±6.4 (2.5-41.3)
Impinged	58	130±61.5 (64-339) y	67	24.5±43.7 (1.8-302.5)
Channel catfish				
Reference	57	122±40.1 (51-220) y	57	14.6±14.5 (0.8-61.0)
Impinged	36	104±44.9 (46-211) z	38	11.2±13.7 (0.5-63.6)
Freshwater drum				
Reference	30	117±44.5 (94-274) y	30	23.0±46.1 (6.3-228.6)
Impinged	89	83±20.3 (49-175) z	92	5.4±6.2 (1.3-43.2)
Threadfin shad				
Reference	97	93±8.6 (73-117) y	97	7.0±2.1 (2.8-14.6)
Impinged	152	72±4.6 (60-85) z	153	2.9±0.7 (1.4-5.3)

Table 3. Sample size (N) and liver somatic index (LSI; mean ±sd) (range in parenthesis) for impinged (*i*) and river (*r*) fish collected at and near Plant Barry during the spring of 2005. Regression equations for each species are presented after the table. No significant differences in the predicted liver weight of impinged fish compared with river fish were detected for any species.

Sample size	*Blue catfish*	*Channel catfish*	*Freshwater drum*
N*r*	50	64	25
N*i*	35	50	16
Liver somatic index			
LSI*r*	1.8 ±0.47 (0.71-3.1)	1.8 ±0.55 (0.51-3.3)	1.5 ±0.63 (0.70-3.3)
LSI*i*	1.9 ±0.47 (0.75-3.0)	2.3 ±1.0 (0.39-5.0)	1.7 ±0.63 (0.64-2.5)

Regression equations:

Blue catfish: Ln liver weight= -4.06 + 1.007(ln body weight) + 0.061(treatment)

Channel catfish: Ln liver weight = -3.587 + 0.833(ln body weight) - 0.007(treatment)

Freshwater drum: Ln liver weight = -3.678 + 0.863(ln body weight) + 0.032(treatment)

Where: Treatment = 0 for reference fish and Treatment = 1 for impinged fish.

Table 4. Sample size (N) and liver somatic index (LSI; mean ±sd) (range in parenthesis) for impinged (*i*) and river (*r*) fish collected at and near Plant Barry during the fall of 2005. Regression equations for each species are presented after the table. No significant differences in the predicted liver weight of impinged fish compared with river fish were detected for any species.

Sample size	*Blue catfish*	*Channel catfish*
N*r*	53	54
N*i*	46	24
Liver somatic index		
LSI*r*	2.5 ±1.7 (0.77-10.8)	1.5 ±0.49 (0.82-2.9)
LSI*i*	2.6 ±2.6 (0.91-13.5)	2.0 ±2.7 (0.77-14.3)

Regression equations:

Blue catfish: Ln liver weight= -3.44 + 0.802(ln body weight) + 0.034(treatment)

Channel catfish: Ln liver weight = -3.907 + 0.836(ln body weight) + 0.101(treatment)

Where: Treatment = 0 for reference fish and Treatment = 1 for impinged fish.

Table 5. Number of fish collected at and near Plant Barry that were necropsied.

	Spring		*Fall*	
Species	Impinged	Reference	Impinged	Reference
Blue catfish	41	51	50	55
Channel catfish	66	66	31	57
Freshwater drum	26	25	67	30
Threadfin shad	76	100	100	97

Table 6. Percentage of fish collected at and near Plant Barry that had lesions on either the skin or gills. Different letters within the same row indicates a significantly ($P \leq 0.05$) higher prevalence.

	Treatment	
Fish species Season	Reference (%)	Impinged (%)
Blue catfish		
Spring	0.0 y	73.2 z
Fall	0.0 y	64.0 z
Channel catfish		
Spring	0.0 y	44.6 z
Fall	1.8 y	38.7 z
Freshwater drum		
Spring	0.0 y	38.5 z
Fall	0.0 y	25.4 z
Threadfin shad		
Spring	0.0 y	14.5 z
Fall	0.0 y	2.0 y

Table 7. Percentage of impinged fish, sampled during the spring and fall of 2005, with lesions that had a pathogen detected in association with the lesion. Pathogens were the protozoan parasite *Ichthyobodo necator* and the bacteria *F. columnare* and *Aeromonas* spp.

Species *Season*	*Number having lesions*	*Number having pathogen*	*Percent with pathogen (%)*
Blue catfish			
Spring	30	28	93
Fall	32	30	94
Channel catfish			
Spring	29	18	62
Fall	12	10	83
Freshwater drum			
Spring	10	8	80
Fall	17	15	88
Threadfin shad			
Spring	11	11	100
Fall	2	2	100

Table 8. Parasites observed on gill or skin of fish collected at and near Plant Barry during the spring 2005. Reference (Ref) and impinged (Imp) fish are compared for each fish species. Sample size is in parentheses.

	Fish Species							
	Blue catfish		Channel catfish		Freshwater drum		Threadfin shad	
Parasite	Ref (51) (%)	Imp (41) (%)	Ref (66) (%)	Imp (66) (%)	Ref (25) (%)	Imp (26) (%)	Ref (100) (%)	Imp (76) (%)
Apiosoma sp.	0	0	0	0	0	3.8	0	0
Henneguya sp.	27.5	31.7	50.0	31.8	0%	3.8	1.0	3.9
Ichthyobodo necator	0	7.3	0	6.1	4.0	50.0*	2.0	9.2*
Monogenea	31.4	31.7	27.3	27.3	0	3.8	0	0
Ambiphrya sp.	1.9	0	0	1.5	0	0	1.0	0
Chilodonella sp.	0	0	0	0	0	0	0	0
Tetrahymena sp.	0	0	1.5	0	0	0	0	1.3
Trichodina sp.	13.7	4.9	4.5	9.1	0	0	0	1.3
Trichophrya sp.	21.6	17.1	1.5	9.1	8.0	11.5	4.0	3.9

* Prevalence significantly ($P \leq 0.05$) greater in impinged fish compared with reference fish..

Table 9. Parasites observed on gill or skin of fish collected at and near Plant Barry during the fall 2005. Reference (Ref) and impinged (Imp) fish are compared for each fish species. Sample size is in parentheses.

	Fish Species							
	Blue catfish		Channel catfish		Freshwater drum		Threadfin shad	
Parasite	Ref (55) (%)	Imp (50) (%)	Ref (57) (%)	Imp (31) (%)	Ref (30) (%)	Imp (67) (%)	Ref (97) (%)	Imp (100) (%)
Apiosoma sp.	0	2.0	0	0	0	0	0	0
Henneguya sp.	5.5	2.0	10.5	6.5	0	0	1.0	3.0
Ichthyobodo necator	0	0	0	0	0	32.8*	0	3.0
Monogenea	18.2	22.0	1.8	6.5	0	0	0	0
Chilodonella sp.	0	0	0	0	0	0	0	14.0*
Trichodina sp.	1.8	4.0	3.5	3.2	6.7	0	0	0
Trichophrya sp.	7.3	14.0	1.8	9.7	10.0	14.9	0	1.0

* Prevalence significantly ($P \leq 0.05$) greater in impinged fish compared with reference fish.

Table 10. Percentage of fish collected during spring 2005 at and near Plant Barry with bacteria isolated and identified. Total bacteria is the percentage of fish with at least one identified bacterial pathogen. Reference (Ref) and impinged (Imp) fish are compared for each fish species. Sample size is in parentheses.

	Fish Species							
	Blue catfish		Channel catfish		Freshwater drum		Threadfin shad	
Bacteria	Ref (51) (%)	Imp (41) (%)	Ref (66) (%)	Imp (66) (%)	Ref (25) (%)	Imp (26) (%)	Ref (100) (%)	Imp (76) (%)
Total bacteria	9.8	75.6*	12.1	39.4*	0	34.6*	4.0	30.3*
Aeromonas spp.	5.9	43.9*	3.0	10.6	0	7.7	1.0	18.4*
Citrobacter sp.	1.9	0	0	0	0	0	0	0
Flavobacterium columnare	0	60.9*	0	30.3*	0	30.8*	0	13.0*
Plesiomonas spp.	1.9	0	7.6	4.5	0	0	1.0	1.3
Staphylococcus spp.	0	0	0	0	0	0	1.0	0
Photobacterium spp.	0	0	0	0	0	0	1.0	0
Pseudomonas spp.	0	0	1.5	1.5	0	0	0	1.3
Serratia spp.	0	0	0	0	0	0	0	1.3
Shigella spp.	0	0	0	1.5	0	0	0	0

* Prevalence significantly ($P \leq 0.05$) greater in impinged fish compared with reference fish.

Table 11. Percentage of fish collected during fall 2005 at and near Plant Barry with bacteria isolated and identified. Total bacteria is the percentage of fish with at least one identified bacterial pathogen. Reference (Ref) and impinged (Imp) fish are compared for each fish species. Sample size is in parentheses.

	Fish Species							
	Blue catfish		Channel catfish		Freshwater drum		Threadfin shad	
Bacteria	Ref (55) (%)	Imp (50) (%)	Ref (57) (%)	Imp (31) (%)	Ref (30) (%)	Imp (67) (%)	Ref (97) (%)	Imp (100) (%)
Total bacteria	32.7	80.0*	33.3	61.3*	3.3	23.9*	0	60.0*
Aeromonas spp.	14.5	18.0	12.3	9.7	3.3	4.5	0	18.0*
Flavobacterium columnare	0	74.0*	0	41.9*	0	16.4*	0	35.0*
Edwardisella spp.	0	2.0	0	3.2	0	0	0	0
Plesiomonas spp.	16.4	6.0	14.0	9.7	0	0	0	7.0*
Staphylococcus spp.	0	0	0	0	0	1.5	0	0
Streptococcus spp.	0	0	1.8	0	0	0	0	0
Enterobacter spp.	0	0	0	0	0	0	0	2.0
Vibrio spp.	1.8	2.0	5.3	6.5	0	3.0	0	1.0

* Prevalence significantly ($P \leq 0.05$) greater in impinged fish compared with reference fish.

Table 12. Total length and weight (mean ±sd; ranges in parentheses) for intake pit and impinged fish species collected during the fall of 2005. Different letters for the same species within columns indicate significant ($P \leq 0.0017$) differences in mean length between treatments.

Species *Treatment*	*Total* N	*Mean Length* *(mm)*	*Total* N	*Mean weight* *(g)*
Blue catfish				
Intake pit	60	98.0±25.8 (59-180) y	60	8.5±8.9 (1.5-47.4)
Impinged	41	136.0±66.1 (64-339) z	50	27.7±49.4 (1.8-302.5)
Channel catfish				
Intake pit	60	77.0±21.5 (52-181) y	60	4.3±5.3 (1.1-39.6)
Impinged	29	106.0±46.5 (46-211) z	31	12.1±14.5 (0.5-63.6)
Threadfin shad				
Intake pit	60	82.0±8.1 (70-99) y	60	3.8±1.5 (1.9-7.4)
Impinged	99	73.0±4.4 (61-85) z	100	2.9±0.7 (1.6-5.3)

Table 13. Total length and weight (mean ±sd; ranges in parentheses) for intake pit and river fish species collected during the fall of 2005. Different letters for the same species within columns indicate significant ($P \leq 0.0017$) differences in mean length between treatments.

Species *Treatment*	*Total* N	*Mean Length* *(mm)*	*Total* N	*Mean weight* *(g)*
Blue catfish				
Intake pit	60	98.0±25.8 (59-180) y	60	8.5±8.9 (1.5-47.4)
River	55	114.0±21.2 (73-190) z	55	10.4±6.4 (2.5-41.3)
Channel catfish				
Intake pit	60	77.0±21.6 (52-181) y	60	4.3±5.3 (1.1-39.6)
River	57	122.0±40.1 (51-220) z	57	14.6±14.5 (0.8-61.0)
Threadfin shad				
Intake pit	60	82.0±8.1 (70-99) y	60	3.8±1.5 (1.9-7.4)
River	97	93.0±8.6 (73-117) z	97	7.0±2.1 (2.8-14.6)

Table 14. Percentage of fish collected at and near Plant Barry for the intake pit study that had lesions on either the skin or gills. Intake pit fish were collected over a 3-d period and compared ($P \leq 0.017$) with impinged and river fish that were necropsied during the fall.

	Treatment		
Species	River reference (%)	Intake pit (%)	Impinged (%)
Blue catfish	0.0	0.0	64.0 *
Channel catfish	1.8	1.7	38.7 *
Threadfin shad	0.0	0.0	2.0

* Values were significantly different than other treatments.

Table 15. Parasites observed on gill or skin of fish collected at and near Plant Barry during the fall 2005. Intake pit fish were collected over a 3-d period and compared with impinged (Imp) fish necropsied in the fall. Sample size is in parentheses.

	Fish Species					
	Blue catfish		Channel catfish		Threadfin shad	
Parasite	Intake (60) (%)	Imp (50) (%)	Intake (60) (%)	Imp (31) (%)	Intake (60) (%)	Imp (100) (%)
Apiosoma sp.	0	2.0	0	0	0	0
Henneguya sp.	0	2.0	5.0	6.5	0	0
Ichthyobodo necator	0	0	0	0	10.0	3.0
Monogenea	43.3*	22.0	3.3	6.5	0	0
Ambiphrya sp.	0	0	1.7	0	0	0
Chilodonella sp.	0	0	0	0	0	14.0*
Teterahymena sp.	0	0	0	0	3.3	0
Trichodina sp.	3.3	4.0	1.7	3.2	0	0
Trichophrya sp.	3.3	14.0	8.3	9.7	1.7	1.0

* Prevalence significantly ($P \leq 0.017$) different.

Table 16. Parasites observed on gill or skin of fish collected at and near Plant Barry during the fall 2005. Intake fish were collected over a 3-d period and compared with river fish necropised in the fall. Sample size is in parentheses.

	Fish Species					
	Blue catfish		Channel catfish		Threadfin shad	
Parasite	Intake (60) (%)	River (55) (%)	Intake (60) (%)	River (57) (%)	Intake (60) (%)	River (97) (%)
Henneguya sp.	0	5.5	5.0	10.5	0	1.0
Ichthyobodo necator	0	0	0	0	10.0*	0
Monogenea	43.3*	18.2	3.3	1.8	0	0
Ambiphrya sp.	0	0	1.7	0	0	0
Tetrahymena sp.	0	0	0	0	3.3	0
Trichodina sp.	3.3	1.8	1.7	3.5	0	0
Trichophrya sp.	3.3	7.3	8.3	1.8	1.7	0

* Prevalence significantly ($P \leq 0.017$) greater in intake compared with river.

Table 17. Bacteria isolated in fish collected from the intake pit at and near Plant Barry compared with fish impinged and necropised during the fall of 2005. Total bacteria is the percentage of fish with at least one identified bacterial pathogen. Sample size is in parentheses.

	Fish Species					
	Blue catfish		Channel catfish		Threadfin shad	
Bacteria	Intake (60) (%)	Imp (50) (%)	Intake (60) (%)	Imp (31) (%)	Intake (60) (%)	Imp (100) (%)
Total bacteria	10.0	78.0*	5.0	61.3*	3.3	50.0*
Aeromonas spp.	0	18.0*	0	9.7	1.7	11.0
Flavobacterium columnare	3.3	74.0*	1.7	41.9*	0	33.0*
Edwardsiella spp.	0	0	0	3.2	0	0
Pleiomonas spp.	3.3	4.0	3.3	9.7	1.7	6.0
Streptococcus spp.	3.3	0	0	0	0	0
Enterobacter spp.	0	0	0	0	0	1.0
Vibrio spp.	0	2.0	0	6.5	0	0

* Prevalence significantly ($P \leq 0.017$) greater in impinged compared with intake..

Table 18. Bacteria isolated in fish collected from the intake pit at and near Plant Barry compared with fish collected from the river and necropised during the fall of 2005. Total bacteria is the percentage of fish with at least one identified bacterial pathogen. Sample size is in parentheses.

	Fish Species					
	Blue catfish		Channel catfish		Threadfin shad	
Bacteria	Intake (60) (%)	River (55) (%)	Intake (66) (%)	River (60) (%)	Intake (60) (%)	River (97) (%)
Total bacteria.	10.0	30.9*	5.0	28.1*	3.3	0
Aeromonas spp.	0	12.7*	0	12.3*	1.7	0
Flavobacterium columnare	3.3	0	1.7	0	0	0
Pleiomonas spp.	3.3	16.4*	3.3	10.5	1.7	0
Streptococcus spp.	3.3	0	0	1.8	0	0
Pseudomonas spp.	0	0	0	0	1.7	0
Vibrio spp.	0	1.8	3.5	0	0	0

* Prevalence significantly ($P \leq 0.017$) greater in river compared with intake.

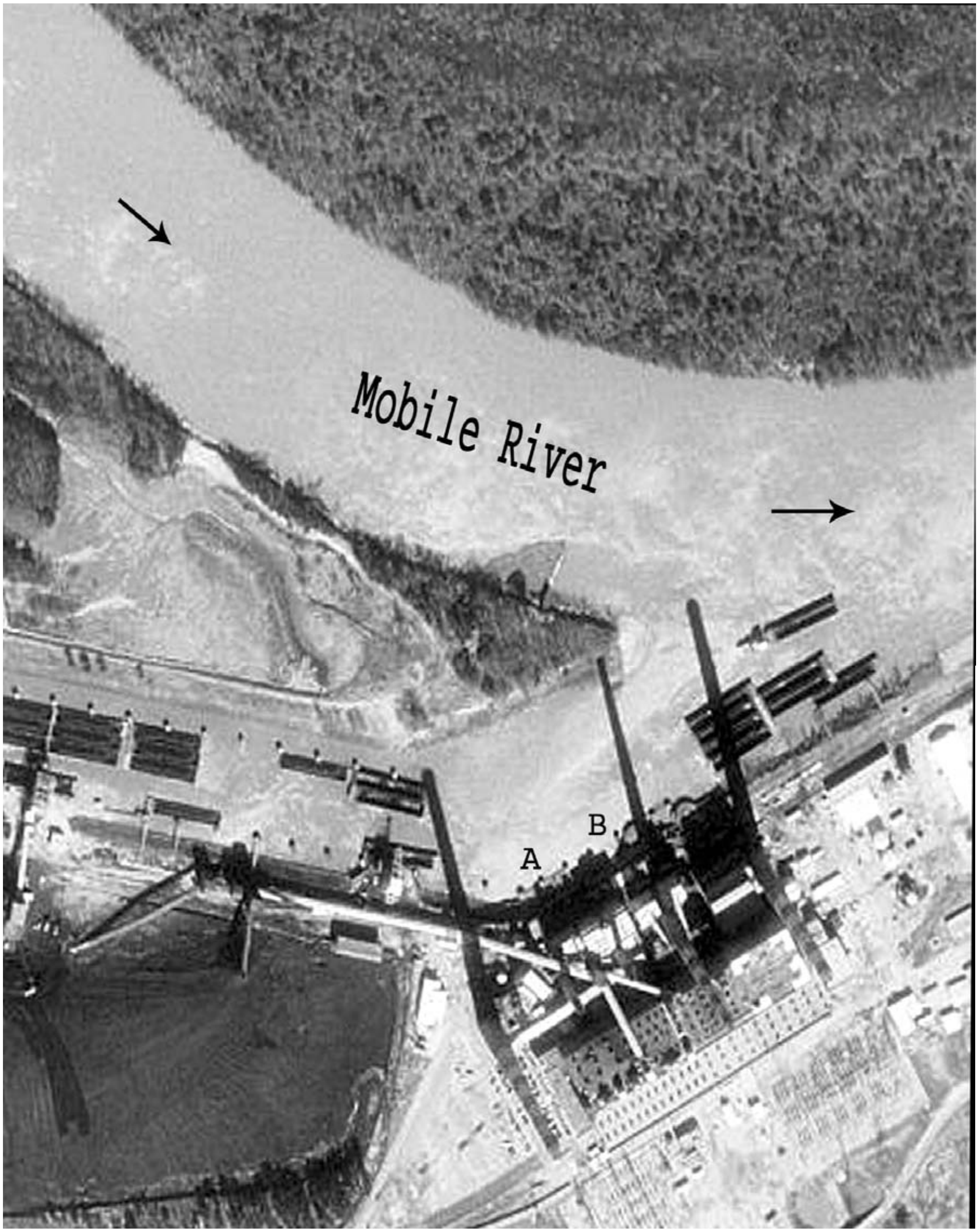

Figure 1. Plant Barry intake structures (A= Units 1-3, B= Units 4-5) and associated intake canal perpendicular to the Mobile River. Arrows indicate direction of river flow.

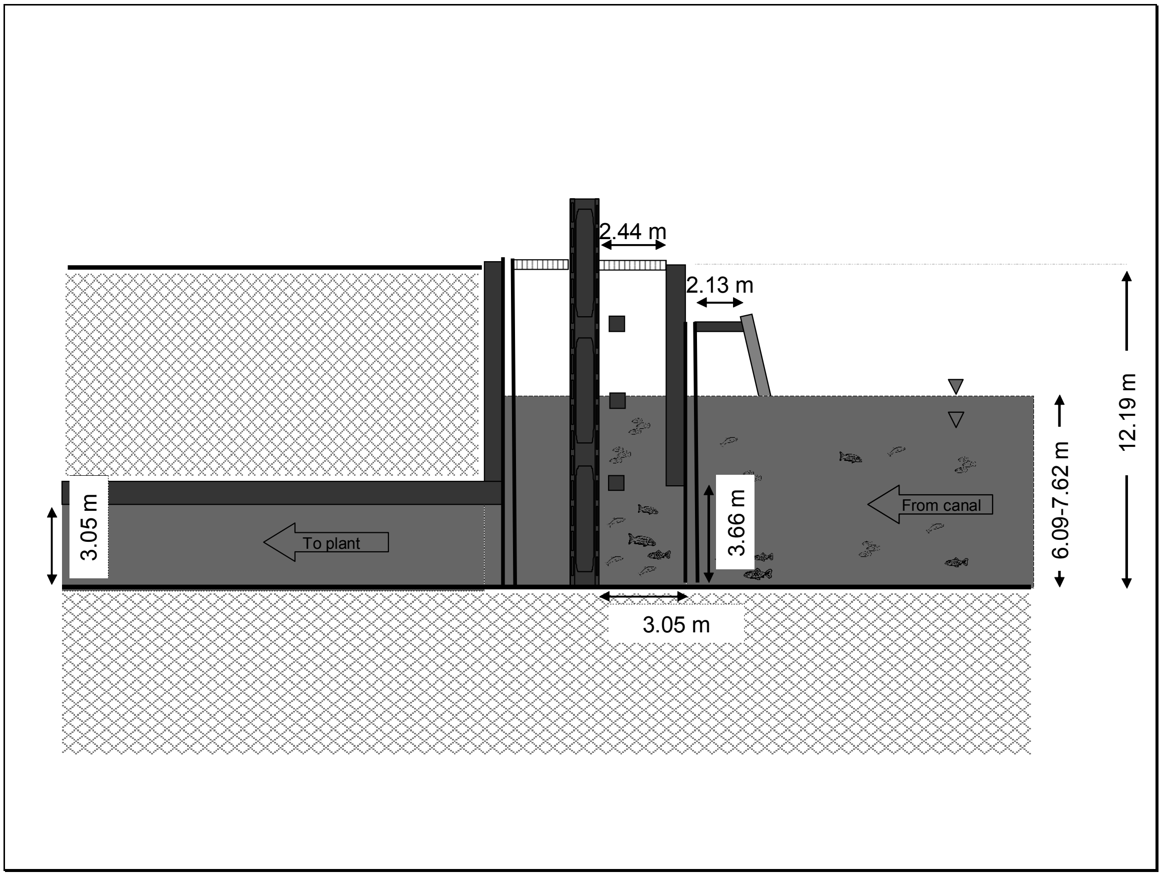

Figure 2. Cooling water intake structure located at Plant Barry intakes. Relevant dimensions are presented in the figure.

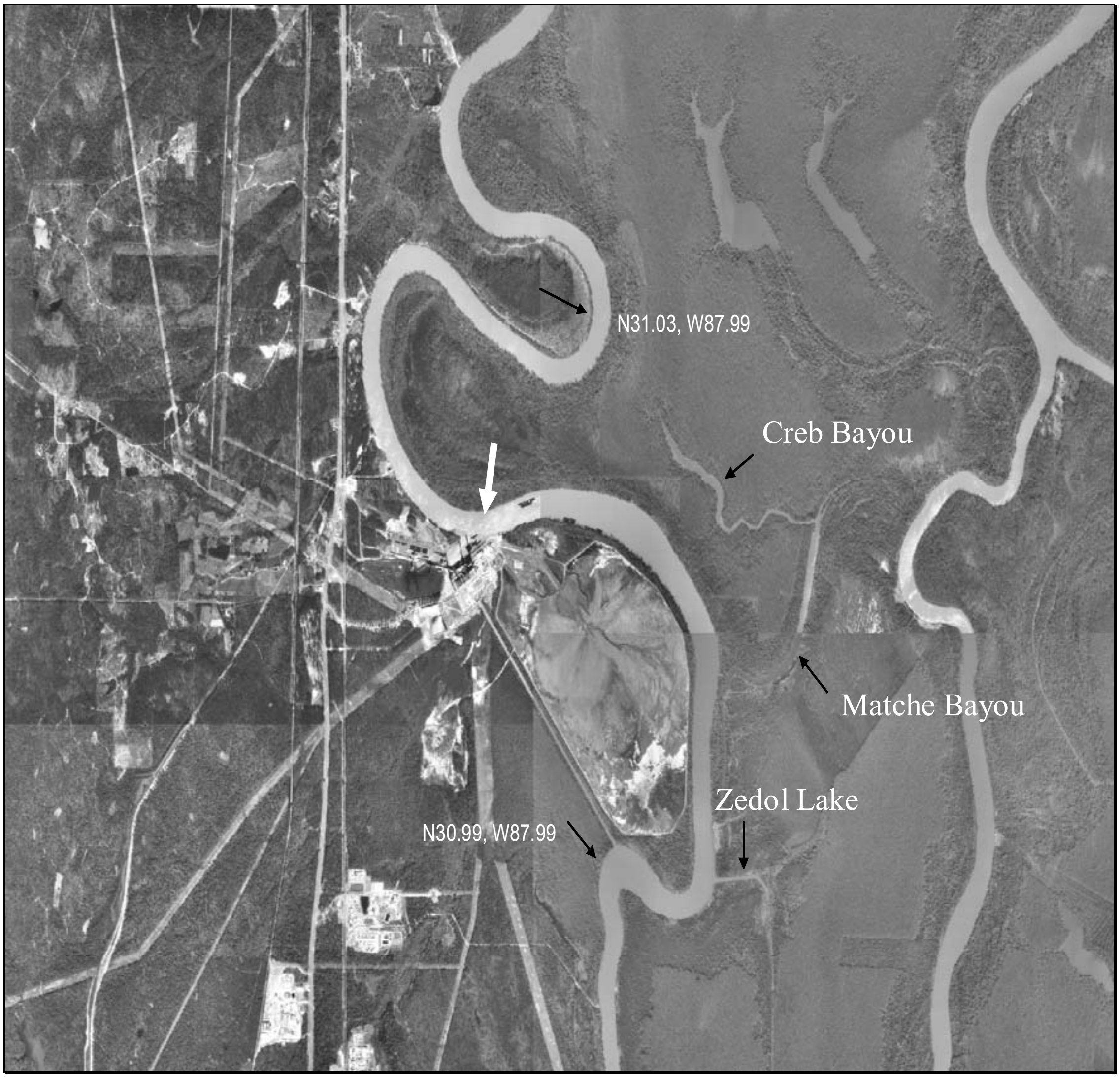

Figure 3. Photo of the Mobile River in the area of Plant Barry. Arrows with latitude longitude coordinates indicate the most upstream and downstream location points for collection of reference fish. Other locations points where reference fish were collected in adjacent water bodies are also indicated with arrows. Plant Barry is indicated with a white arrow.

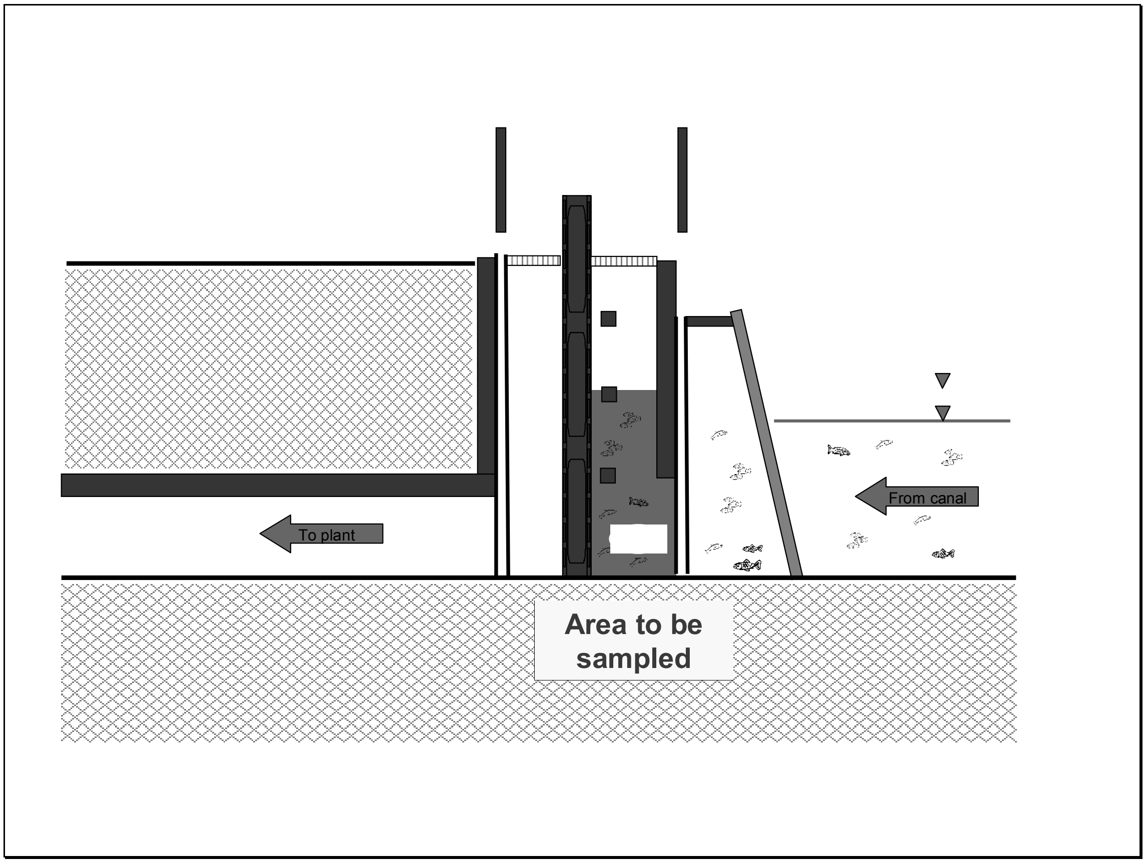

Figure 4. Cross sectional view of an intake structure at Plant Barry Units 4 and 5. The intake pit area that was sampled is shaded.

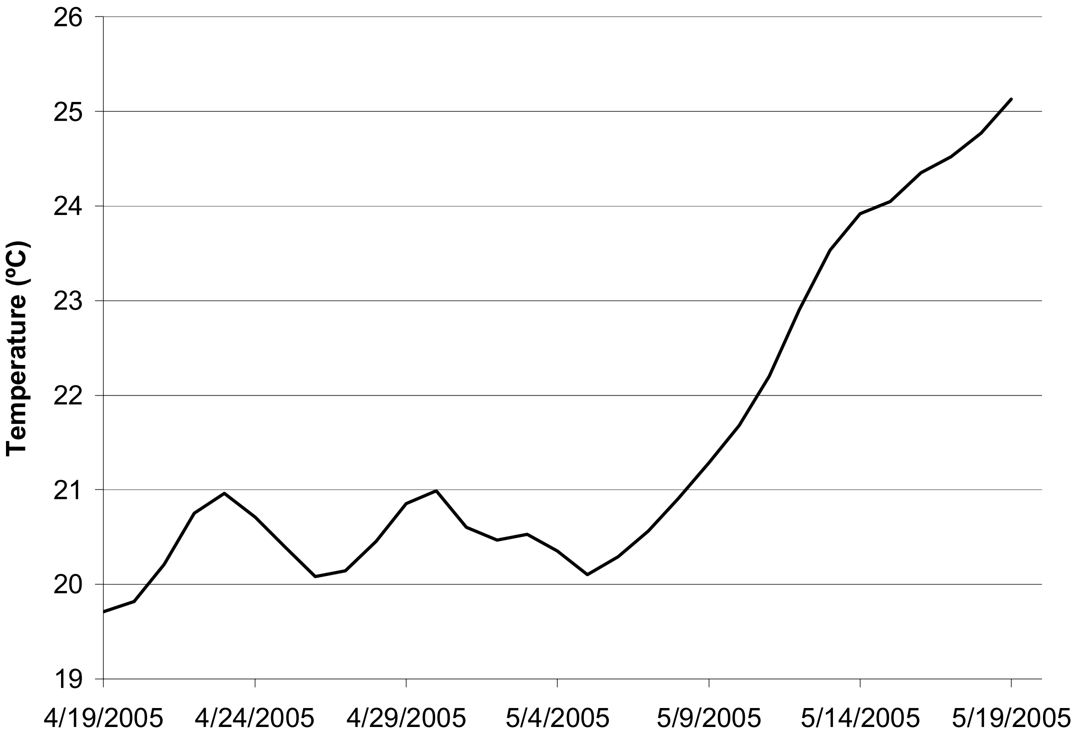

Figure 5. Temperatures for the spring 2005 sample period as measured one meter under the surface of the water in front of the intake structures.

Figure 6. Temperatures for the fall 2005 sample period as measured one meter under the surface of the water in front of the intake structures.

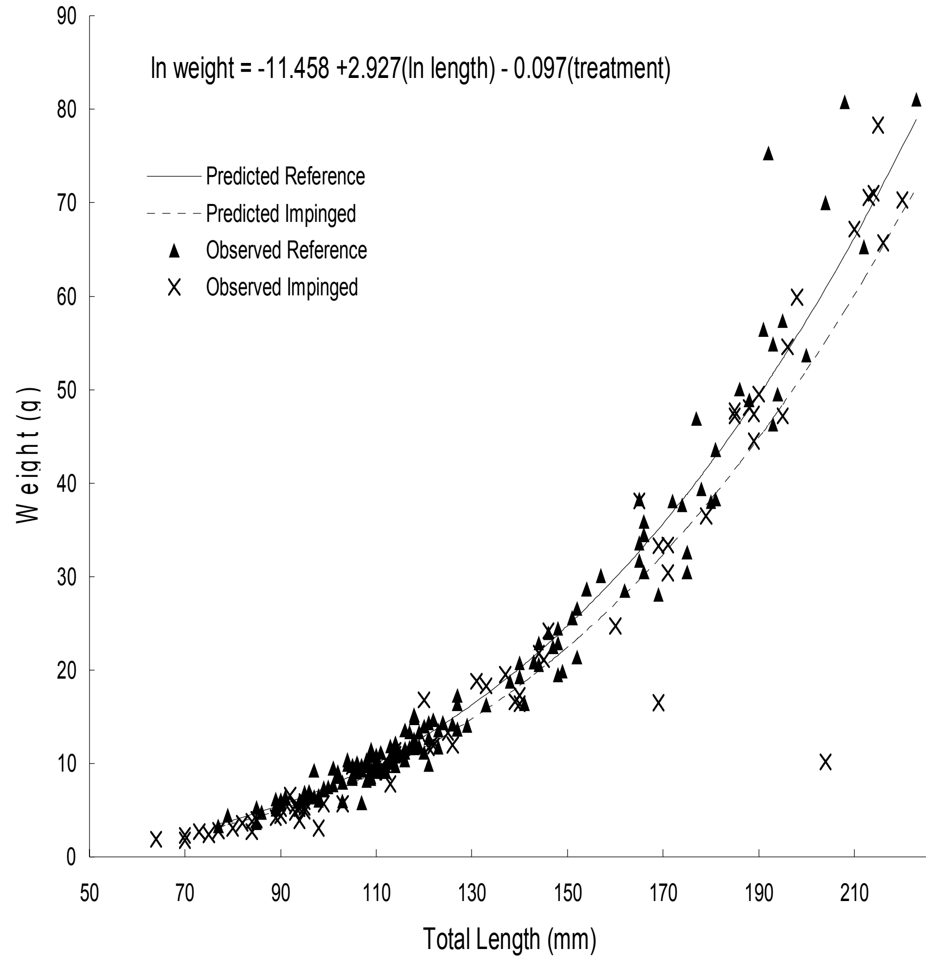

Figure 7. Predicted and observed weights for reference and impinged blue catfish collected at Plant Barry during spring 2005. Predicted weights of impinged fish were significantly different ($P \leq 0.006$) than reference fish.

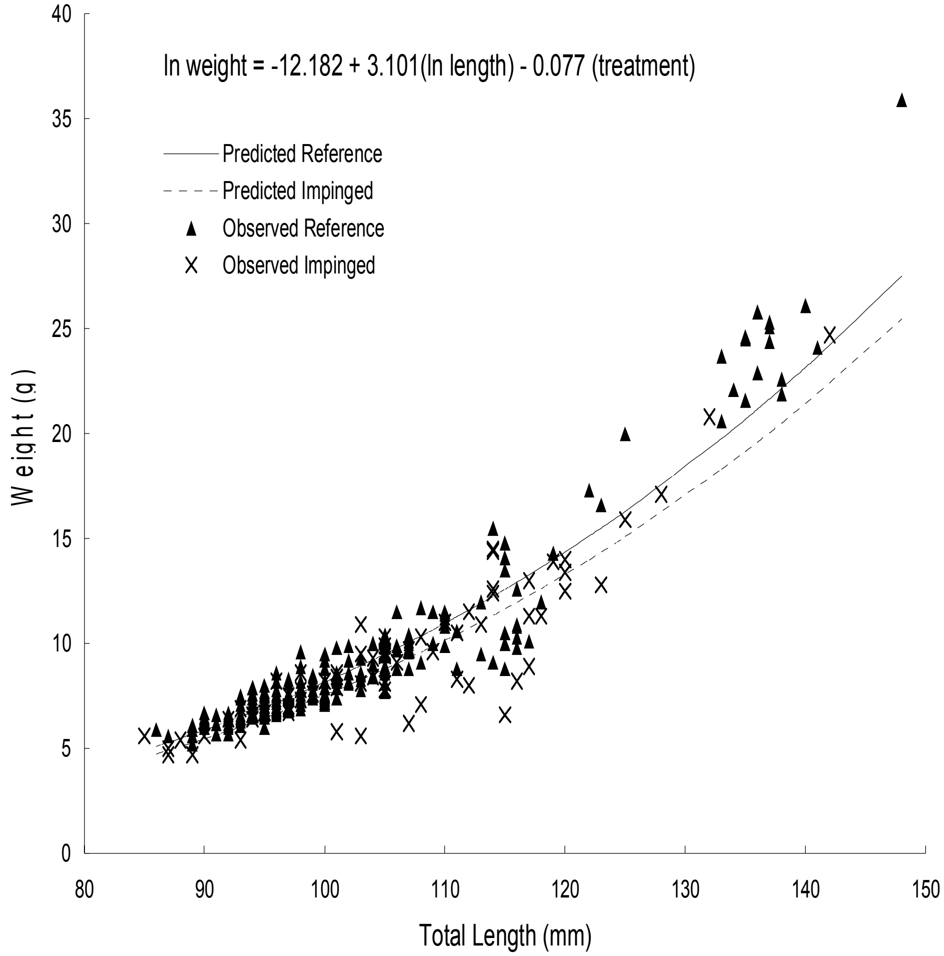

Figure 8. Predicted and observed weights for reference and impinged threadfin shad collected at Plant Barry during spring 2005. Predicted weights of impinged fish were significantly different ($P \leq 0.0001$) than reference fish.

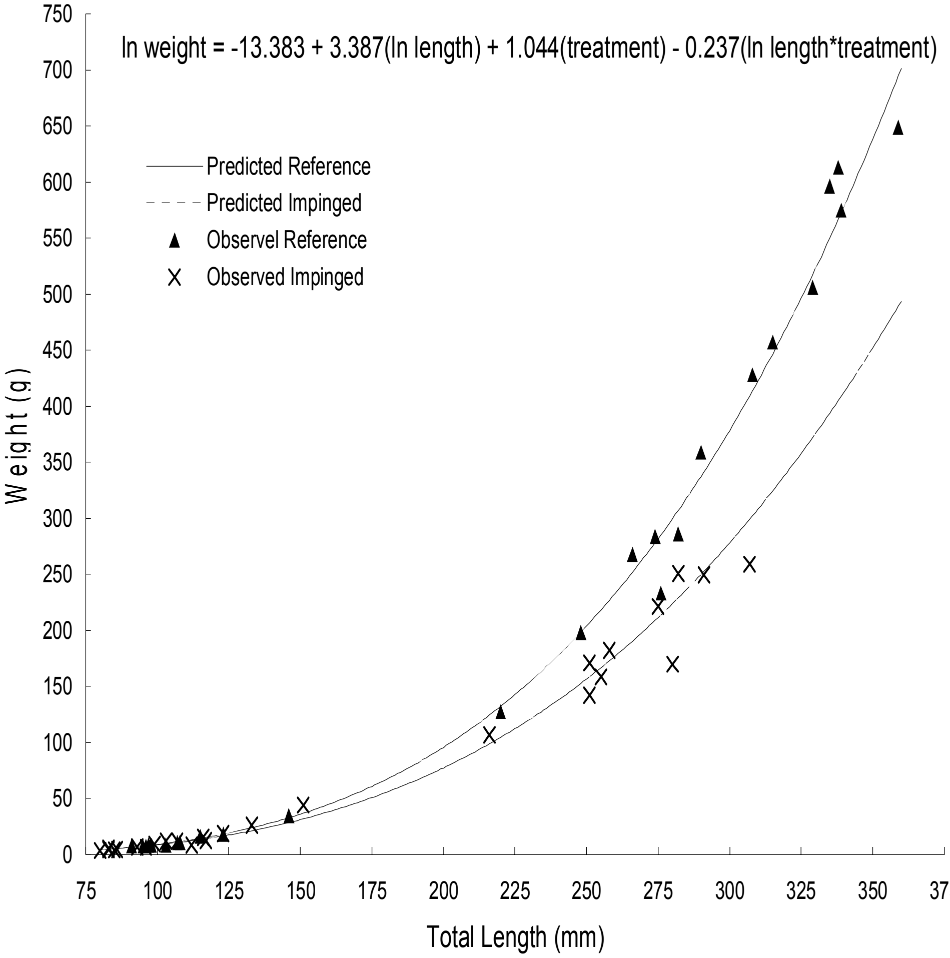

Figure 9. Predicted and observed weights for reference and impinged freshwater drum collected at Plant Barry during spring 2005. Predicted weights of impinged fish were significantly different ($P \leq 0.002$) than reference fish.

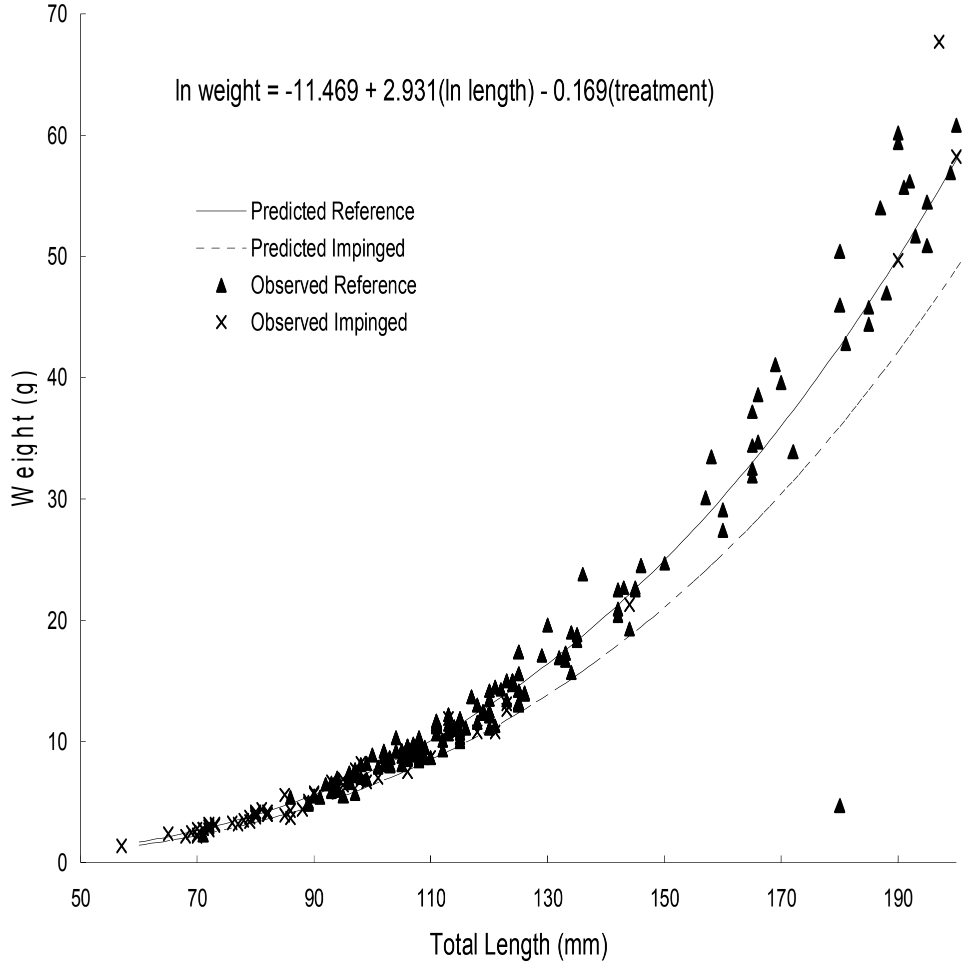

Figure 10. Predicted and observed weights for reference and impinged channel catfish collected at Plant Barry during spring 2005. Treatment was not considered significant in the regression model.

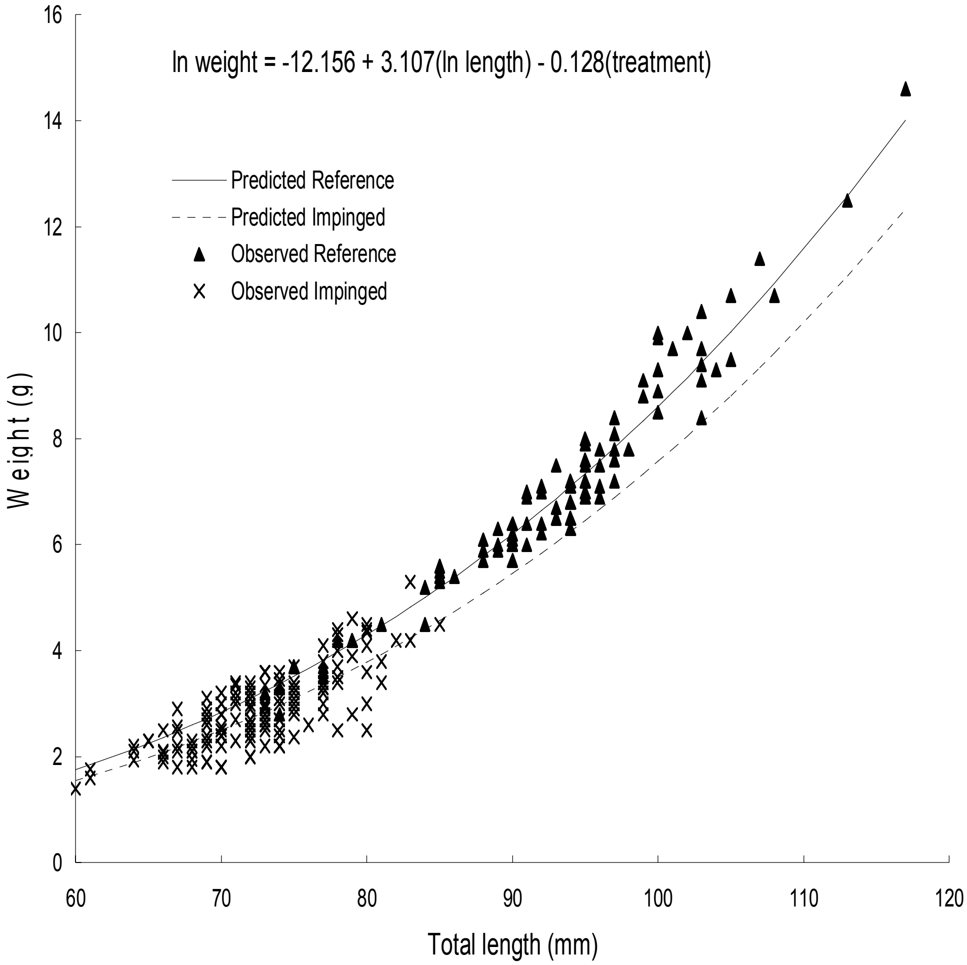

Figure 11. Predicted and observed weights for reference and impinged threadfin shad collected at Plant Barry during fall 2005. Predicted weights of impinged fish were significantly different ($P \leq 0.0001$) than reference fish.

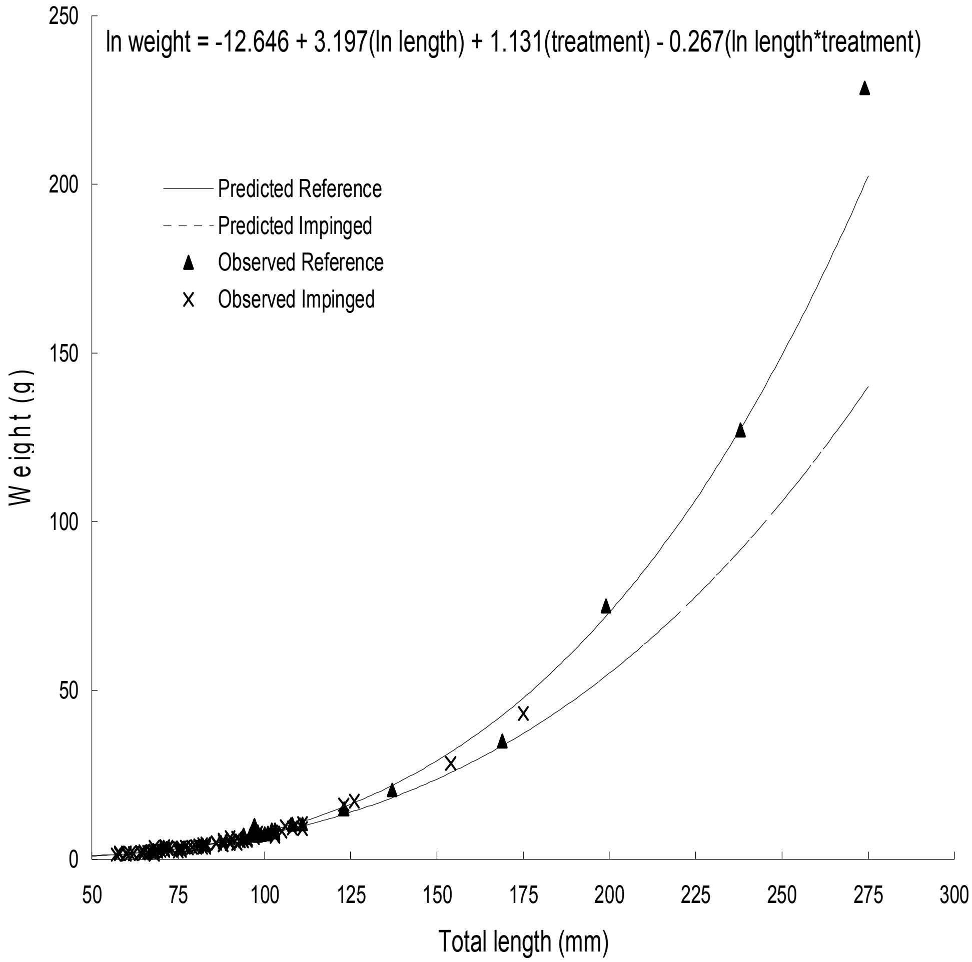

Figure 12. Predicted and observed weights for reference and impinged freshwater drum collected at Plant Barry during fall 2005. Predicted weights of impinged fish were significantly different ($P \leq 0.013$) than reference fish.

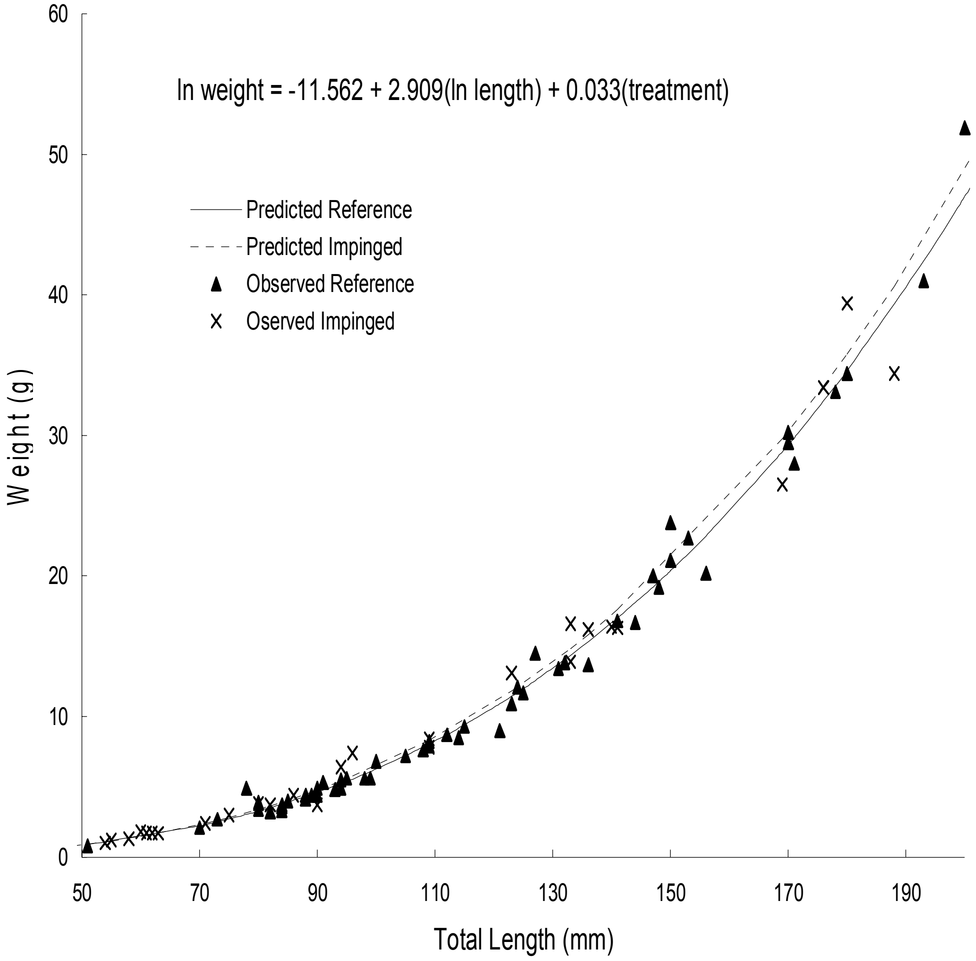

Figure 13. Predicted and observed weights for reference and impinged channel catfish collected at Plant Barry during fall 2005. Treatment was not considered significant in the regression model.

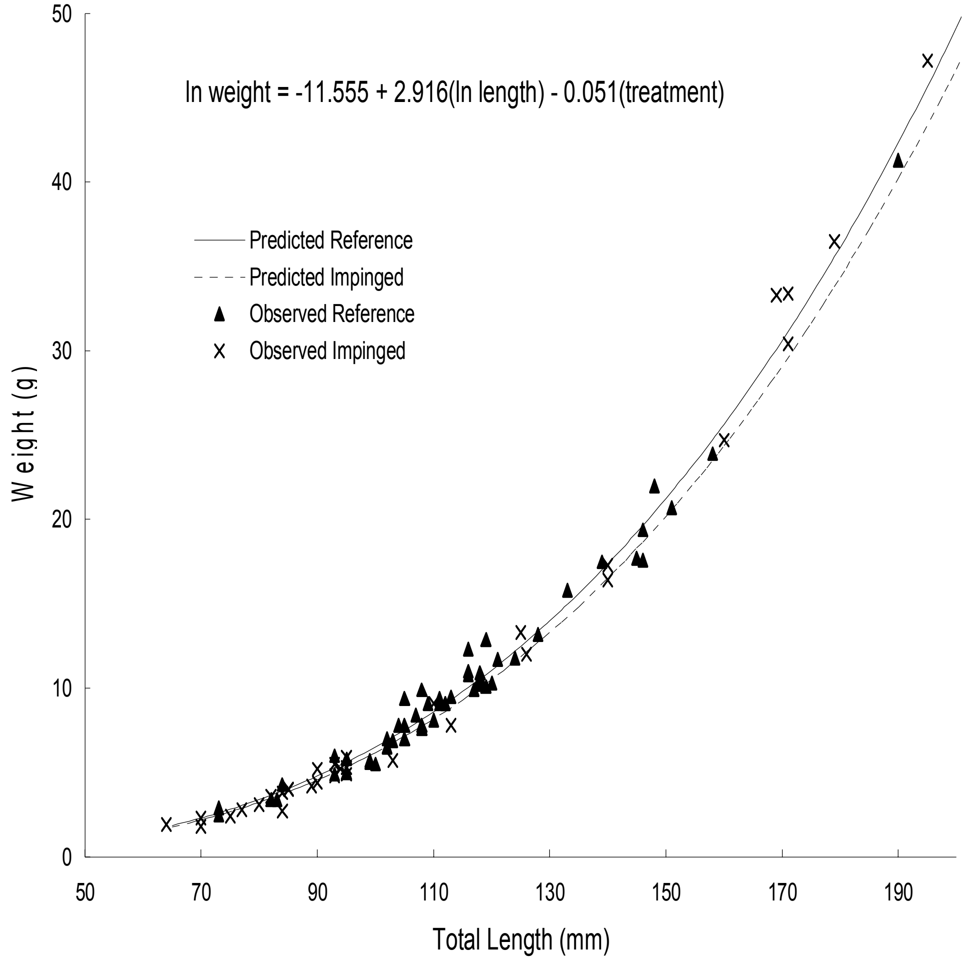

Figure 14. Predicted and observed weights for reference and impinged blue catfish collected at Plant Barry during fall 2005. Treatment was not considered significant in the regression model.

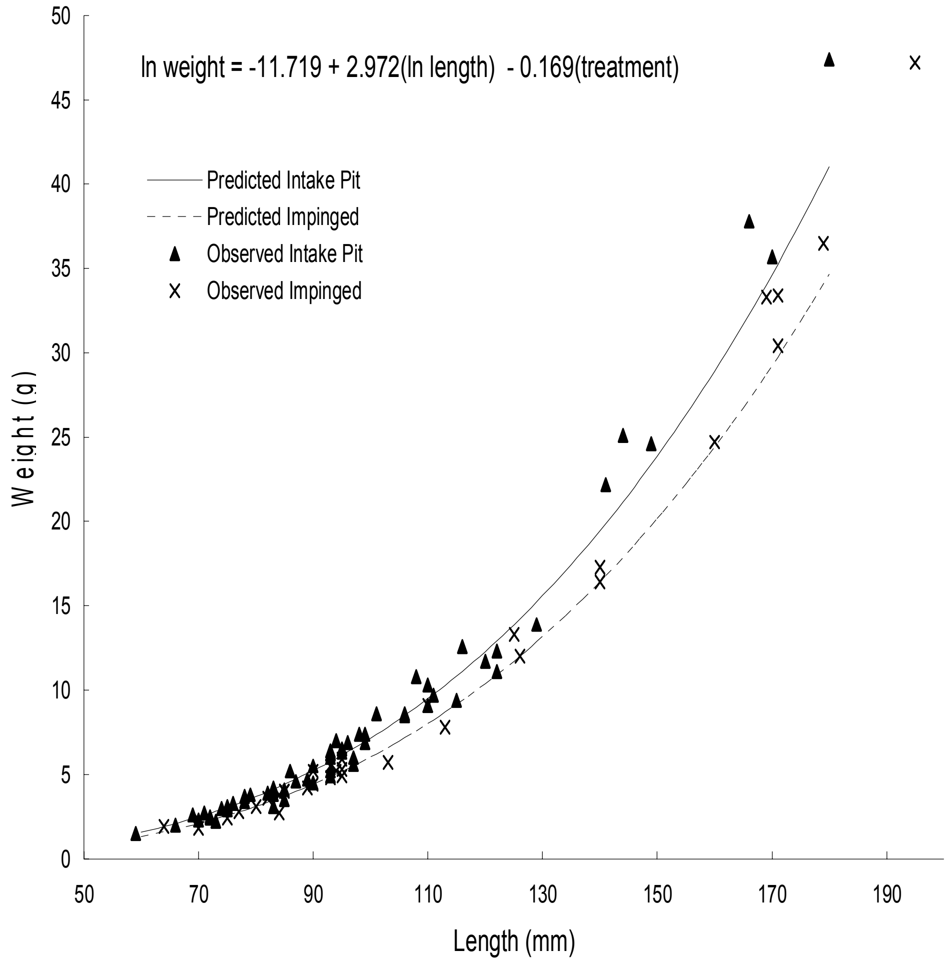

Figure 15. Predicted and observed weights for intake pit and impinged blue catfish collected at Plant Barry during fall 2005. Predicted weights of impinged fish were significantly different (*P* ≤ 0.002) than fish from the intake pit.

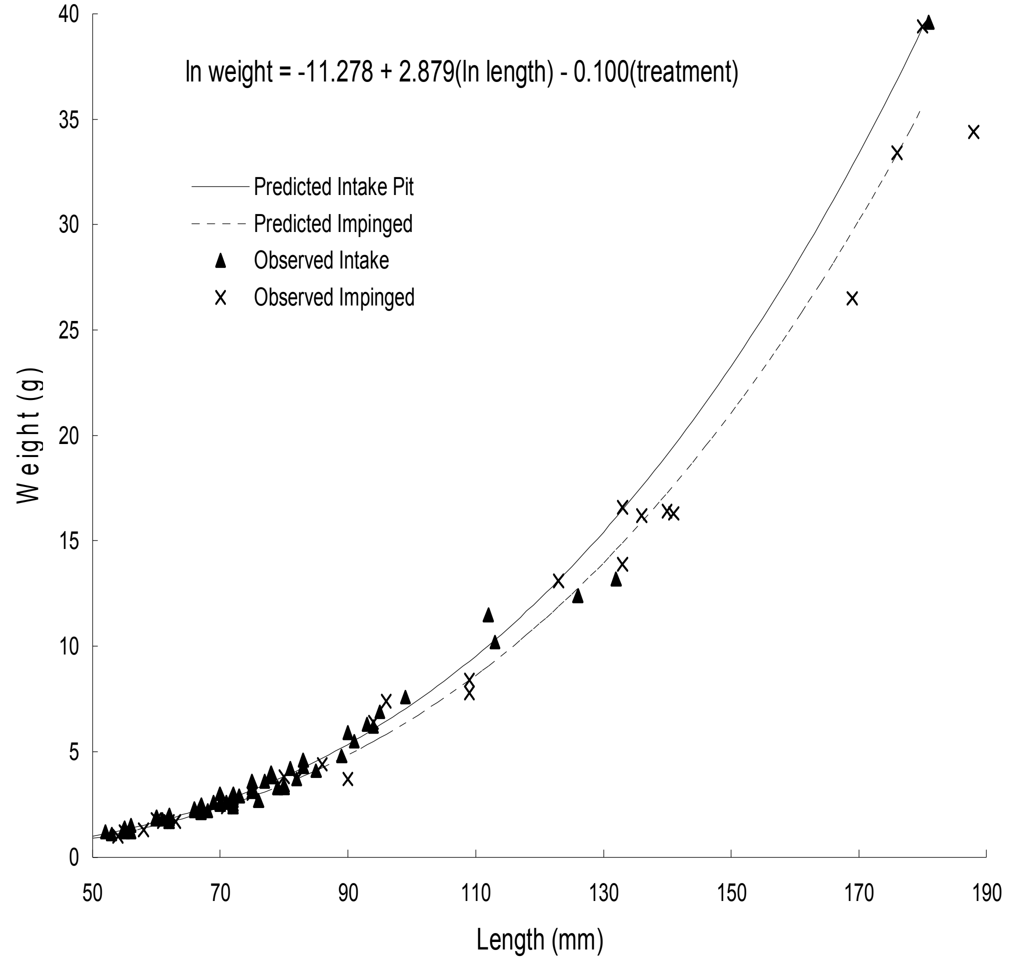

Figure 16. Predicted and observed weights for intake pit and impinged channel catfish collected at Plant Barry during fall 2005. Predicted weights of impinged fish were significantly different ($P \leq 0.001$) than fish from the intake pit.

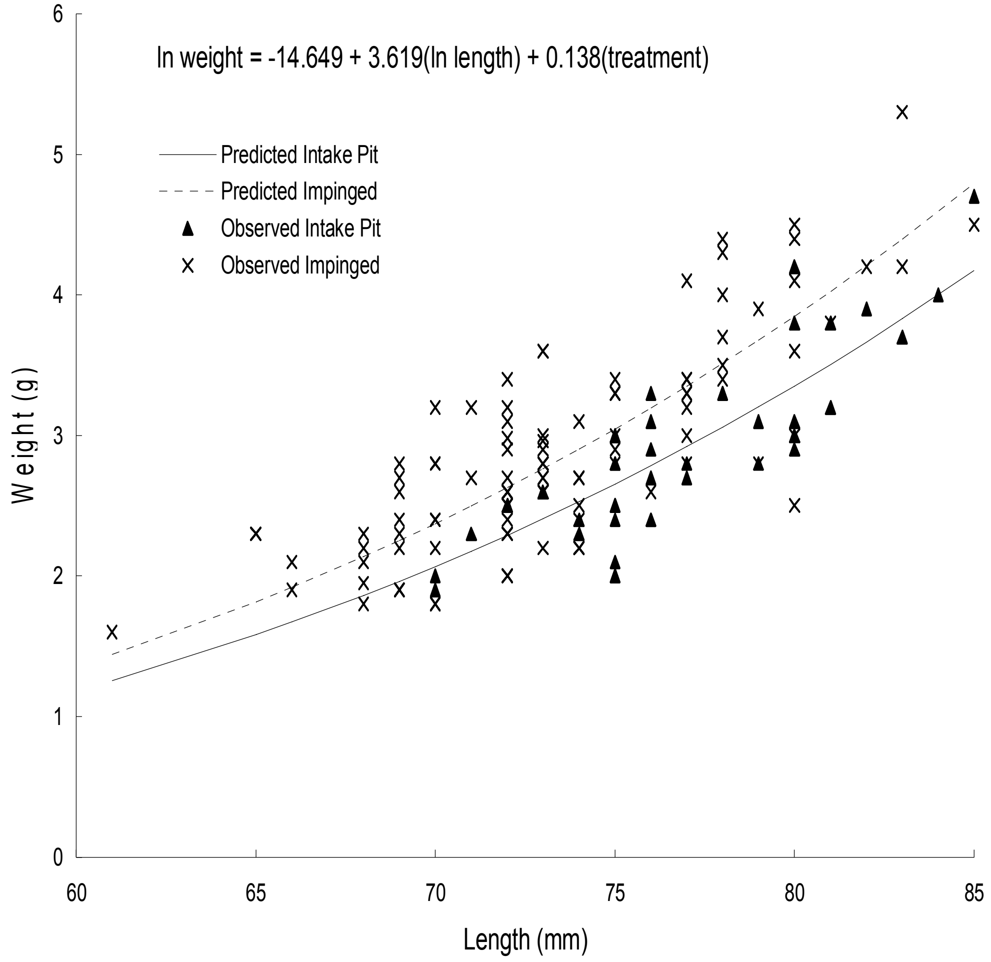

Figure 17. Predicted and observed weights for intake pit and impinged threadfin shad collected at Plant Barry during fall 2005. Predicted weights of impinged fish were significantly different ($P \leq 0.0001$) than fish from the intake pit.

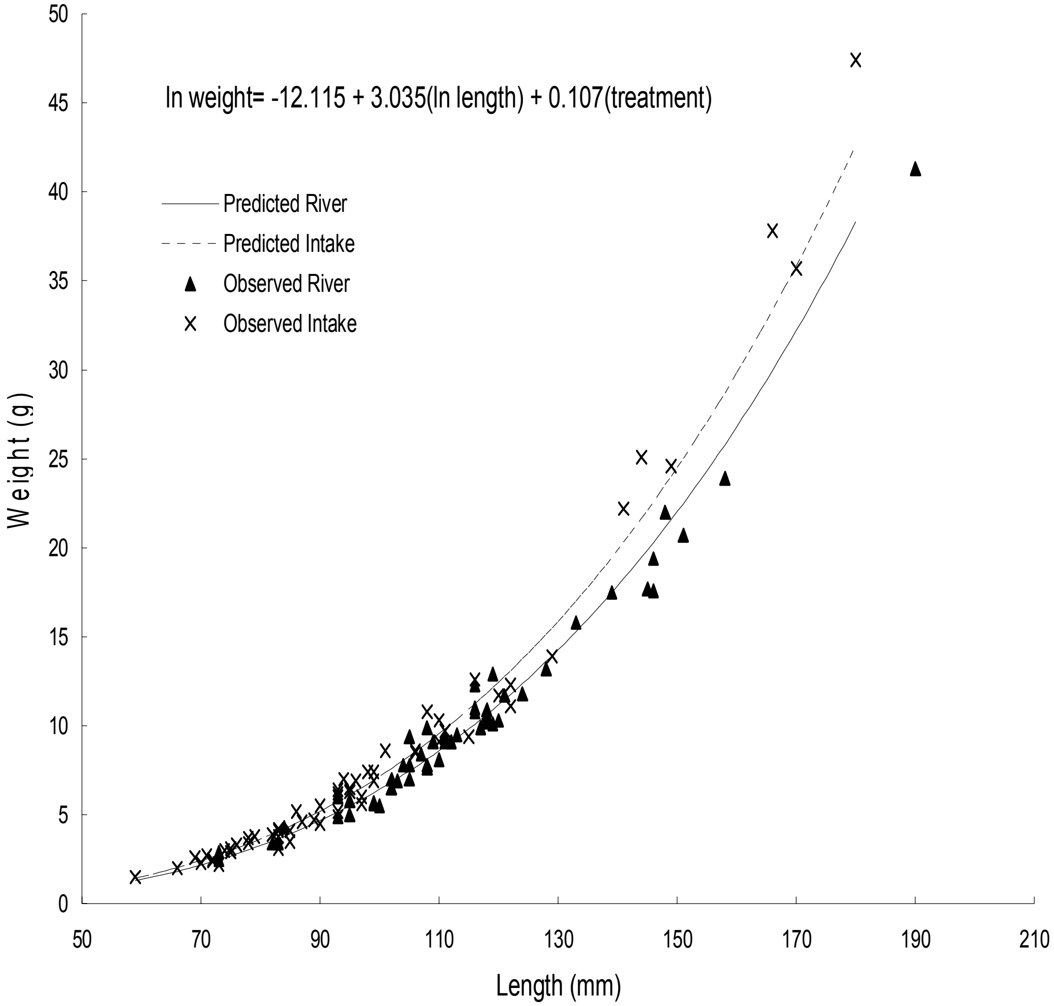

Figure 18. Predicted and observed weights for intake pit and river blue catfish collected at Plant Barry during fall 2005. Predicted weights of reference fish were significantly different ($P \leq 0.0001$) than fish from the intake pit.

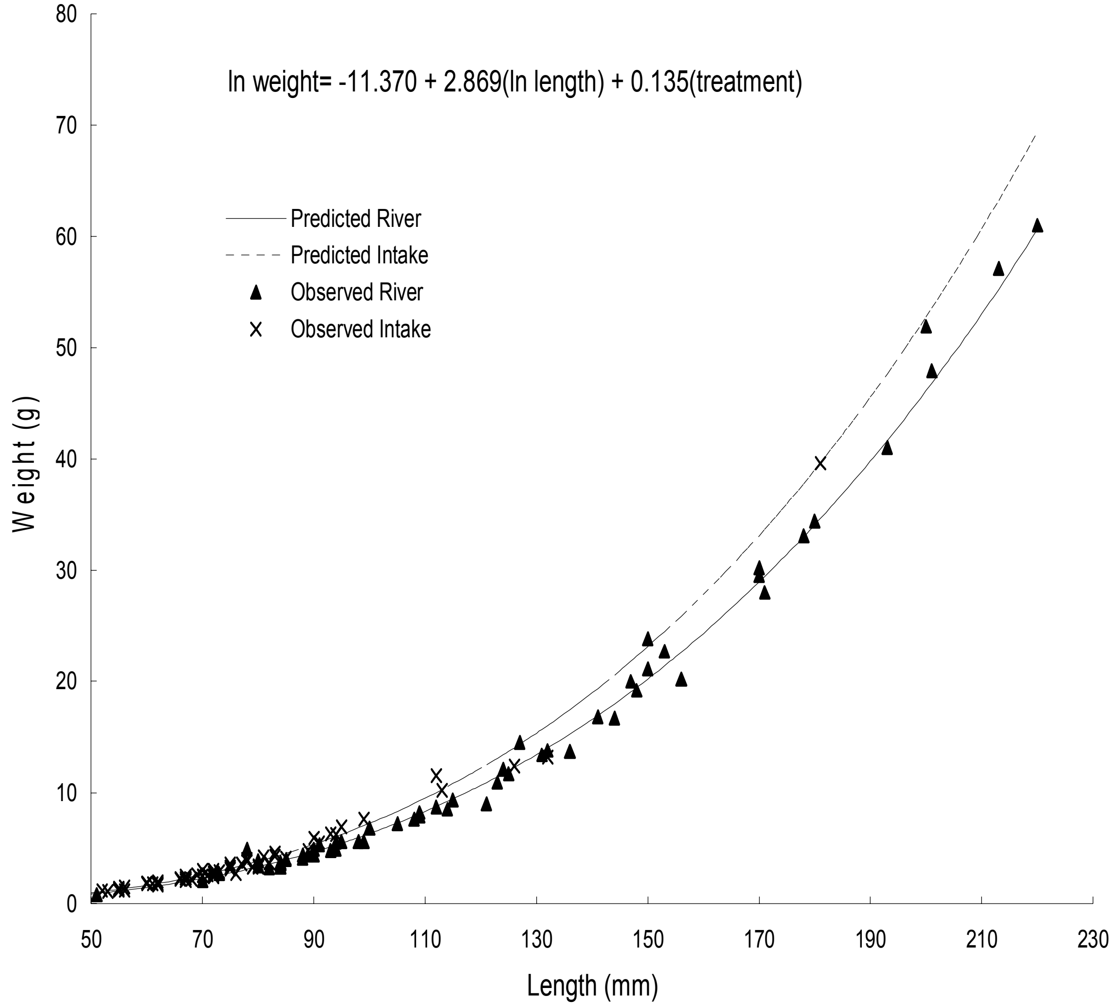

Figure 19. Predicted and observed weights for intake pit and river channel catfish collected at Plant Barry during fall 2005. Predicted weights of reference fish were significantly different ($P \leq 0.0001$) than fish from the intake pit.

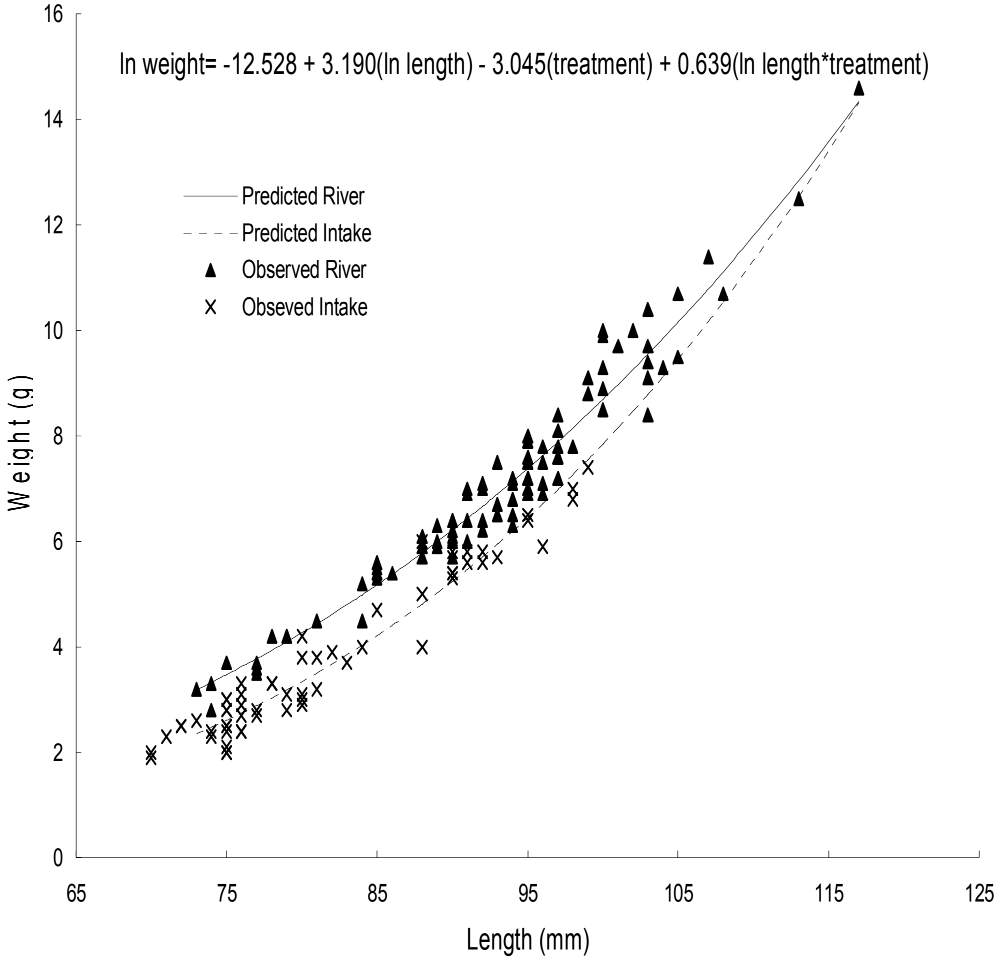

Figure 20. Predicted and observed weights for intake pit and river threadfin shad collected at Plant Barry during fall 2005. Predicted weights of reference fish were significantly different ($P \leq 0.0001$) than fish from the intake pit.

APPENDIX

Table A.1. The API-20E profiles obtained from isolates cultured during the spring 2005. Included is the number of isolates per profile. Isolates were only reported to the genus level.

Genus	*Profile*	*Number of isolates*
Aeromonas	7047124	8
Aeromonas	7047127	5
Aeromonas	7047105	5
Aeromonas	7047105	4
Aeromonas	7047126	2
Aeromonas	7047107	1
Aeromonas	7047137	1
Aeromonas	7067124	4
Aeromonas	7067104	1
Aeromonas	7007127	3
Aeromonas	7067127	1
Aeromonas	7247125	2
Aeromonas	7247144	1
Aeromonas	7247124	3
Aeromonas	7246165	1
Aeromonas	7267124	1
Aeromonas	7067125	1
Aeromonas	6047104	1
Aeromonas	7047123*	1

Table A.1. Continued.

Genus	*Profile*	*Number of isolates*
Aeromonas	7047121*	1
Pseudomonas	2000004	2
Pseudomonas	2202000	1
Pseudomonas	2204004	1
Pseudomonas	2206044	1
Pseudomonas	2041004	1
Plesiomonas	7144204	10
Plesiomonas	7344204	1
Plesiomonas	7104204	1
Plesiomonas	6144200	1
Shigella	0004000	1
Serratia	5317761	1
Citrobacter	1604572	1
Photobacterium	2015000	1
Non-fermenter	0000004	4
Unidentified	2204040	1
Unidentified	3046125	1
Unidentified	0000000	4

* Isolate was cytochrome oxidase negative. Isolate was not used in calculation of prevalence rates because species within this genus are cytochrome oxidase positive.