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I am submitting herewith a thesis written by Brooks Ashley Fost entitled "Physiological & Behavioral Indicators of Shad Susceptibility to Impingement at Water Intakes." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Wildlife and Fisheries Science.

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Physiological & Behavioral Indicators of Shad Susceptibility to
Impingement at Water Intakes

A Thesis

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Master of Science

Degree

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Brooks Ashley Fost

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Lastly, I would like to thank my fiancé, family, and friends who supported me daily and whose encouragement made the entire process so much easier.

Abstract

To address Clean Water Act regulations concerning reduction of fish impingement at water intakes (316b) I investigated approaches for identifying moribund or impaired shad under stressors of cold shock and reduced ration. In preliminary experiments, we determined the initial point of loss of equilibrium (LOE) for threadfin shad to be 7.2°C and for gizzard shad to be less than 3.5°C. I exposed shad to increasing levels of cold shock and reduced ration and then measured physiological and behavioral responses. Gizzard and threadfin shad exhibited reduced swimming performance at temperatures slightly above LOE. Cortisol and chloride showed a linear correlation with swimming performance in gizzard shad but not threadfin shad. Cortisol increased as a response to cold shock and declined after an acclimation period at a cold temperature. The expected change in serum chloride in relation to cortisol occurred in threadfin shad but not gizzard shad. I observed few differences in swimming performance and physiological indicators among ration treatments for either species. However, field observations suggest that the duration of reduced ration I used, 21-days, was not sufficient to reproduce the condition of shad in late winter. The nutritional status indicators, hematocrit and condition factor, demonstrated a declining trend. These indicators could be measured easily in the field to determine the susceptibility of shad if further testing shows a relationship with decreased swimming performance. The results of this study indicate that the term moribund does not apply well to all shad impacted by natural environmental stressors and its application should be reevaluated. Threadfin and gizzard shad are susceptible to impingement before moribundity occurs. The use of physiological indicators as predictors of impingement susceptibility appears promising.

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I. Introduction

In 2004, the United States Environmental Protection Agency (USEPA) published the Clean Water Act §316(b) phase II rule pertaining to existing power generation facilities. The phase II rule established performance standards for cooling water intake structures that required a large reduction in impingement mortality relative to the calculation baseline. This baseline refers to the impingement mortality that would theoretically occur if the facility had a shoreline, near-surface intake with a standard 3/8-inch mesh traveling screen with its face oriented parallel to the shoreline, but no other measures to reduce impingement mortality. The rule states that a reduction by 80 to 95 percent from the calculation baseline must be achieved regardless of the type of source water body.

Studies have shown natural mortality of shad in lakes and reservoirs in winter corresponds to peak impingement periods (Griffith and Tomljanovich 1975; Loar et al. 1978; Griffith 1978; McLean et al. 1980, 1982; Adams et al. 1985; White et al. 1986). The USEPA has recognized the need to identify naturally moribund (dying) fish and requires, as part of a verification monitoring plan that impingement has been reduced, a proposal on how naturally moribund fish that enter the cooling water intake structure will be identified and quantified when determining if performance standards of 80-95% reduction have been met. Two factors, cold shock and reduced ration, are related to the natural mortality of shad.

In the Southeast US, over 90% of fish impinged on cooling-water intake screens of thermal power stations are threadfin shad (*Dorosoma petenense*) or gizzard shad (*D. cepedianum*) (EPRI, 2005). Threadfin shad are abundant forage fish found in lakes and

reservoirs of the southeast (Figures 1 and 2). The species' natural range in gulf coast states has been expanded through stocking by fisheries agencies, which sought small, highly prolific prey species for game fish such as largemouth bass (*Micropterus salmoides*). The populations occurring in the species' current northern range are subject to cold temperatures that can cause loss of equilibrium (LOE) or death. A range of temperatures causing loss of equilibrium and mortality have been reported for threadfin shad. Griffith (1978) found mortality to occur at temperatures as high as 9°C and 100% mortality by 4°C. Impingement of threadfin shad increases significantly when water temperatures drop below 7°C (McLean et al. 1985). Gizzard shad are an abundant forage fish that range from the Midwest to the East coast and as far north as the Great Lakes region (Figures 3 and 4). Gizzard shad account for a large percentage of impingement in the Great Lakes region where temperatures often reach the reported LOE temperature for this species. A range of LOE temperatures has been reported for gizzard shad. Cox and Coutant (1976) showed the LOE to be lower than 6.5°C. Neumann et al. (1977) reported the species can survive at temperatures below 1°C for a short period. Heidinger (1983) suggested that mortality occurs in gizzard shad at temperatures between 4 and 0°C.

Impingement likely increases when shad are subjected to temperatures that affect their physiological function and performance. Susceptibility to impingement occurs at some point above the LOE temperature for both species and is the preface to natural mortality probably induced by cold shock. The premise for this research is that the amount of acute and chronic stress prior to LOE can be quantified using bioindicators of stress. The use of plasma cortisol and chloride to quantify sub lethal stress responses in fish is well established (Barton et al. 2002, Strange and Schreck 1978). White et al.

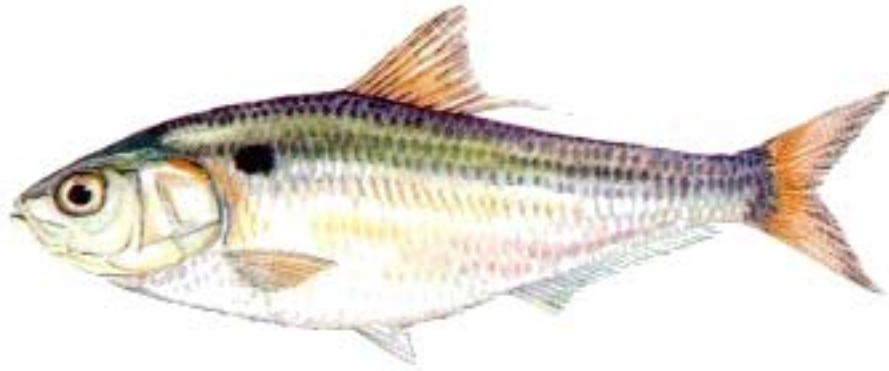


Figure 1. - Threadfin shad (NatureServe 2005).

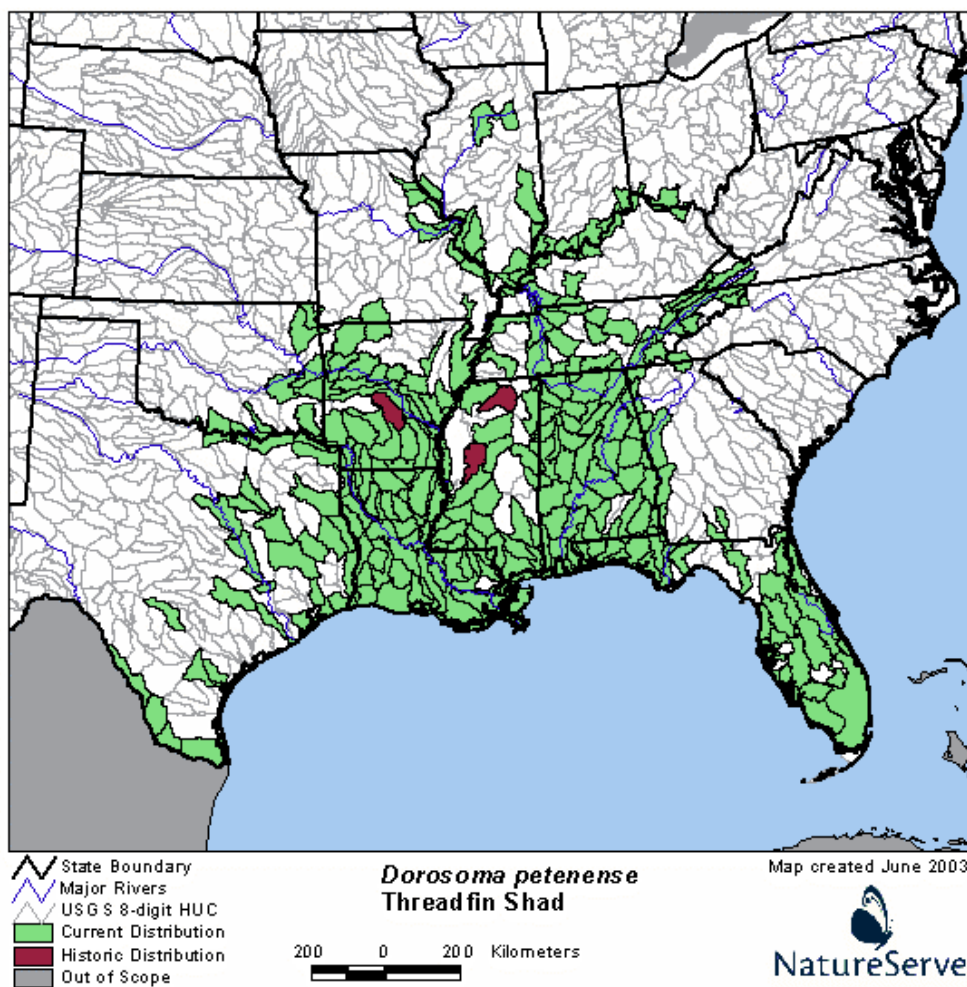


Figure 2. - The current (green) distribution of threadfin shad in the U.S. (NatureServe 2005).

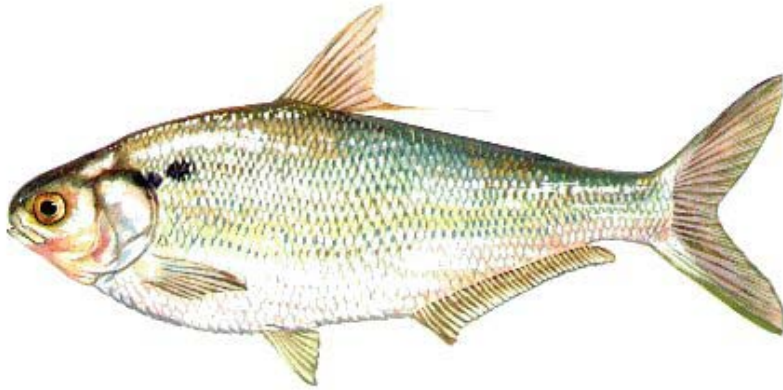


Figure 3. - Gizzard shad (NatureServe 2005).

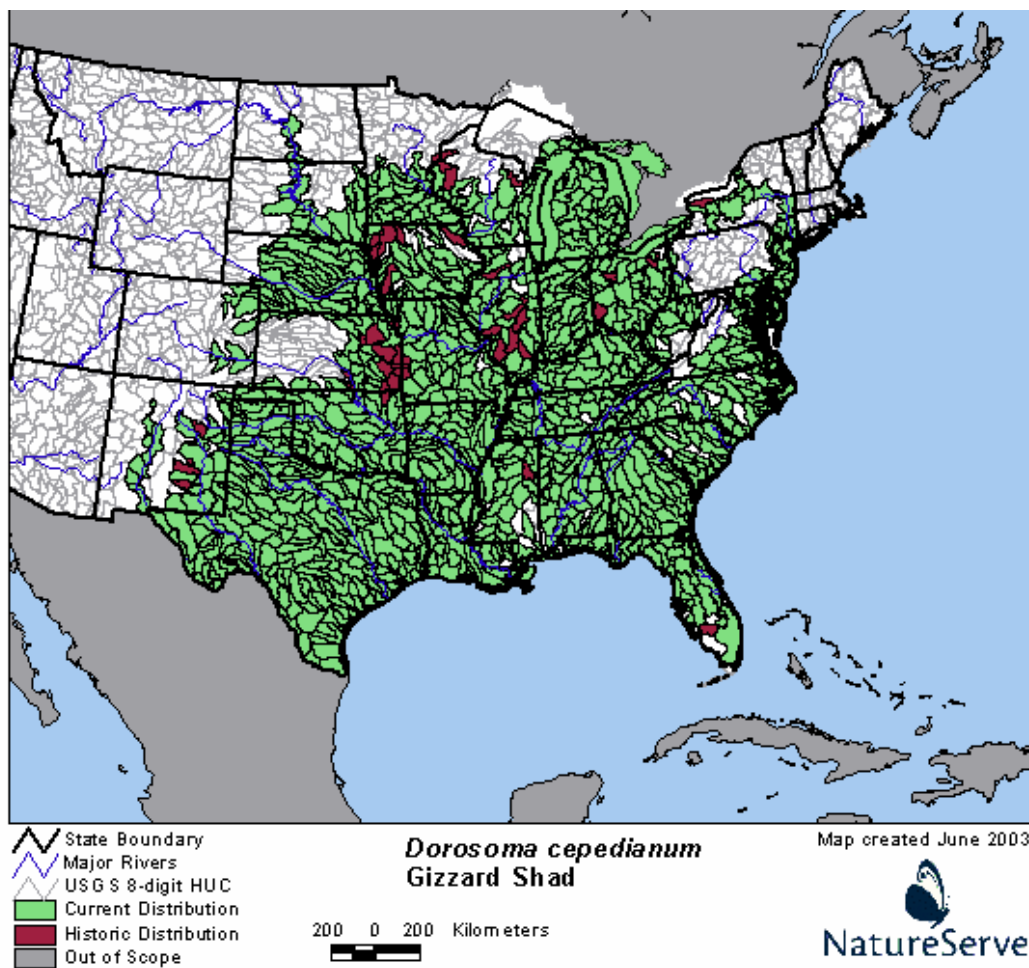


Figure 4. - The current (green) distribution of gizzard shad in the U.S. (NatureServe 2005).

(1986) used plasma cortisol and chloride to quantify stress response to cold shock in gizzard shad. Reduced ration has also been shown to affect the susceptibility to natural mortality in gizzard shad (Adams et al. 1985). The role of reduced ration in threadfin shad natural mortality has not been investigated, particularly in colder periods. Lipids are typically stored during periods of high food availability (summer and fall) and utilized during periods of low food availability or nonfeeding periods (winter and early spring) (Adams 1999). The influence of feeding at cold temperatures and duration of starvation on susceptibility to impingement has not been quantified. Bodola (1966) reported gizzard shad to discontinue feeding at 11°C. Both species remain active but lethargic during periods of cold temperatures, requiring the utilization of energy reserves to maintain physiological homeostasis. The physiological condition of fish, quantified using bioindicators or condition indices, could reveal the role of ration in natural mortality. Hematocrit, triglycerides, and total protein have been used as general indicators of nutrition and starvation (Adams et al. 1985, 1992; Barton et al. 2002). Condition factor (K), an index that relates weight and length, has been used as an integration of energy storage and metabolism due to starvation (Dutil et al. 2003).

Swimming performance is important for relating physiological condition to impingement. Griffith and Tomljanovich (1975) used swimming performance to determine the ability of cold shocked threadfin shad to avoid impingement and found temperatures below 8°C caused high impingement mortality. Martinez et al. (2004) demonstrated that Atlantic cod (*Gadus morhua*) that were starved had a reduced swimming performance compared to cod that were fed. However, the combined affects of ration and cold shock on swimming performance has not been investigated.

The challenge for power station operators is to determine whether fish impinged on intake screens would have died anyway because of environmental conditions. A rapid and simple procedure for assaying fish is desired, either using fish already impinged or those collected in the intake forebay.

The primary purposes of this study were to: 1) identify the critical points where cold shock and reduced ration affect the ability of fish to escape impingement and 2) identify physiological & performance indicators for identifying susceptibility of fish to impingement. I first determined the temperature that caused a near-lethal response by subjecting shad to a cold shock (starting at 15°C and reduced by 0.5°C/Hour) and observing individual LOE. Shad were then cold shocked using the same decline rate over a range of temperatures slightly above the LOE temperature to quantify stress and swimming performance response. A lab study combining full and reduced ration groups paired with a single cold shock regime to quantify stress and swimming performance response was also performed. Blood was withdrawn from individuals to identify physiological indicators of stressed fish. Swimming performance of individual fish was assessed using a stream channel with a velocity similar to that deemed acceptable by USEPA for a power plant intake (~0.15 meters/sec).

II. Methods and Results

General Methods

Gizzard and threadfin shad were collected by electrofishing from August to October 2005 on the Clinch River, Tennessee, United States. Gizzard shad were collected from a single cove (near Clinch River kilometer 15, Figure 5) and threadfin shad from the discharge area of the Tennessee Valley Authority's Melton Hill Dam (Clinch River kilometer 23, Figure 6). Water temperatures ranged from 20-28°C.

I transported live shad to Oak Ridge National Laboratory in 151 L barrels equipped with two battery operated aerators and treated with 400 g of sodium chloride. Shad were then held at 24°C for 3 to 5 days in 889 L circular tanks. The tanks measured 122 cm in diameter and 76 cm in height. Each tank was equipped with an aerator and about 0.6 L/min flow through. Shad were acclimated to feeding of frozen brine shrimp during this period.

Test groups of shad were held in one of four 530 L rectangular tanks receiving 0.25 L/min of flow. This allowed testing of 4 groups at a time. Test groups of gizzard shad numbered 34 individuals with mean length of 153 mm (131-177 mm) and weight of 30 g (17-47g). Test groups of threadfin shad included 45 individuals with mean length of 134 mm (107-158 mm) and weight of 30 g (9-30g). The number of individuals in each test group exceeded the number required for testing to allow for holding mortality. Portable refrigeration units paired with temperature controllers were used to regulate exposure temperatures within $\pm 0.2^{\circ}\text{C}$. Each test group was acclimated for one week at $15 \pm 0.2^{\circ}\text{C}$ prior to testing in one of two experiments. The protocol used for the two experiments is illustrated in Figure 7. A single test group for each experiment was

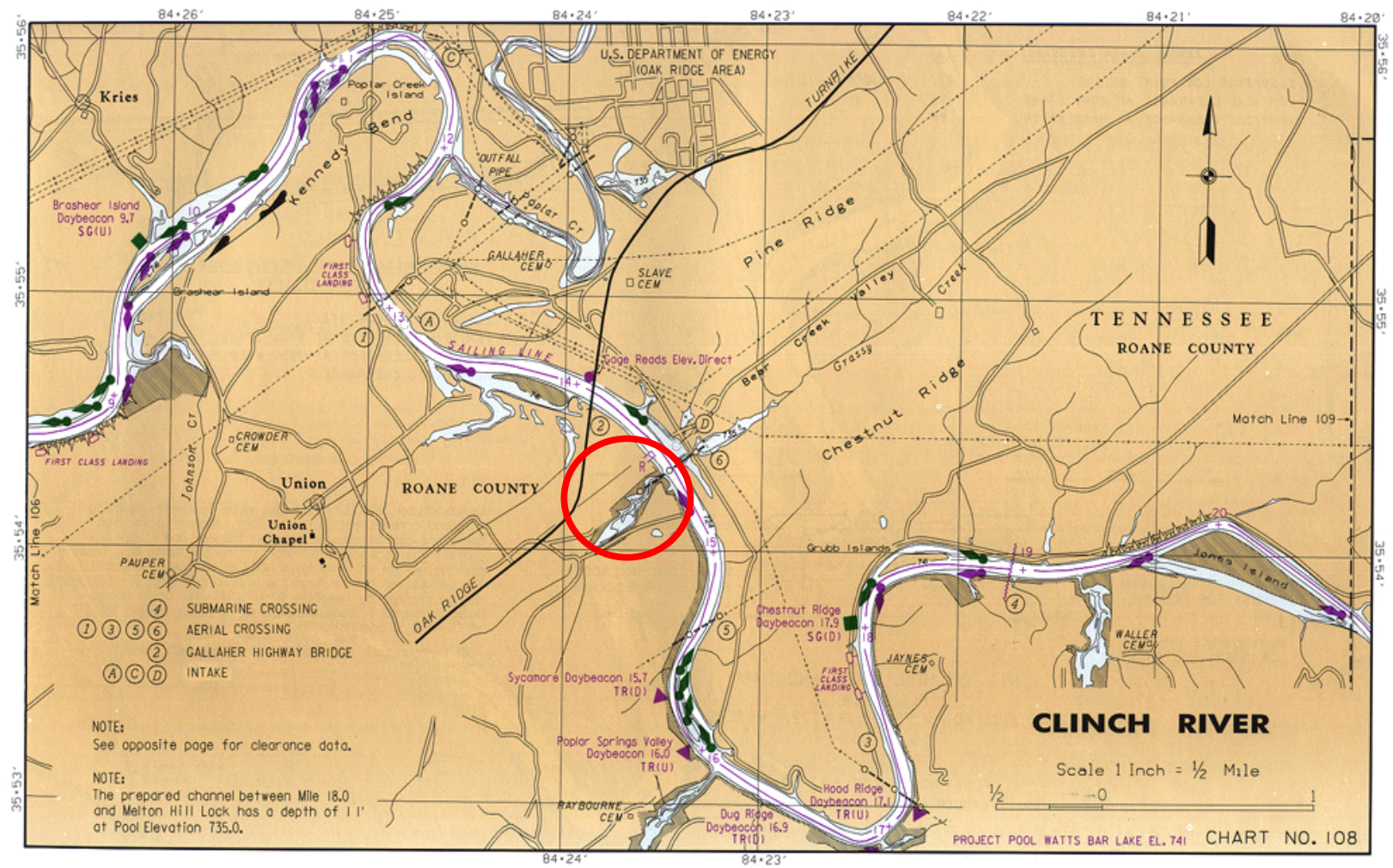


Figure 5. - The gizzard shad collection site, circled in red, near Clinch River km 15 (U.S. Army Corps of Engineers 2004).

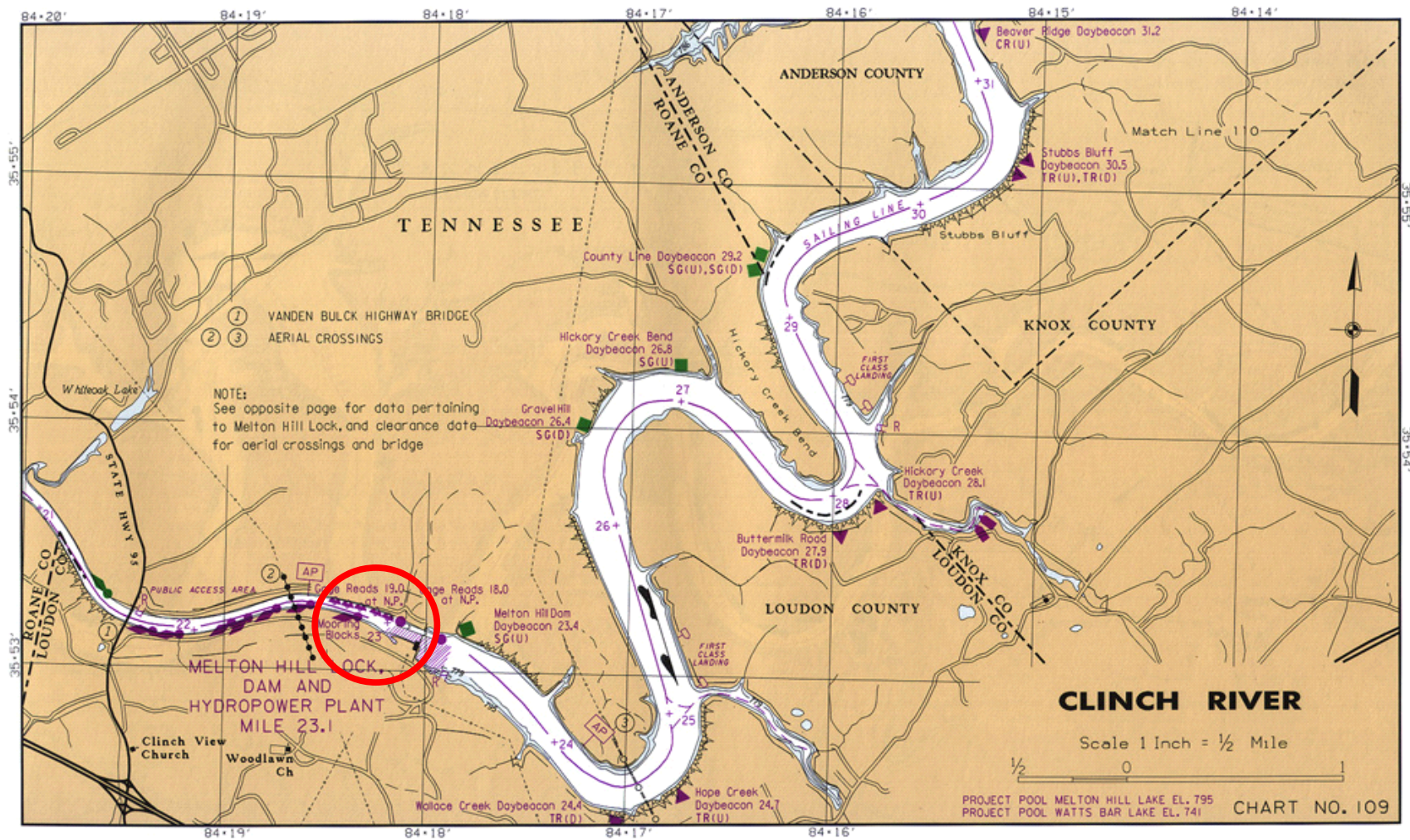


Figure 6. - The threadfin shad collection site, circled in red, at Clinch River km 23, Tennessee (U.S. Army Corps of Engineers 2004).

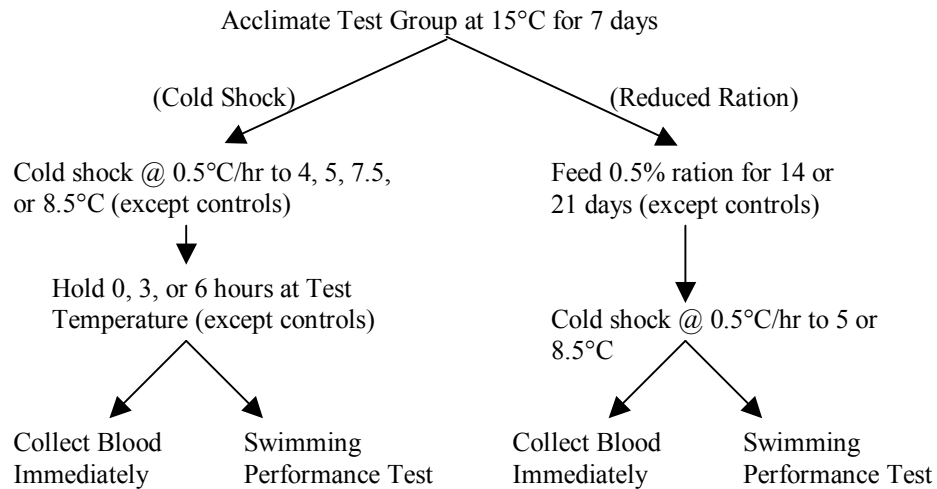


Figure 7. - Summary of the protocol used during the course of cold shock and reduced ration experiments for gizzard and threadfin shad. Procedures that are specific to a particular experiment or not performed on controls are labeled in parentheses.

repeated for each species. Repeated cold shock experiments were conducted 4 weeks after the initial test group for gizzard shad and three weeks later for threadfin shad. The repeated reduced ration groups were conducted one day later than the initial 21-day test group.

Eighteen gizzard or 24 threadfin shad were removed from their exposure temperature prior to collection of blood for serum analysis. I removed shad from the exposure tanks with small dip nets that allowed for quick removal of individuals to minimize handling stress and immediately anesthetized in a solution of tricaine (MS-222). Only three shad were removed at a time and bled within 2 to 4 minutes of being placed in MS-222. Fish were bled using 21G1 Vacutainer™ needles paired with 13 X 75 mm heparinized Kendall™ collection tubes. I measured total length (mm) and weight (g) of each individual. Blood samples were randomly pooled, due to the low volume of serum derived from individuals, to form six pool groups of 3 gizzard or 4 threadfin shad for each treatment. Hematocrit was measured for each pooled sample. Plasma was

separated from whole blood by centrifugation, transferred to 1.5-mL cryotubes, and stored in liquid nitrogen for later analysis. Plasma cortisol concentration was determined via Coat-A-Count® solid-phase ¹²⁵I-cortisol radioimmunoassay. Plasma chloride was determined using a spectrophotometric assay by Pointe Scientific™.

For swimming performance tests, groups of ten gizzard or threadfin shad were removed from their treatment tanks (the same tanks from which the fish to be bled were taken) and placed into the corral area of the swimming performance channel (Figure 8). The dimensions of the test channel and methods of the test allowed 5 fish to be tested simultaneously. The swim channel was maintained at the temperature fish were exposed to within their treatment using portable refrigeration units. The flow (~0.15 meters/sec) in the test channel was produced by a ¾ hp centrifugal pump. Water was pumped from the corral zone and introduced to the upper end of the test channel through a series of increasing-diameter pipes and a 0.32 cm mesh screen to even the flow distribution within the test zone of the channel. The performance test channel was 10.8 cm wide X 122 cm long. Water depth was held at 14.6 cm. The power to the pump was surged 3-4 times to allow the fish to orient upstream and gain swimming balance prior to initiating full flow velocity. Each individual was observed during a maximum 1 hour period to determine if impingement occurred at the rear screen for > 15 sec. The total swim time (≤ 1 hour), total length, and weight of each individual was recorded. Condition factor (weight / (length³) * 1000) was calculated for individuals.

Statistical analyses on all data were performed using SAS, version 9.1, and SPSS, version 14. A value of P<0.05 was considered significant for all tests and simultaneous

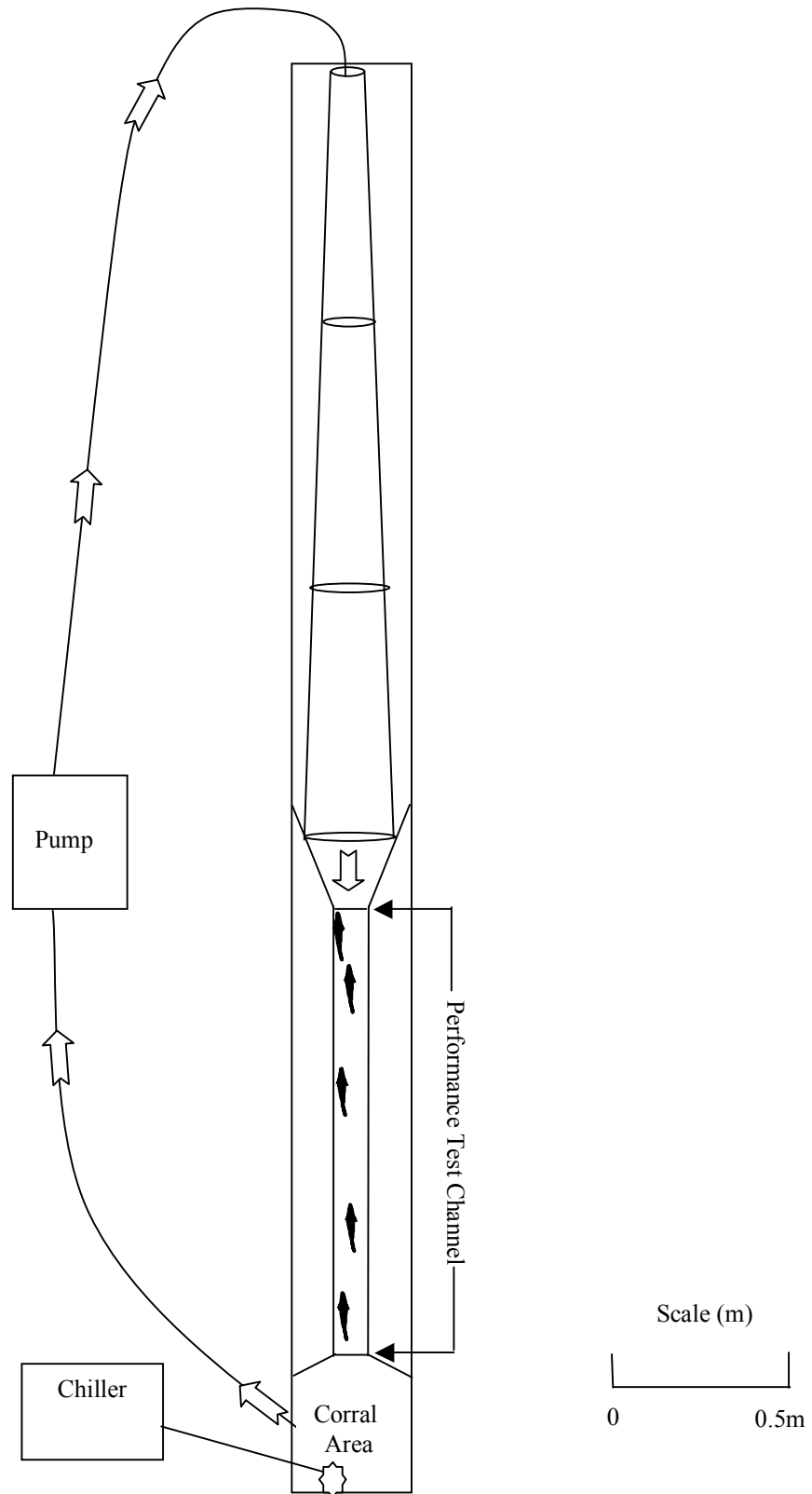


Figure 8. - Schematic of the swimming performance channel from the top down perspective. Fish were confined to the 10.8 cm wide by 122 cm long area during testing.

confidence was held at $P=0.05$ for all post hoc tests. Correlations between variables were investigated using Pearson correlation coefficients. Differences between test groups in swimming performance and physiological indicators were analyzed with analysis of variance (ANOVA). The Shapiro-Wilk statistic was used to test the assumption of normally distributed errors. If data were not normal, a natural log transformation of the dependent variable or ranked data was used in the ANOVA. Homogeneity of variance between treatments was assessed with Levene's test. If significant differences in mean values were indicated by the ANOVA F test, paired means were evaluated using the least significant difference (LSD) test. Dunnett's mean separation test for unequal group variances was used when heterogeneous group variances exceeded a 3-fold difference between any treatment pair (van Belle 2002). The results from repeated groups were: 1) averaged with the original test group when comparing treatment effects and 2) compared to the initial test group for differences. The word repeated is used because the experiments did not occur at the same time and therefore are not replicates.

Two sets of experiments were conducted, (1) to identify physiological and swimming performance indicators of cold shock, and (2) indicators as functions of reduced ration. The methods and results of these two sets of tests are described here.

Indicators of Cold Shock and Effects on Swimming Performance

Methods

A preliminary experiment incorporating declining temperatures was conducted to determine the temperature of LOE for each species. This information was necessary to determine the temperatures to be used in cold shock treatments. I gave the shad (24 threadfin and 18 gizzard) an acute cold shock by decreasing temperature 0.5°C/hr

beginning at the acclimation temperature of 15°C. The temperature and time since 15°C were recorded for each individual when LOE occurred. The loss of swimming equilibrium was used to determine LOE. Gizzard shad did not lose equilibrium during testing at 3.4°C and were removed because the chiller equipment was not capable of chilling the volume of water any colder. The temperatures used in the following cold shock experiments were based on the highest temperature (7.2°C for threadfin) each species experienced LOE.

Cold shock experimentation began with an acute cold shock (0.5°C/hr) to gizzard shad terminating at test temperatures of 4 and 5°C. I also tested gizzard shad that were held for 6 hours at the 4 or 5°C test temperature to determine the effect of prolonged exposure at those temperatures after temperature decline (Figure 9). The 5°C test group

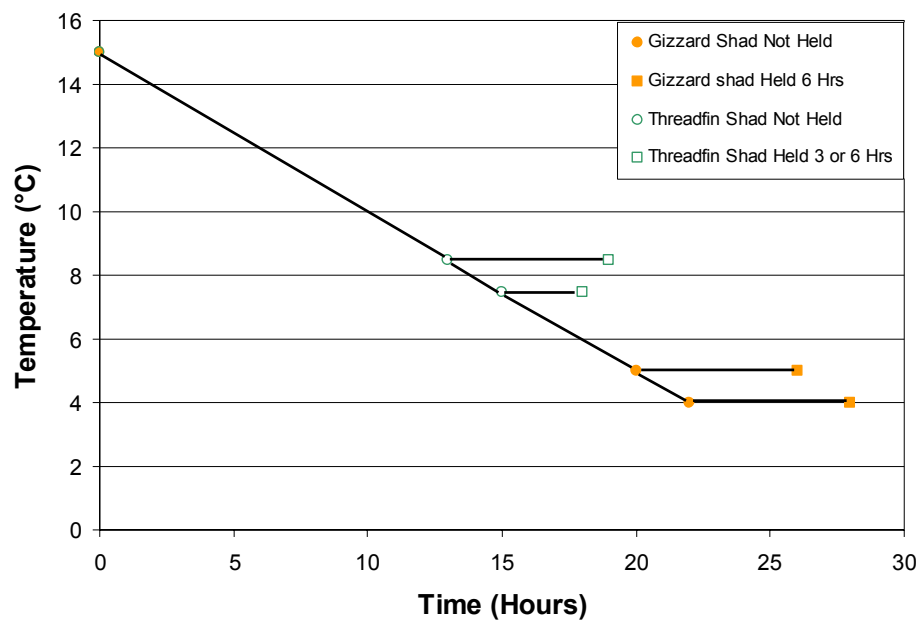


Figure 9. - Treatment graph displaying the points (circles and squares) where gizzard and threadfin shad were sampled during 0.5°C/hour cold shock experiments. Circles indicate groups sampled immediately when the test temperature was reached. Squares indicate groups sampled 3 or 6 hours after reaching the test temperature.

was repeated 4 weeks after the initial. Threadfin shad were given an acute cold shock to test temperatures of 7.5 and 8.5°C. Threadfin shad were also held for 6 hours at 8.5°C and 3 hours at 7.5°C. I repeated the 8.5°C test group held for 6 hours three weeks after the initial. Controls for both species were sampled at the acclimation temperature of 15°C. The test groups were monitored for abnormal behavior or signs of distress during the cold exposure. The plasma cortisol (ng/ml) and chloride (mEq/liter) levels in each pooled group were measured to determine acute stress response to the test temperatures. Condition factor was calculated for each individual to determine if a significant difference occurred between groups or if swimming performance was correlated with condition. Significance was tested at $P=0.05$ across all variables and test groups.

Results

The mean LOE temperature for threadfin shad exposed to cold shock at 0.5°C/hr was 5.8°C. LOE experienced by threadfin shad ranges from 7.2°C to 5°C. Only one threadfin shad had not experienced LOE prior to 4.7°C at the time experiment was terminated. No gizzard shad experienced LOE prior to 3.4°C at the time experiment was terminated (Figure 10). Using these preliminary results, the testing protocols were established for cold shock testing.

Gizzard Shad

I observed signs of distress (abnormal response) in gizzard shad during the cold shock treatments. Activity levels decreased as temperatures approached 5°C and fish became totally lethargic by 4°C. There was little response to vibration in the water and netting at 4°C. Gizzard shad experiencing cold shock had significantly lower mean swimming times than the control ($P=0.0005$) (Figure 11, Table A1). Mean swimming

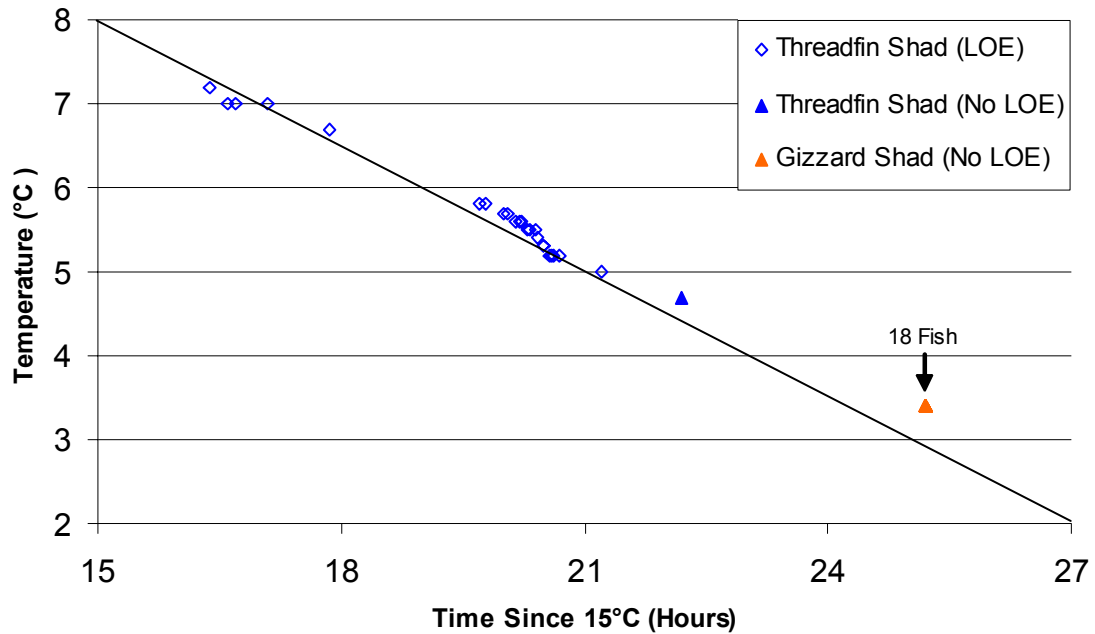


Figure 10. – Loss of equilibrium, as a function of time and temperature, for gizzard and threadfin shad subjected to a cold shock treatment that began at 15°C and declined at a rate of 0.5°C/hour. The intended temperature decline is indicated by the line. Open diamonds are for shad that lost equilibrium. Triangles are for shad that were removed prior to LOE.

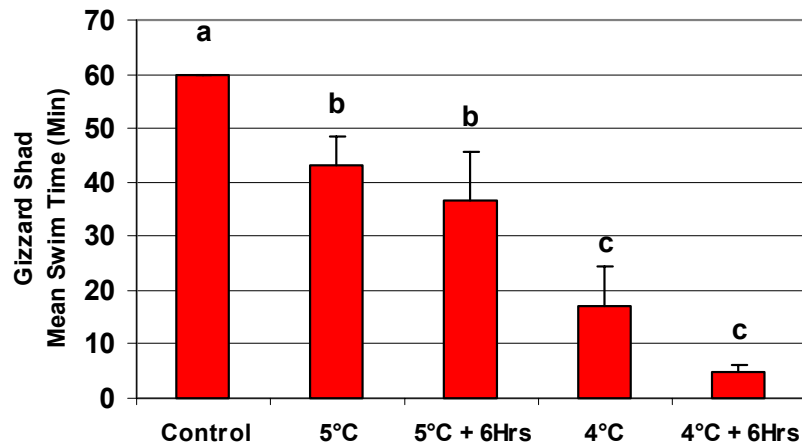


Figure 11. - Mean (+1 SE) swimming time of gizzard shad exposed to cold shock treatment beginning at 15°C and declining at a rate of 0.5°C/hour to the test temperature. Groups were tested at 15°C (control), 5°C, after 6 hours at 5°C (5C + 6Hrs), 4°C, and after 6 hours at 4°C (4°C + 6 hours). Treatments that are statistically different ($P < 0.05$) have different letters.

performance time was less in gizzard shad cold shocked to 4°C than at 5°C. Mean swimming performance of gizzard shad held for an additional 6 hours at their test temperature was not different statistically from fish not held for an additional 6 hours. Mean condition factor was not different between test groups or control (Figure 12). There was no correlation between condition factor and mean swimming performance for gizzard shad. The repeated group did not differ in swimming performance compared to the initial group (Table A3).

Cold shock affected cortisol and chloride ($P < 0.0001$) in gizzard shad. Mean plasma cortisol and chloride were significantly higher in all cold shocked groups compared to controls (Figure 13, Table A1). Mean plasma cortisol also was significantly less among groups cold shocked at 5°C, 4°C, and 4°C held for 3 hours. A significant correlation was found between swimming performance and cortisol ($R^2 = 0.7588$) and chloride ($R^2 = 0.7561$) (Figure 14, 15). Mean cortisol was significantly lower in the repeated group compared to the initial group (Table A3). Mean plasma chloride did not differ among cold shock treatments. The repeated group did not differ in mean chloride compared to the initial group (Table A3).

Threadfin Shad

I observed signs of distress in threadfin shad during the cold shock treatments at temperatures 2-3°C above the LOE identified in the preliminary experiment. Individuals began to swim out of sequence rather than in a school, often swimming into the side of the tank as if searching for warmer water. The activity level of these fish appeared to

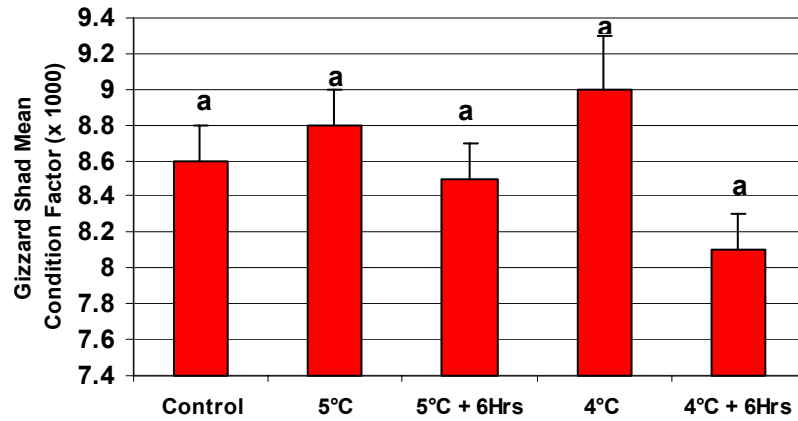


Figure 12. - Mean (+1 SE) condition factor of gizzard shad exposed to cold shock treatment beginning at 15°C and declining at a rate of 0.5°C/hour to the test temperature. Treatments that are statistically different ($P < 0.05$) have different letters.

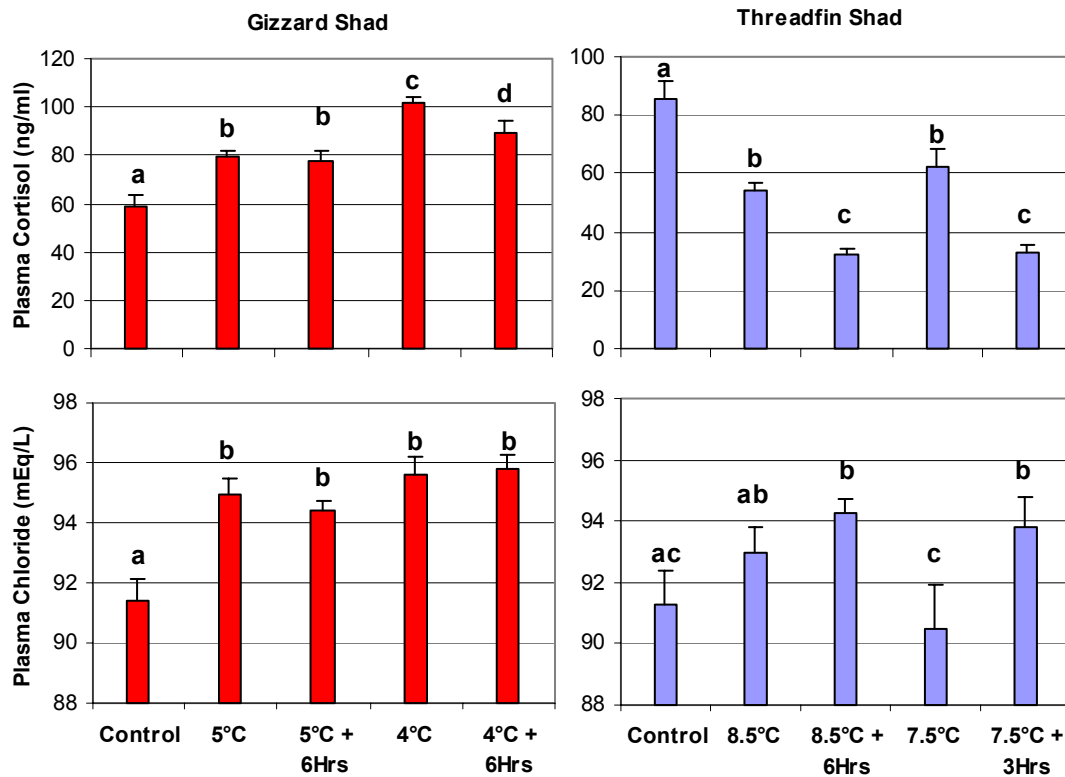


Figure 13. - Mean (+1 SE) plasma cortisol and chloride of gizzard and threadfin shad exposed to cold shock treatment beginning at 15°C and declining at a rate of 0.5°C/hour to the test temperature. Treatments that are statistically different ($P < 0.05$) have different letters.

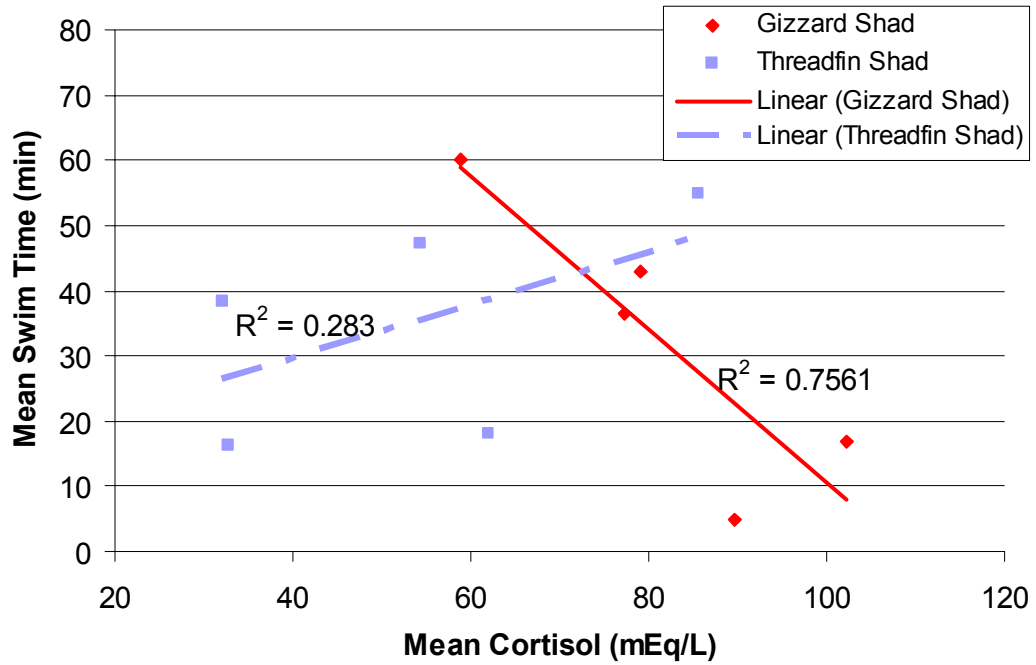


Figure 14. - Linear correlation of mean cortisol to mean swim time of threadfin and gizzard shad.

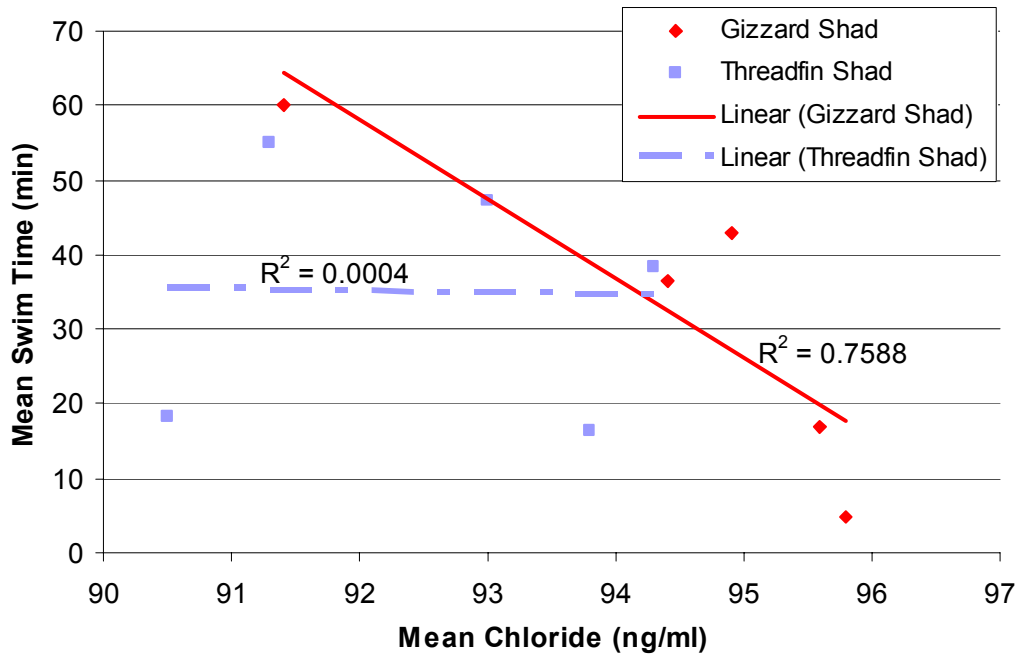


Figure 15. - Linear correlation of mean chloride to mean swim time of threadfin and gizzard shad.

increase as temperatures decreased. There was little response to vibration and netting at 8.5°C.

Cold shock had a significant effect on swimming performance of threadfin shad ($P < 0.01$). Threadfin shad experiencing cold shock at declining temperatures to 7.5°C and held for an additional 0 or 3 hours had significantly shorter times for mean swimming performance than controls and the 8.5°C test group (Figure 16, Table A1). The test group experiencing a decline to 8.5°C + 6 hour holding was different only from the 7.5°C + 3 hour group (Figure 16, Table A1). Mean condition factor did not differ among test groups, but two groups (the 8.5°C and 8.5°C + 6 h) were less than the control (Figure 17). There was no correlation between condition factor and mean swimming performance for threadfin shad. The repeated group had a significantly longer mean swimming performance compared to the initial group (Table A3).

Mean plasma cortisol was significantly lower in all threadfin shad test groups compared to the control ($P < 0.0001$) (Figure 13, Table A1). Test groups held for an additional 3 or 6 hours at their test temperature were significantly different from those sampled immediately (Figure 13, Table A1). No significant correlation was found between swimming performance and cortisol ($R^2 = 0.283$) and chloride ($R^2 = 0.0004$) (Figure 14, 15). The repeated group had significantly lower mean cortisol compared to the initial group (Table A3). Mean plasma chloride was significantly different in only the 8.5°C + 6 hour and 7.5°C + 3 hour test group compared to controls ($P = 0.0034$) (Figure 13, Table A1). The 7.5°C test group had significantly lower mean chloride values compared to all other test groups (Figure 13, Table A1). The repeated group did not differ in mean chloride compared to the initial group (Table A3).

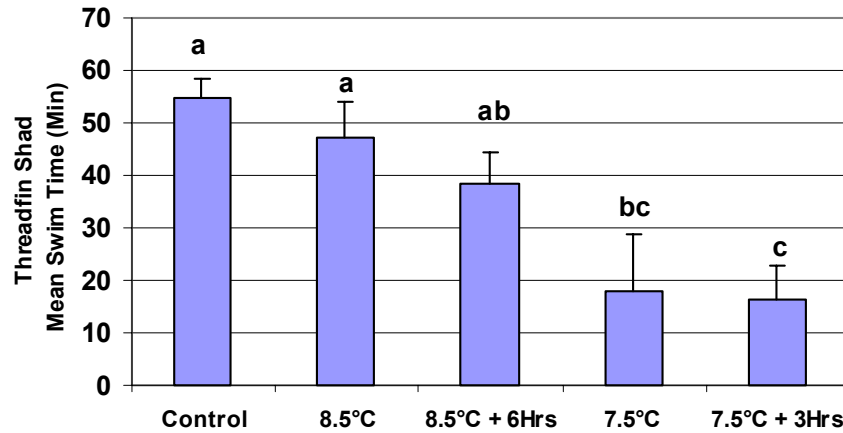


Figure 16. - Mean (+1 SE) swimming time of threadfin shad exposed to cold shock treatment beginning at 15°C and declining at a rate of 0.5°C/hour to the test temperature. Groups were tested at 15°C (control), 8.5°C, 8.5°C + 6 hours, 7.5°C, and 7.5°C + 3 hours. Treatments that are statistically different ($P < 0.05$) have different letters.

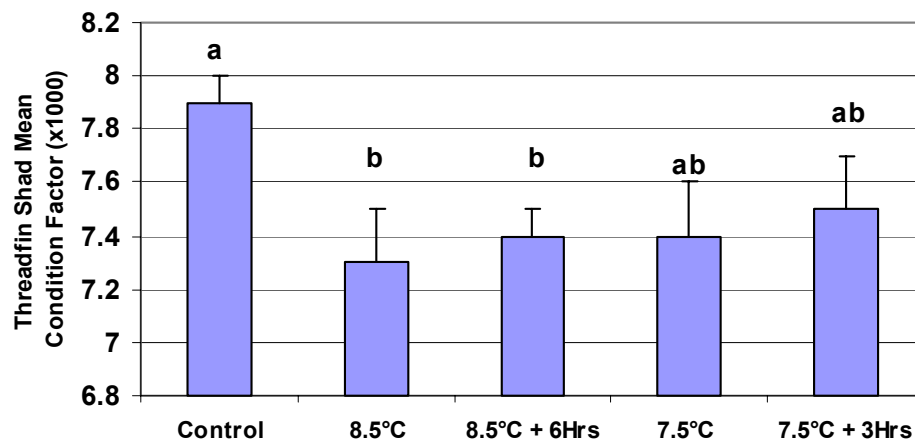


Figure 17. - Mean (+1 SE) condition factor of threadfin shad exposed to cold shock treatment beginning at 15°C and declining at a rate of 0.5°C/hour to the test temperature. Treatments that are statistically different ($P < 0.05$) have different letters.

Indicators of Combined Coldshock and Reduced Ration and Effects on Swimming Performance

Methods

I fed each test group a reduced ration of 0.5% of their mass in frozen brine shrimp for 14 and 21 days. The control groups were fed 5% of their mass in frozen brine shrimp for 14 days. I then cold shocked gizzard shad to a temperature of 5°C and threadfin shad at a temperature of 8.5°C at a rate of 0.5°C/hr. The 21-day reduced ration group was repeated for each species 1 day after the initial had begun. The test groups were observed for changes in swimming activity during the reduced ration period. Fish were collected in March of 2006 to determine if the level of ration observed after 21-days was similar to that found in fish in late winter.

Following the reduced ration period and cold shock, blood was withdrawn and total length (mm) and weight (g) was measured for each individual. I measured plasma cortisol (ng/ml), chloride (mEq/liter), total protein (mg/dl), triglycerides (mg/dl), and hematocrit (%) level in each pooled group. Total protein and triglycerides were determined using a centrifical fast analyzer (Cobas brand). The Cobas is an automated spectrophotometer that operates as a centrifical fast analyzer for testing that can be programmed to test multiple samples at once.

Results

Gizzard Shad

Gizzard shad remained active during the reduced ration periods. Groups fed a reduced ration did not have significantly different mean swimming performance than

controls ($P=0.6943$) (Figure 18, Table A2). A treatment effect of reduced ration was observed in both cortisol and chloride ($P<0.0001$). The mean cortisol level of the 14-day reduced ration group was higher compared to the 21-day reduced ration group and control (Figure 19, Table A2). The mean plasma chloride levels of both reduced ration groups was different from controls. The 14-day and 21-day ration groups also differed in mean plasma chloride. Treatment effects were also observed in total protein ($P<0.01$), triglycerides ($P<0.0001$), hematocrit ($P<0.01$), and condition factor ($P<0.01$). Mean plasma total protein was significantly higher in the 21-day reduced-ration group than the 14-day reduced-ration group (Figure 20, Table A1). Mean plasma triglycerides decreased between 14 and 21 days of reduced ration. Mean hematocrit and condition factor was lower only in the 21-day test group compared to the control; the 14-day reduced ration group did not differ from the controls for either. Mean condition factor was lower in the 21-day group and control. Gizzard shad collected in March of 2006 had lower mean condition factors ($K=7.4$) compared to the condition resulting from 21-days of reduced-ration treatment in this study ($K=8.1$).

Mean swimming performance and mean cortisol were significantly lower in the repeated 21-day reduced ration group compared to the initial 21-day reduced ration group (Table A3). No other differences were found between the repeated and initial group.

Threadfin Shad

Threadfin shad schooled and remained active during the reduced-ration test period. Groups fed a reduced ration did not have significantly different mean swimming performance compared to controls ($P=0.6051$) (Figure 18, Table A2). Treatment effects were not found in cortisol ($P=0.5975$) or chloride ($P=0.0845$) (Figure 19, Table A2).

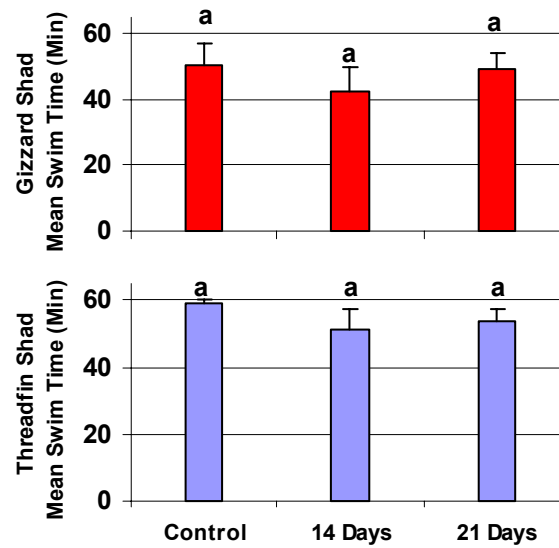


Figure 18. - Mean (+1 SE) swimming time of gizzard and threadfin shad exposed to cold shock after one of three protocols: 14 days of full ration, 14 days of reduced ration, or 21 days of reduced ration. Treatments that are statistically different ($P < 0.05$) have different letters.

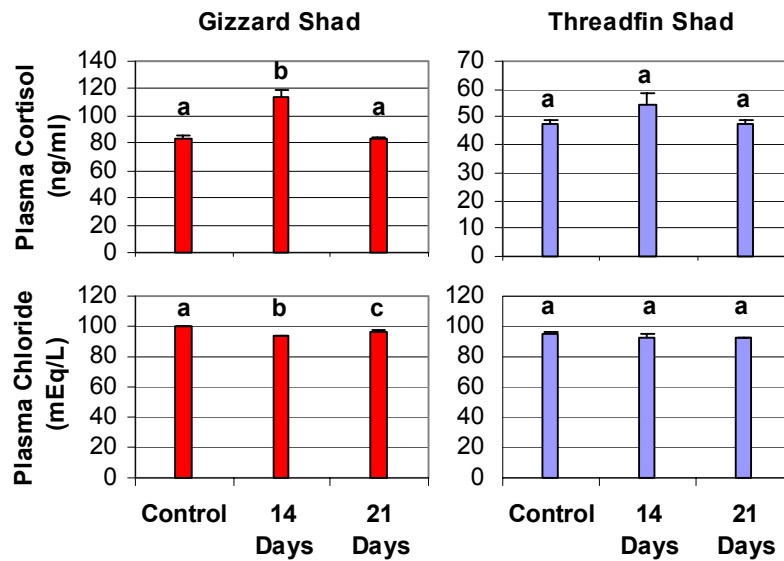


Figure 19. - Mean (+1 SE) plasma cortisol and chloride of gizzard and threadfin shad exposed to cold shock after one of three ration treatments: 14 days of full ration, 14 days of reduced ration, or 21 days of reduced ration. Treatments that are statistically different ($P < 0.05$) have different letters.

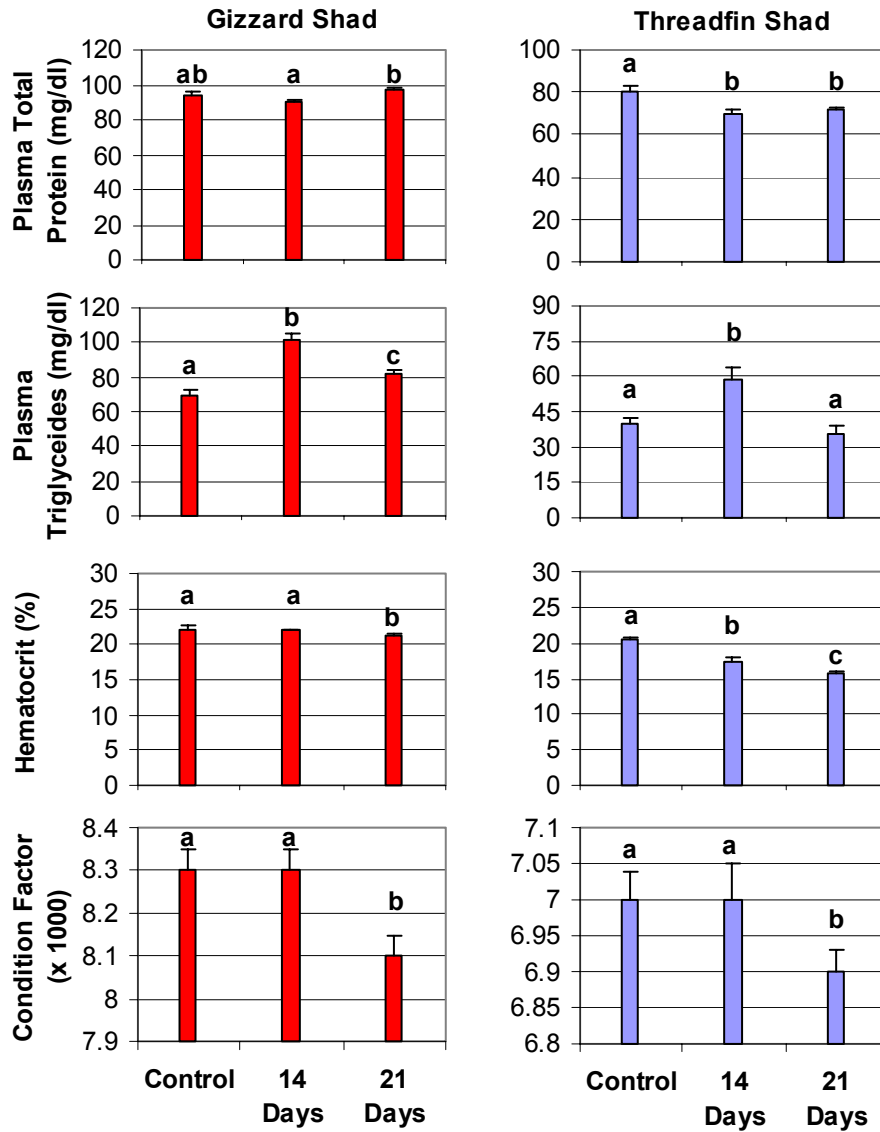


Figure 20. - Mean (+1 SE) condition factor, hematocrit, plasma total protein, and plasma triglycerides of gizzard and threadfin shad exposed to cold shock after one of three ration treatments: 14 days of full ration, 14 days of reduced ration, or 21 days of reduced ration. Treatments that are statistically different ($P < 0.05$) have different letters.

Treatment effects were observed in total protein ($P=0.0001$), triglycerides ($P<0.0001$), hematocrit ($P<0.0001$), and condition factor ($P<0.01$). Mean plasma total protein was significantly lower in the reduced ration groups than in the control, but there was no difference between the two reduced ration groups. Mean plasma triglycerides were significantly higher in the 14-day reduced-ration group than controls and 21-day reduced-ration group. Hematocrit was significantly lower in both reduced ration groups than the control and mean hematocrit was lower for the 21-day reduced-ration group than the 14-day reduced-ration group. Mean condition factor was significantly lower in the 21-day reduced-ration group compared to 14-day reduced-ration group and control which were not different (Figure 20, Table A1). Higher mean cortisol and lower mean triglycerides were the only differences between the repeated and initial 21-day reduced ration groups. Gizzard shad collected in March of 2006 had lower mean condition factors ($K=6.7$) compared to the condition resulting from 21-days of reduced-ration treatment in this study ($K=6.9$).

III. Discussion

The preliminary experiment yielded LOE temperatures within the reported range of previous studies for both species. None of the 18 gizzard shad lost equilibrium by the time 3.4°C was reached. The experiment was ended at this point due to time and equipment limitations. Since the initial LOE temperature for gizzard shad was not determined, we could not establish equivalent degrees above LOE for our further testing of both species. More testing is needed to identify the point of LOE of gizzard shad but the results for both species show similar changes in physiological and behavioral indicators at the temperatures I tested.

Signs of behavioral distress during cold shock prior to LOE or death in threadfin shad have been reported by others. Threadfin shad exposed to acute temperature declines began showing signs of behavioral distress as much as 5°C prior to mortality and a lack of response to movement and vibration 6-7°C above lethal temperatures (Griffith 1978). Moribund threadfin shad, exposed to 1-4°C temperature declines in 4 hours, swam individually rather than in schools prior to LOE (Griffith and Tomljanovich 1975). Studies reporting gizzard shad showing signs of distress or response prior to LOE were not found.

The swimming performance, cortisol, and chloride levels of gizzard shad changed in relation to cold shock treatment. I found that water temperatures of 5°C and less affect the swimming performance of gizzard shad and render this species more susceptible to impingement. Holding gizzard shad for an additional six hours at a test temperature did not result in a significantly lower mean swimming performance, but a response of

reduced performance with increased exposure is strongly suggested. Condition factor did not differ between any given groups of gizzard shad used in the swimming performance test. This is important because I collected gizzard shad over a three month period during which environmental factors could have influenced condition. Cortisol and chloride were positively correlated with swimming performance and could be potential indicators of susceptibility to impingement. Cortisol was expected to increase as a response to cold shock induced stress, which has been observed in similar studies. Hyvarinen et al. (2004) found increasing cortisol levels when brown trout were cold shocked in an ice bath. In most species, chloride changes are the inverse of cortisol but in this case chloride response was similar to cortisol and most likely the correlation found is not valid. Davis (2004) also documented the trend of increasing cortisol and decreasing chloride as a response to a stressor (acute confinement). All test groups of gizzard shad experienced increases in both cortisol and chloride. The cortisol level in the control group was higher than resting in most fish species (0-50 ng/ml), however no published research involving shad cortisol levels was found for comparison. The chloride values were lower in all groups of gizzard shad than the published range (100-130 mEq/liter) for unstressed fish (Barton et al. 2002). The acclimation period of one week to 15°C may not have been sufficient to stabilize osmoregulatory function in this species.

The swimming performance, cortisol, and chloride levels of threadfin shad responded to the cold shock treatment. The swimming performance of threadfin shad was similar to that of gizzard shad when exposed to cold shock treatments. Water temperatures of 7.5°C and less reduced the swimming performance of threadfin shad and possibly renders this species more susceptible to impingement than gizzard shad.

Similarly, a study by Griffith and Tomljanovich (1975) showed the ability of threadfin shad to resist impingement was severely impaired at temperatures below 8°C, but at higher temperatures impingement was slightly or not at all impaired. One individual in the control group did not swim the 60 minute maximum, which caused the 8.5°C test groups' means to not differ from the control. If fish were allowed to swim greater than 60 minutes, the 8.5°C mean probably would have differed from the control. Holding threadfin shad for an additional 3 or 6 hours at their test temperature did not result in a significantly lower mean swimming performance, but a response of reduced performance with increased exposure is strongly suggested. Condition factor differed between several groups of threadfin shad used in the swimming performance tests. However, condition factor was not correlated with mean swim time. Cortisol and chloride were not correlated with swimming performance. Cortisol was expected to increase as a response to cold shock induced stress, but I found the opposite response. The unexpected cortisol changes could be due to: 1) a reduced ability of shad to mount a stress response under abnormally cold temperatures or 2) the control group experienced unknown stressors such as reduced water quality or effects due to handling stress that resulted in higher cortisol levels. Strange (1980) documented the reduced ability of channel catfish to mount a stress response at cold temperatures. Davis (2004) also showed that fish held at colder temperatures had delayed responses in cortisol and chloride in comparison to those fish held at warmer temperatures. However, it is most likely that the control group was stressed for an unknown reason. If the cortisol levels were in the normal resting range of most fish species the expected changes (increase in response to stressor and decrease during acclimation) in cortisol would be present. The groups held for an additional 3 or 6

hours showed decreases in cortisol compared to those not held which could represent a recovery or acclimation to the cold temperatures. Strange (1980) showed that channel catfish became acclimated to stress after several days and cortisol declined. Similarly, Strange and Schreck (1978) showed that cortisol levels began to decrease 3.5 hours after a stressor was presented to juvenile Chinook salmon. The expected chloride relationship, an inverse of the cortisol response, was found in threadfin shad. All test groups experienced increases in chloride when cortisol decreased and vice versa. Similar to gizzard shad, the chloride values are lower in all groups than the published range (100-130 mEq/liter) for unstressed fish (Barton et al. 2002). The acclimation period of one week at 15°C may not have been sufficient to stabilize osmoregulatory function in this species.

The repeated cold shock test groups showed that cortisol levels were ~ 20 ng/ml lower in both species compared to the initial groups. The lower mean cortisol observed in repeated cold shock group of gizzard shad apparently did not affect their swimming performance because levels were still above normal resting values. However, the mean swimming performance of the repeated cold shock group of threadfin shad was affected by the decrease in cortisol and is possibly related to the lower cortisol value being similar to resting levels. Johansen et al. (1994) found that when cortisol levels of rainbow trout increased above resting levels due to a stressor (toxicant), swimming performance decreased. The drop in cortisol in both species could be related to the difference in time that tests were performed. The initial test groups were performed on shad that were collected at 27-28°C, whereas the repeated groups were collected when water temperatures were closer to 20°C. The experimental methods included a one week

acclimation period to ambient laboratory temperature of about 22°C followed by a week at 15°C, but this may not have been long enough to offset the difference in field acclimation temperature. This supports the need for more research involving acclimation temperature.

Previous studies on a variety of fish species indicated that swimming performance, total protein, triglycerides, hematocrit, and condition factor would decline as the duration of the reduced ration increased. Martinez et al. (2004) demonstrated that Atlantic cod (*Gadus morhua*) that were starved had a reduced swimming performance compared to cod that were fed. McMillan and Houlihan (1991) reported rainbow trout having reduced serum total protein after several days of fasting. Ruane et al. (2002) compared triglyceride levels in common carp (*Cyprinus carpio*) fed a high ration to those fed a low ration and found that triglyceride levels were lower in those fed a low ration. Adams et al. (1985) reported lower hematocrit and condition factor levels in stressed gizzard shad compared to unstressed shad. Gizzard and threadfin shad showed little response in mean swim time or physiological indicators after 14 and 21 days of reduced ration when subjected to cold shock. It is possible that a stress response occurred and the shad adapted to the lack of feeding or that the period of starvation was not long enough to illicit an affect. Since these species typically experience periods of low food availability in winter, possible adaptation to periods of reduced feeding could occur, therefore helping to explain, in part, the lack of a clear response in the lab to reduced ration. The repeated group (21-day reduced ration) for gizzard shad showed decreased cortisol levels and a decrease in swimming performance. However, the cortisol levels were still above normal resting values. The repeated group (21-day reduced ration) for

threadfin shad differed only in cortisol (which is probably not biologically significant with mean cortisol differences of only 6 ng/ml) and triglycerides.

To better understand the implications of reduced ration, I collected gizzard and threadfin shad from the Clinch River in March 2006. These fish had significantly lower condition factors, (7.4 for gizzard and 6.7 for threadfin shad) than those held for 21 days under reduced ration (8.1 for gizzard and 6.9 for threadfin shad). Therefore, even though the reduced ration period of 21 days did result in poorer condition of fish compared to controls, condition of fish in the lab did not approximate that of shad collected from the reservoir in late winter. If the condition of fish in the lab had been similar to that of shad collected in the field in late winter, the expected declines in nutritional status indicators and swimming performance may have occurred in those fish subjected to the cold shock treatment. Changes in hematocrit and condition factor were significant in threadfin shad after 21-days of reduced ration. This differential response between species is likely due to gizzard shad storing proportionately greater amounts of lipids than threadfin shad (Adams 1999).

Hematocrit and condition factor are relatively simple indicators that could be used in the field to rapidly determine susceptibility of shad to impingement. These are the types of rapid assessment indicators that would be necessary to demonstrate fish were impaired prior to impingement in order to meet EPA protocol requirements. However, the applicability of these measures and other easily and rapidly applied indicators of susceptibility should be further assessed by investigating physiology and swimming performance of shad whose condition simulates the condition of shad collected from the reservoir in late winter.

Several hybrid shad were found during the study. They closely resembled a threadfin shad with the exception of a blunt snout overhanging the lower jaw that is typical of a gizzard shad (Figures A4, A5). This is not uncommon: Minckley and Krumholz (1960) and Shelton and Grinstead (1973) documented cases of hybridization between these species. Data collected from these fish were not used in the analyses. Testing hybrid shad may be needed to determine if they respond differently to cold shock and reduced ration.

Practical Implications and Future Studies

The overall results of this study indicate that cold temperatures, particularly those above the temperatures of LOE, render gizzard and threadfin shad more susceptible to impingement. Potential indicators of susceptibility to impingement have been identified in this study (e.g., hematocrit and condition factor) and could be performed on fish deemed susceptible to impingement in the vicinity of intake structures. The use of multiple indicators of stress aids in accounting for or helping to explain confounding stressors which may be present in natural ecosystems.

This study has shown that gizzard and threadfin shad susceptible to impingement could be classified as either impaired or moribund. It is assumed that moribund fish would not recover and die regardless of impingement. Impaired shad could have recovered if environmental conditions improved and therefore would not have died if not impinged. However, impaired shad are more susceptible than healthy shad to natural predation. In comparison to natural predation, power plants could be considered selective predators that are removing the weak individuals from the population that would likely have been predated. Several studies have related cold shock to increased predation rates

in fish (Coutant et al. 1974; Coutant et al. 1976; Wolters and Coutant 1976). The current USEPA regulations state that accounting for moribund fish can be used to meet impingement reductions of 80-95% at existing intakes. The USEPA could consider revising the moribund classification of fish to include environmentally impaired shad as well, based on temperatures a few degrees above the species' LOE temperatures. However, changing the classification of fish that can be used to meet impingement reductions must be supported with strong scientific evidence of the predation relationship. The issue of impaired fish versus moribund fish would be more significant at existing intakes located in warmer climate areas where moribundity is less likely.

Using physiological and performance-level indicators to assess impingement susceptibility appears promising, but further studies are necessary to evaluate the relative importance of cold shock regimes and nutritional status to impingement susceptibility. More testing is needed for both species on the effects of: 1) rate of temperature decline relative to the acclimation temperature, 2) lower acclimation temperatures relative to the cold shock test temperatures, and 3) a longer acclimation period prior to testing. Further research addressing the role and importance of nutritional status should include: 1) longer reduced ration treatments using fish collected in late summer, 2) combining several different cold shock temperatures with the reduced ration treatment, 3) the recovery of fish held under reduced ration and cold shocked. This type of information would clarify the relationship of the physiological indicators to the susceptibility of impingement of shad.

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Appendix

Table A1. - Mean ($\pm$ SE) swimming performance, plasma cortisol, and plasma chloride of gizzard and threadfin subjected to cold shock. Groups of 34 gizzard shad were cold shocked at 0.5°C per hour to one of the following sampling temperatures: 5°C and held for 0 hours, 5°C and held for 6 hours, 4°C and held for 0 hours, or 4°C and held for 6 hours. Groups of 24 threadfin shad were cold shocked at 0.5°C per hour to one of the following sampling temperatures: 8.5°C and held for 0 hours, 8.5°C and held for 6 hours, 7.5°C and held for 0 hours, or 7.5°C and held for 3 hours. Controls for both species were sampled at their acclimation temperature of 15°C. Treatments that are statistically different ($P < 0.05$) have different letters.

Treatment Group	Swimming Performance (minutes)		Plasma cortisol (ng/ml)		Plasma chloride (mEq/liter)	
Gizzard Shad						
Control	60.0 $\pm$ 0.0	a	58.8 $\pm$ 5.3	a	91.4 $\pm$ 0.7	a
5°C + 0Hrs	43.0 $\pm$ 5.4	b	79.1 $\pm$ 2.5	b	94.9 $\pm$ 0.6	b
5°C + 6Hrs	36.6 $\pm$ 8.9	b	77.4 $\pm$ 4.8	b	94.4 $\pm$ 0.3	b
4°C + 0Hrs	17.0 $\pm$ 7.5	c	102.2 $\pm$ 1.9	c	95.6 $\pm$ 0.6	b
4°C + 6Hrs	4.9 $\pm$ 1.1	c	89.7 $\pm$ 4.8	d	95.8 $\pm$ 0.5	b
Threadfin Shad						
Control	54.9 $\pm$ 3.5	a	85.6 $\pm$ 5.9	a	91.3 $\pm$ 1.1	ac
8.5°C + 0Hrs	47.1 $\pm$ 6.7	a	54.4 $\pm$ 2.6	b	93.0 $\pm$ 0.8	ab
8.5°C + 6Hrs	38.4 $\pm$ 6.1	ab	32.1 $\pm$ 2.4	c	94.3 $\pm$ 0.4	b
7.5°C + 0Hrs	18.2 $\pm$ 10.8	bc	62.0 $\pm$ 6.6	b	90.5 $\pm$ 1.4	c
7.5°C + 3Hrs	16.4 $\pm$ 6.5	c	32.9 $\pm$ 2.5	c	93.8 $\pm$ 1.0	b

Table A2. - Mean (SE) condition factor, plasma cortisol, chloride, total protein, and triglycerides of gizzard and threadfin shad fed reduced rations and cold shocked. Groups of 18 gizzard shad or 24 threadfin shad were fed a 0.5% body weight ration per day for 14 or 21 days. Gizzard shad were then cold shocked at 0.5°C per hour to 5°C and held for 0 hours. Threadfin shad were then cold shocked at 0.5°C per hour to 8.5°C and held for 0 hours. Controls for both species were fed a full ration. Treatments that are statistically different (P<0.05) have different letters.

Treatment Group	Swimming Performance (minutes)	Plasma cortisol (ng/ml)	Plasma chloride (mEq/liter)	Plasma Total Protein (mg/dl)	Plasma Triglycerides (mg/dl)	Hematocrit (%)	Condition Factor
Gizzard Shad							
Control	50.3 ± 6.5 a	83.3 ± 2.6 a	99.8 ± 0.9 a	94.2 ± 1.9 a b	69.6 ± 2.9 a	22.2 ± 0.4 a	8.3 ± 0.05 a
14 Days	42.5 ± 7.2 a	113.2 ± 5.6 b	93.5 ± 0.8 b	90.0 ± 1.4 a	101.7 ± 3.2 b	22.0 ± 0.2 a	8.3 ± 0.05 a
21 Days	48.9 ± 5.1 a	82.5 ± 2.5 a	96.4 ± 0.7 c	96.7 ± 1.1 b	82.4 ± 1.8 c	21.2 ± 0.2 b	8.1 ± 0.05 b
Threadfin Shad							
Control	59.2 ± 0.9 a	47.5 ± 1.6 a	95.4 ± 0.9 a	80.0 ± 2.6 a	39.9 ± 2.6 a	20.5 ± 0.3 a	7.0 ± 0.04 a
14 Days	51.0 ± 6.4 a	54.6 ± 4.1 a	93.0 ± 1.4 a	70.0 ± 1.7 b	58.3 ± 5.0 b	17.5 ± 0.4 b	7.0 ± 0.05 a
21 Days	53.3 ± 3.6 a	47.5 ± 1.5 a	92.4 ± 0.7 a	71.3 ± 0.9 b	36.0 ± 2.8 a	15.8 ± 0.2 c	6.9 ± 0.03 b

Table A3. – A comparison of dependent variable means of original and repeated test groups. The 4°C test group (cold shock) and the 21 day test group (reduced ration and cold shock) were repeated in gizzard shad. The 8.5°C + 6 hrs test group (cold shock) and the 21 day test group (reduced ration and cold shock) were repeated in threadfin shad. Significant differences ($P < 0.05$) in means are indicated by asterisks with the p-value in parentheses.

Dependent Variables (test group repeated)	Original Treatment	Repeat Treatment	Significance ($P < 0.05$)
Cold Shock			
Gizzard Shad (4°C test group)			
Swim Time	42.33	43.60	(0.9382)
Cortisol	88.60	69.52	*(< 0.0001)
Chloride	95.92	93.90	(0.0871)
Threadfin Shad (8.5°C + 6 hrs test group)			
Swim Time	22.32	54.56	*(0.0049)
Cortisol	42.63	21.64	*(0.0001)
Chloride	94.28	94.25	(0.9568)
Reduced Ration + Cold Shock			
Gizzard Shad (21 day test group)			
Swim Time	60.00	37.85	*(0.0250)
Cortisol	93.95	71.08	*(< 0.0001)
Chloride	96.50	96.23	(0.8498)
Total Protein	95.83	97.50	(0.4605)
Triglycerides	83.42	81.42	(0.5877)
Hematocrit	21.33	21.00	(0.3960)
Condition Factor	8.64	8.35	(0.8743)
Threadfin Shad (21 day test group)			
Swim Time	51.28	55.40	(0.3722)
Cortisol	44.54	50.51	*(0.0400)
Chloride	93.42	91.44	(0.1453)
Total Protein	70.00	72.50	(0.1670)
Triglycerides	45.08	27.00	*(0.0008)
Hematocrit	15.67	16.00	(0.2891)
Condition Factor	7.24	7.21	(0.1248)



Figure A4. - Two hybrid shad at top of picture followed by three threadfin shad and a single gizzard shad. Notice the similarity in fin coloration but different snout shape. The hybrid also had the long dorsal fin filament (not visible in picture) characteristic of a threadfin shad.



Figure A5. – Gizzard shad (top) compared to two hybrid shad (middle and bottom). Notice the yellow fin coloration, rounded overhanging snout, and long dorsal filament.

VITA

Brooks Fost was born in Warfordsburg, PA on October 3, 1981. He was raised there and attended elementary and high school at Southern Fulton. He graduated from Southern Fulton High School in 1999 with strong interests in the environment and wildlife. From there, he went to the University of Tennessee, Knoxville and received a B.A. in Wildlife and Fisheries Sciences with a minor in forestry and a M.A. in fisheries in 2006.

Brooks is currently employed by Oak Ridge National Laboratory and is searching for a doctoral project opening in fisheries. He is continuing his shad research until a doctorate position is assumed.